Dopamine D₅ Receptors in Nucleus Accumbens Contribute to the Detection of Cocaine in Rats

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Dopamine D₁/D₅ receptor antagonism has been shown to block the euphoric and stimulatory effects of cocaine in humans and rats. In the present study, rats trained to discriminate the presence of cocaine (10 mg/kg) from its absence were used to analyze the functional contribution of D₁ (D₁R) versus D₅ (D₅R) receptors in the nucleus accumbens, an important neural site for the actions of cocaine. Bilateral microinfusion into the nucleus accumbens of an antisense oligonucleotide directed at the D₅R (0.75 nmol/0.3 μ l per side, two times per day for 3 d) elicited a downward shift in the dose–effect curve for cocaine with a suppression of peak efficacy; the dose of cocaine estimated to elicit 50% drug-lever responding (ED₅₀) was 6.71 mg/kg when assessed 12 hr after the D₅R antisense oligonucleotide compared to the control ED₅₀ of 1.83 mg/kg and to the ED₅₀ of 1.75 mg/kg established 7 d after the last D₅R antisense

oligonucleotide infusion. The D₁R antisense and scrambled oligonucleotide (0.75 nmol/0.3 μ l per side, two times per day for 3 d) were both ineffective. Thus, using drug discrimination techniques that model the subjective effects of cocaine, we show that responsiveness to cocaine is dramatically attenuated after interference with the process of translation of the D₅R mRNA to its protein product. These findings suggest that D₅R is a functionally important target site for the indirect actions of cocaine and that rigorous investigations of the function of D₅R may help guide the discovery of strategies for pharmacotherapy in cocaine dependence.

Key words: behavior; cocaine; D₁ receptor; D₅ receptor; D1a receptor; D1b receptor; discriminative stimulus effects; dopamine

Cocaine abuse continues to impose serious medical, psychological, and criminal challenges for society. A thorough understanding of the neural basis underlying the effects of cocaine is critical to the development of science-based treatment protocols for cocaine dependence. One primary target for the actions of cocaine is the mesocorticolimbic dopamine circuit (Callahan et al., 1997; McBride et al., 1999), which has been implicated in reward, emotional, and motivational processes, as well as in psychosis and mania (Kalivas and Nemeroff, 1988). Dopamine acts at D₁- and D₂-like receptors, each of which is comprised of at least two receptor subtypes. The D₁-like receptor family includes the D₁ receptor (D₁R; also known as D1a) and D₅ receptor (D₅R; also known as D1b), which are separate gene products, exhibiting differential anatomical localization, but pharmacologically indiscriminable transduction pathways and agonist/antagonist profiles (Baldessarini and Tarazi, 1996). Oral administration of the D₁R/D₅R antagonist ecopipam (SCH 39166) was shown to significantly attenuate the euphoric and stimulatory effects of cocaine in humans (Romach et al., 1999). This recent observation in humans is consistent with findings that systemic administration of the D₁R/D₅R antagonist SCH 23390 blocked the locomotor stimulant, discriminative stimulus and reinforcing effects of cocaine in rodents (Callahan et al., 1991; Caine and Koob, 1994; Tella, 1994). In addition, intra-accumbens microinfusion of SCH 23390 completely antagonized the interoceptive (Callahan et al., 1997) and reinforcing effects of systemically administered cocaine (Caine et al., 1993; McGregor and Roberts, 1993). However,

because SCH 23390, as well as other nonselective D₁-like receptor antagonists, has almost equal affinity for D₁R and D₅R, the contribution of each receptor subtype to the behavioral effects of cocaine has received little attention. In fact, because of the lack of selective ligands, the relative functional contributions of the D₁R versus D₅R remain uncharacterized *in vivo*. With the cloning of both the D₁R and D₅R genes (Monsma et al., 1990; Sunahara et al., 1991), the application of molecular biological tools to this question has been possible, in the forms of analysis of D₁R knock-out mice (Xu et al., 1994) and the use of antisense oligonucleotides directed at the translation initiation sequences of the rat D₁R and D₅R genes (Zhang et al., 1994; Dziewczapolski et al., 1998).

In the present study, we used the drug discrimination technique as a rodent model of the subjective effects of cocaine in humans (Schuster and Johanson, 1988; Drummond et al., 1995). In this assay, saline or cocaine (10 mg/kg) is injected and the rat must press one of two levers in the operant chamber to obtain a water reinforcer based on the recognition of the preceding injection. For example, when completion of the fixed ratio of 20 presses on the cocaine-associated lever follows the cocaine injection, the animal is “correct”, and a water reinforcer is delivered. On saline

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sessions, completion of a fixed ratio of 20 presses on the saline-appropriate lever is reinforced with water. To analyze the relative role of D₁R versus D₅R in the discriminative stimulus effects of cocaine (10 mg/kg), a cumulative dose–response relationship (Schechter, 1997) for cocaine was established before and after microinfusion of antisense oligonucleotides directed at either D₁R or D₅R mRNA into the shell of the nucleus accumbens, which has been shown to be particularly sensitive to the actions of cocaine (Pontieri et al., 1995; McBride et al., 1999). We found that a selective and reversible loss of recognition of cocaine resulted from intra-nucleus accumbens pretreatment with the D₅R (but not D₁R) antisense oligonucleotide.

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MATERIALS AND METHODS

Animals. Adult male Sprague Dawley rats ($n = 34$; Harlan, Houston, TX) weighing between 300 and 350 gm at the beginning of the study were used. The rats were housed in pairs in a colony room that was maintained at a constant temperature (21–23°C) and humidity (40–50%); lighting was maintained on a 12 hr light/dark cycle (7:00 A.M.–7:00 P.M.). The amount of water each animal received during drug discrimination studies was restricted to that given during operant training sessions, after test sessions (10–15 min), and on weekends (36 hr). All experiments were conducted during the light phase (between 9:00 A.M. and 3:00 P.M.). All experiments were performed in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Apparatus. The procedures were conducted in commercially available, two-lever operant chambers (model 80001; Lafayette Instrument, Lafayette, IN). Each chamber, housed in a light- and sound-attenuating cubicle (model 80015; Lafayette Instrument), was equipped with a water-filled dispenser mounted equidistant between two response levers on one wall. A 28 V house light provided illumination, and a blower supplied ventilation and masking noise. An interface (MedAssociates, St. Albans, VT) connected the chambers to a computer that controlled and recorded all experimental events.

Procedures. Standard two-lever, water-reinforced drug discrimination procedures were used (Cunningham et al., 1985). Rats were injected intraperitoneally with cocaine (10 mg/kg) or saline (1 ml/kg) 15 min before daily sessions. During this phase, only the stimulus-appropriate (drug or saline) lever was present. Training began under a fixed ratio 1 (FR 1) schedule of water reinforcement, and the FR requirement was incremented until all animals were responding reliably under an FR 20 schedule for each experimental condition. Both levers were then presented simultaneously, and rats were required to respond on the stimulus-appropriate lever to obtain reinforcement (“discrimination” training).

Test protocols. When rats demonstrated individual accuracies of at least 80% correct responses before the first reinforcer for 10 consecutive sessions, test sessions were initiated, and training sessions were run during the intervening days to maintain discrimination accuracy. Because only a narrow time frame was available in which to assess oligonucleotide-evoked alterations, we used a cumulative dose–response procedure (Schechter, 1997). The series began with an injection of saline and introduction to the operant chamber 10 min later. After completion of 20 responses on either lever (occurring within a few seconds), a single reinforcer was delivered, and the house lights were turned off; as the rat was removed from the chamber, the first dose of cocaine (0.625 mg/kg) was administered. Access to the operant chamber occurred 10 min later, and rats were again removed after accumulation of 20 responses on either lever. The series continued with another injection of cocaine (0.625 mg/kg) and testing; thus, the sequential cocaine dosing schedule was 0 (saline), 0.625, 0.625, 1.25, 2.5, and 5 mg/kg tested at 10 min intervals to achieve doses of 0, 0.625, 1.25, 2.5, 5, and 10 mg/kg of cocaine. The dose–response curve for cocaine established using the cumulative dosing procedure did not differ from that initially determined (after acquisition) with bolus injections of cocaine (0.625, 1.25, 2.5, 5, or 10 mg/kg) administered 15 min before rats were tested for lever selection (data not shown) (Callahan et al., 1991). After testing, the rats were returned to the colony and allowed *ad libitum* access to water for 10 min beginning 30 min after the end of each test.

Surgery and cannulae implantation. Rats were anesthetized with an intramuscular injection of 8.6 mg/kg of xylazine, 1.5 mg/kg of acepromazine, and 43 mg/kg of ketamine in physiological saline (0.9% NaCl). Bilateral guide cannulae (26 gauge) were stereotactically implanted 2 mm above the nucleus accumbens shell (anteroposterior, -1.7 mm from bregma; mediolateral, ± 0.75 mm; dorsoventral, -6 mm) (Paxinos and Watson, 1998). After surgery, animals were injected twice with penicillin (10,000 U/kg, i.m.) and were allowed a 1 week recovery period during which each rat was handled and weighed daily.

Microinfusion protocols. After recovery, discrimination training was reinstated for several weeks, and the cumulative dose–response curve for cocaine was reestablished and did not differ from that established before surgery (data not shown); the postsurgical dose–response curve served as control in the present experiment. During that period, rats were handled for 10–15 min each day for 5 d to familiarize them with the microinfusion procedure. Intracranial infusions (0.75 nmol/0.3 μ l per side, two times per day for 3 d) were administered through 33 gauge internal cannulae that extended 2 mm below the tips of guide cannulae into the shell of the nucleus accumbens. The injection volume of 0.3 μ l per side was infused over a 3 min period at 0.1 μ l per min using a micropump (HoneyBee Pump; BAS, West Lafayette, IN). Injection cannulae remained in place for an additional 1 min to allow for diffusion away from the cannulae tips. Twelve hours and 7 d after the intracranial infusion procedure was completed, the cumulative dose–response curve for cocaine was reestablished. At the completion of the study, rats were killed by an overdose of chloral hydrate (800 mg/kg, i.p.), and tissue sections were processed for localization of cannulae. Only those animals whose cannulae were within the shell of the nucleus accumbens were included for statistical analysis (D₁R rats, $n = 7$; D₅R rats, $n = 8$; SCR rats, $n = 8$). No significant tissue damage was evident after histological examination of sections.

Drugs. Cocaine HCl (National Institute of Drug Abuse, Rockville, MD) was dissolved in saline (0.9% NaCl). Antisense oligonucleotides (Life Technologies, Gaithersburg, MD) were designed against the translation initiation sequences of the rat D₁R (5'-TAGGAGCCATCTTCCAG-3') and D₅R sequences (5'-CAGCATGTGCGCTGAGT-3'). A scrambled oligonucleotide (SCR; 5'-ATACTTCACGCCGATGG-3') was designed that had thermodynamic properties similar to these antisense oligonucleotides. Based on a FastA search of GenBank, these oligonucleotides did not have significant homologies with any relevant, known cDNA sequences. The HPLC-purified oligonucleotides were dissolved in sterile saline.

Statistical analyses. Performance in the drug discrimination task was expressed as the percentage of drug-appropriate responses to total responses before delivery of the first reinforcer, and the response rate was calculated as the total number of responses on either lever divided by the number of minutes taken to complete the FR 20. Only data from animals that completed the FR 20 during the test sessions were used. A two-way ANOVA for repeated measures was used to analyze the effects of oligonucleotide pretreatment and cocaine dose on the percentage of drug-lever responding and response rate. Because treatments with D₁R antisense and scrambled oligonucleotide were not associated with any significant main effects or interactions, the results of these ANOVAs are not presented. The methods of Litchfield and Wilcoxon (1949) were used to estimate the dose of cocaine predicted to elicit 50% cocaine-appropriate responding (ED₅₀) and its 95% confidence intervals (Tallarida and Murray, 1986).

RESULTS

Assessment of the dose–effect curve for cocaine 12 hr after the last injection of the antisense oligonucleotide targeted to the D₅R (0.75 nmol/0.3 μ l per side, two times daily for 3 d) indicated that the recognition of cocaine was significantly impaired (Fig. 1*B*, gray squares) in comparison to the control dose–effect curve for cocaine (Fig. 1*B*, open circles). Two-way ANOVA indicated a significant effect of treatment ($F_{(2,21)} = 3.94$; $p = 0.035$), dose ($F_{(5,105)} = 34.3$; $p = 0.001$) and a significant treatment $\times$ dose interaction on cocaine-lever responding ($F_{(10,105)} = 3.07$; $p = 0.002$); no significant differences were observed for the response rate measure. A complete recovery was observed as shown by the fact that the cumulative dose–effect curve for cocaine assessed 7 d after the termination of the D₅R antisense oligonucleotide treatment (Fig. 1*B*, filled triangles) did not differ from the control dose–effect curve. The cocaine dose estimated to elicit 50% drug-lever responding (ED₅₀) was 6.71 mg/kg when assessed 12 hr after the D₅R antisense oligonucleotide compared to the control ED₅₀ of 1.83 mg/kg and to the ED₅₀ established 7 d after the last D₅R antisense oligonucleotide infusion (1.75 mg/kg; Table 1). The effects elicited by the D₅R antisense oligonucleotide appear to be specific, because neither the D₁R antisense (Fig. 1*A*) nor the scrambled oligonucleotide (Fig. 1*C*) altered the discriminative stimulus effects of cocaine (Table 1).

DISCUSSION

Impairment of the translation of D₅R mRNA to its protein product in the nucleus accumbens resulted in the failure of trained rats to recognize the subjective effects of systemically

Figure 1. D₅R antisense oligonucleotide pretreatment evokes a rightward shift in the dose–response relationship for cocaine. The cumulative dose–response curve for cocaine (intraperitoneally) is shown for rats before the start of oligonucleotide infusion (Control; open circles), 12 hr (Oligo; gray squares), and 7 d after the last infusion of the oligonucleotide (Recovery; filled triangles). Symbols connected by solid lines denote the mean percentage of cocaine-appropriate responses during the test session ($\pm$ SEM; left ordinate); symbols connected by dotted lines denote the mean response rate per minute ($\pm$ SEM; right ordinate). For comparison, the percentage of cocaine-lever responding (SAL, left of each panel) and response rate (SAL, right of each panel) observed after saline test administered immediately before the start of cumulative dose–response testing are included. Pretreatment with the D₁R (A) and SCR (C) did not significantly alter cocaine-lever responding or response rates (results of analyses not shown).

Table 1. Pretreatment with D₁R and D₅R antisense and SCR oligonucleotides: ED₅₀ values for cocaine

Oligonucleotide	Control ED ₅₀ (CI)	Treatment ED ₅₀ (CI)	Recovery ED ₅₀ (CI)
D ₁ R	1.81 (0.85–3.84)	1.67 (0.79–3.55)	1.80 (0.92–3.51)
D ₅ R	1.83 (0.86–3.94)	6.71 (3.28–13.76)	1.75 (0.81–3.79)
SCR	1.67 (0.92–2.99)	1.75 (0.84–3.63)	1.65 (0.82–3.34)

The dose (mg/kg) predicted to elicit 50% drug-lever responding and its 95% confidence intervals (CI) were calculated using the methods of Litchfield and Wilcoxon (Tallarida and Murray, 1986).

administered cocaine. Our findings suggest that actions at D₅R may underlie the efficacy of D₁-like receptor antagonists to block the euphoric and stimulatory effects of cocaine in humans (Romach et al., 1999) and animals (Callahan et al., 1991; Caine and Koob, 1994; Tella, 1994), particularly those effects mediated by the nucleus accumbens (Caine et al., 1993; McGregor and Roberts, 1993; Callahan et al., 1997). However, although our findings support an important role for D₅R in the behavioral effects of

cocaine, the failure of the D₁R antisense oligonucleotide to modify the stimulus effects of cocaine does not categorically rule out a role for D₁R; even in the face of a knockdown of D₁R, a population of spare D₁R in the nucleus accumbens might be capable of maintaining normal D₁R function.

A D₁R antisense oligonucleotide similar to that used in the present study was shown to inhibit both grooming behavior evoked by the D₁R-like agonist SKF 38393 in intact mice (Zhang et al., 1994) and SKF 38393-evoked rotational behavior in either mice (Zhang et al., 1994) or rats (Dziewczapolski et al., 1998) with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway. An opposite role for D₅R in the control of locomotor activity was suggested by the recent finding that SKF 38393-evoked rotational behavior in 6-OHDA-lesioned rats was facilitated after intracerebroventricular infusion of a D₅R antisense oligonucleotide (Dziewczapolski et al., 1998). In the present study, an important role for the D₅R in mediating the stimulus effects of cocaine is suggested by the selective loss of recognition of cocaine after pretreatment with the D₅R antisense oligonucleotide into the nucleus accumbens shell and a subsequent complete recovery. We did not analyze presumed loss of D₁R and D₅R protein after oligonucleotide administration because of the limited availability of selective D₁R and D₅R antibodies and the noted lack of correlation between functional parameters and reductions in protein levels. For example, both behavioral and physiological responses have been shown to be altered after knockdown conditions in the absence of significant changes in receptor density (Wahlestedt et al., 1993; Zhou et al., 1994). In fact, a previous study had demonstrated that D₁R and D₅R antisense oligonucleotide administration significantly altered behaviors evoked by the D₁R/D₅R agonist SKF 38393 in the absence of a detectable loss of receptor protein (Dziewczapolski et al., 1998). In contrast, a recent analysis of D₁R and D₅R-like immunoreactivity in the ventral tegmental area after intraparenchymal infusion of a similar D₁R or the identical D₅R antisense oligonucleotide to those used here identified a significant loss of D₁R and D₅R protein in this region, respectively (Frye and Vongher, 1999).

Our evidence indicates that the D₅R in nucleus accumbens plays an important role in the actions of cocaine. The abundance of D₅R mRNA and protein in the striatal complex of rats and primates, however, has been described as relatively low (Tiberi et al., 1991; Meador-Woodruff et al., 1992; Bergson et al., 1995; Ariano et al., 1999; Luedtke et al., 1999). Interestingly, selective protein–protein coupling between the D₅R and GABA_A-ligand-gated channel has been identified and shown to enable mutually inhibitory functional interactions in cultured hippocampal cells (Liu et al., 2000). Such an interaction may underlie the observation that stimulation of a D₁-like receptor modulates GABA_A receptor-mediated synaptic activity in striatal neurons (Yan and Surmeier, 1997). Furthermore, numerous recent observations suggest that GABAergic control of striatal function is integral in the neural response to cocaine administration (Gerasimov et al., 1999; Jung et al., 1999; Resnick et al., 1999). Thus, the attenuation of cocaine-induced behavior seen after D₅R knockdown may be amplified by an operational disruption of the coupled function between D₅R and GABA_A receptor channels, allowing the apparently limited number of D₅R in striatal regions to play an important role in the control of behavior.

In summary, using drug discrimination techniques that model the subjective effects of cocaine, we show that responsiveness to cocaine is dramatically attenuated by knockdown of the D₅R in the nucleus accumbens. These findings suggest that D₅R is a functionally important target site for the indirect actions of cocaine. Based on the known parallel between the discriminative stimulus properties of cocaine in animals and its interoceptive effects in humans as well as the prominent role of interoceptive stimuli in the initiation and maintenance of drug-seeking behavior,

ior (Schuster and Johanson, 1988; Drummond et al., 1995), the present data suggest that rigorous investigations of the role of D₅R in drug dependence and psychiatric disorders are warranted and may help guide the discovery of D₅R-targeted strategies for effective pharmacotherapies.

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