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ORIGINAL INVESTIGATION

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Cocaine-induced locomotor activity and cocaine discrimination in dopamine D_4 receptor mutant mice

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Abstract *Rationale:* Previous studies have found a role for dopamine D_2-like receptors in many of the behavioral effects of cocaine, including its stimulation of locomotor activity and interoceptive discriminative-stimulus effects. However, given the lack of selectivity of most of the available pharmacological tools among D_2, D_3 and D_4 dopamine receptors, the roles of these specific receptors remain unclear. *Objectives:* The roles of specific dopamine D_4 receptors in the behavioral effects of cocaine, including its locomotor stimulant and interoceptive discriminative-stimulus effects were investigated using dopamine D_4 receptor knockout (DA D_4R KO) and wild-type (WT) mice. *Methods:* The mice were trained in daily sessions to discriminate IP injections of saline from cocaine (10 mg/kg). Responses on one of two response keys intermittently produced a food pellet; one response was reinforced in sessions following cocaine injection (10 mg/kg), and the other response was reinforced in sessions following saline injection. Each 20th response produced a food pellet (fixed-ratio, or FR20 schedule of reinforcement). The dose-effects of cocaine and its interaction with the D_2-like antagonist, raclopride, were assessed. Horizontal locomotor activity was also assessed in each genotype. *Results:* As previously shown), cocaine was a more potent stimulant of locomotor activity in the DA D_4R KO mice compared to WT littermate mice. In addition, cocaine was more potent in producing discriminative-stimulus effects in DA D_4R KO mice (ED_{50} value=0.50 mg/kg) compared to their WT littermates (ED_{50} value=2.6 mg/kg). Raclopride shifted the cocaine dose-effect curve in both DA D_4R KO and WT mice, though the shift was greater for the DA D_4R KO mice. *Conclusions:* The present results on the stimulation of activity and interoceptive/subjective effects of cocaine are consistent with the previously reported disregulation of dopamine synthesis in DA D_4R KO mice, and further suggest a role of the DA D_4R in vulnerability to stimulant abuse.

Keywords Cocaine · Locomotor activity · Discrimination · Dopamine receptors · Mouse

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Introduction

The role of subtypes of dopamine receptors in drug abuse has been a subject of interest, both from the basic and applied perspectives. Pharmacological tools have been available for some time to differentiate between the major groups of D_1-like and D_2-like dopamine receptors; however within these groups, there has been difficulty in discerning respective roles due to a lack of exquisitely selective agonists and antagonists.

Genetic engineering promises to provide an alternative approach that, when coupled with pharmacology, can provide answers to questions regarding the respective roles of dopamine receptor subtypes in various behavioral effects of cocaine related to its abuse. For example, the role of D_2 dopamine receptors in the locomotor activating, subjective (Chausmer et al. 2002), and reinforcing (Caine

et al. 2002) effects of cocaine has been recently examined. These studies suggest that the D_2 receptor is related in a fundamental way to both the locomotor stimulant effects of cocaine and its subjective effects. However, its role in the reinforcing effects of cocaine is more difficult to discern, due to the complex changes in the cocaine dose-effect curve (Caine et al 2002).

The present study examined the role of the DA D_4R in the locomotor stimulant and discriminative stimulus effects of cocaine. Several previous studies suggest a role for the D_4 DA receptor in drug abuse. For example, an association between polymorphisms of the DA D_4R gene and a trait for "novelty seeking" has been reported (e.g. Benjamin et al. 1996), and this trait has been suggested by some to underlie drug abuse and other forms of risk-taking behavior in humans. Not unexpectedly, a genetic linkage between novelty seeking and drug abuse in humans remains controversial (e.g. Vandenberg et al. 1997). Of more direct relevance, an association has been reported between DA D_4R and drug abuse. For example, there is an association between DA D_4R polymorphisms and alcohol (George et al. (1993) and opiate abuse (Kotler et al. 1997). However, the linkage, if any, between these genes and a complex behavior is not a simple one (e.g. Comings et al. 1999), and a study in mice has found no difference in alcohol consumption between DA D_4R KO and WT mice (Falzone et al. 2002).

Preclinical data also suggest some effects of the DA D_4R that may be related to drug abuse. The patterns of DA D_4R localization indicate that sites related to drug abuse, e.g. nucleus accumbens, contain DA D_4R (e.g. Defagot et al. 2000), although the pattern of D_4R distribution differs from other DA receptors (e.g. Ariano et al. 1997). A study by Dulawa et al. (1999) shows that DA D_4R KO mice avoid novel objects placed in a familiar open field. These preclinical data are consistent with a hypothesis relating the DA D_4R and novelty seeking. In addition, DA D_4R KO mice are more sensitive to the locomotor stimulating effects of cocaine than WT littermates (Rubinstein et al. 1997). These changes in the effects of cocaine are accompanied by elevations in DA synthesis in D_4R KO mice without changes in D_1-like and D_2-like binding parameters in striatum compared to littermate controls (Rubinstein et al. 1997).

The present study sought to compare further the effects of cocaine in DA D_4R KO mice and their WT littermate controls. Like Rubinstein et al., we examined the stimulation of locomotor activity produced by acute doses of cocaine, and in addition, we also examined the subjective effects of cocaine, as indicated by training mice with cocaine as a discriminative stimulus (e.g. Chausmer et al. 2002). These effects of cocaine are considered by many to be related to its abuse liability. In the cocaine discrimination procedure, the interactions of cocaine and the D_2-like antagonist, raclopride were assessed. This antagonist was chosen for its difference in affinity between DA D_2 and D_4 receptors.

Materials and methods

Subjects

The mice were at least 8 weeks of age at the start of the study and were derived from the mating of DA D_4R heterozygotes (129/Ola×C57Bl/6J) for more than ten generations (Rubinstein et al. 1997). Male DA D_4R WT and DA D_4R KO offspring from matings of these heterozygotes weighed 32.4±0.81 and 28.3±0.48 g, respectively. The subjects were deprived of food, and fed a daily ration of Purina rodent chow to maintain them at 85% of these unrestricted-feeding weights. Body weights were increased by 5% every 30 days to account for normal growth. Water was freely accessible at all times except during testing. Subjects were individually housed in a temperature- and humidity-controlled vivarium, with a 12 h light/dark cycle (lights on 0700 hours).

Locomotor activity testing

Subjects were tested daily, 5 days per week in 40 cm^3 clear acrylic chambers. The chambers were placed inside monitors (Omnitech Electronics, Columbus, Ohio, USA) that were equipped with light sensitive detectors, spaced 2.5 cm apart along two perpendicular walls. Mounted on the opposing walls were infrared light sources that were directed at the detectors. One count of horizontal activity was registered each time the subject interrupted a single beam. Mice were allowed to habituate to the acrylic chamber and saline injections during daily 1-h sessions over a 5-day period before drug testing was initiated. Subjects were injected (IP) and immediately placed in the apparatus for 60 min, with horizontal activity counts collected every 10 min. Cocaine was administered no more frequently than twice per week, with at least 2 days between successive doses. The subjects used in these studies had been used previously in the cocaine discrimination procedure and were similarly food deprived during this phase of the study. All of the subjects received all of the doses, which were administered in a mixed sequence.

Cocaine discrimination

Subjects were tested 5 days per week in operant-conditioning chambers (modified Med Associates, Inc., St Albans, Vt., USA), which were contained within light- and sound-attenuating enclosures. White noise was present throughout testing to mask extraneous sounds. Ambient illumination was provided by a lamp mounted at the top of the front panel of the chamber. Two response keys (levers) were set 7 cm apart, with three stimulus lights above each. A force of 1 N through 1 mm was required to register a response, and each response produced an audible click from a relay mounted behind the front panel of the chamber. Reinforced responses produced one 20-mg food pellet (BioServe, Frenchtown, N.J., USA) delivered from a dispenser mounted behind the front panel into a tray located centrally between the response keys.

Experimentally naive subjects were trained to press the two keys with food reinforcement at the beginning of the study. After this initial training, the cocaine discrimination procedure was implemented. Cocaine was administered IP at a dose of 10 mg/kg and responses on only one of the two keys were reinforced; following saline injections, responses on the alternate key were reinforced. The assignment of cocaine- and saline-appropriate keys was counterbalanced across mice. Immediately after injection, subjects were placed inside the experimental chambers. After a 5-min time-out period, during which all stimulus lamps were off and responding had no scheduled consequences other than feedback clicks, lamps above the keys and the one providing overall illumination were turned on and responses on the appropriate key were reinforced. The number of responses required for reinforcement (fixed-ratio or FR value) was increased to 20 over several training sessions. Responses on the inappropriate key reset the FR response requirement on the appropriate key.

Each food presentation was followed by a 20-s time-out period during which all lamps were off, and responding had no scheduled consequences other than the feedback clicks. Sessions ended after 20 food presentations or 15 min, whichever occurred first. As the FR value reached 20, training sessions for which cocaine (C) and saline (S) injections were administered were ordered in a CSSCCS... sequence, with test sessions conducted after consecutive SC or CS training sessions.

On test sessions, different doses of cocaine, raclopride, or their combination were administered before sessions. Cocaine doses were administered in a mixed sequence, and a complete cocaine dose-effect curve was completed before studying each ascending dose of raclopride. A test session was conducted if the subject achieved criteria on both of the immediately preceding saline and cocaine training sessions. The criteria were at least 85% cocaine- or saline-appropriate responding overall and during the first FR of the session. Test sessions were identical to training sessions, with the exception that 20 consecutive responses on either key were reinforced.

Data collection and analysis

Because cocaine has a relatively short duration of action, locomotor activity data from the first 30 min after injection were selected for presentation. Analyses indicated that data from the second 30 min were qualitatively similar. For the cocaine discrimination, the overall response rate on both keys and the percentage of responses occurring on the cocaine-appropriate key were calculated for each subject. The mean values were calculated for each measure at each drug dose tested. If less than half of the subjects responded at a particular dose, no mean value was calculated for percentage of cocaine-appropriate responding at that dose.

Each dose-effect curve was analyzed using two-way (genotype, dose) analysis of variance (ANOVA). The ED_{50} values and their 95% confidence limits (Snedecor and Cochran 1967) were calculated by linear regression based on half of the obtained maximum stimulation. In order to assess relative potency of drugs in saline- and cocaine-treated rats, the dose-effect data were also analyzed by standard parallel-line bioassay techniques as described by Finney (1964). A significant relative potency difference is indicated when the 95% confidence limits for that ratio do not include 1.0. For these analyses, points on the linear portions of the dose-effect curve were used.

Results

Stimulation of locomotor activity

Control levels of horizontal activity in DA D_4R WT and KO mice were 5624±365 and 6170±852 counts per 30 min, respectively and are shown as the unconnected points in Fig. 1. There were no significant differences between genotypes for these values. Cocaine produced a dose-dependent increase in horizontal locomotor activity

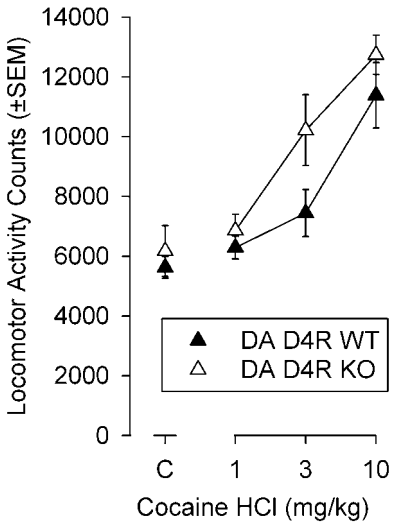

Fig. 1 Locomotor responses of DA D_4R WT and KO littermates after vehicle injection (points above *C*), and after administration of various doses of cocaine. The locomotor activity is expressed as number of horizontal activity counts per minute. Values are the means of five WT and four KO subjects. The *filled symbols* represent data from DA D_4R WT mice; *open symbols* represent data from DA D_4R KO mice. *Vertical bars* around points represent 1 SEM

counts [$F(3,32)=28.366$, $P<0.001$; Fig. 1] in both genotypes. The effects of genotype were also significant [$F(1,28)=6.285$, $P=0.018$], with KO mice displaying a greater potency of cocaine compared to WT littermates, which is reflected in ED_{50} values and a significantly greater relative potency (Table 1). The interaction of cocaine and genotype was not significant.

Discriminative stimulus effects

Both genotypes acquired the discrimination of 10 mg/kg cocaine from saline. The number of sessions to first test (±SEM) for DA D_4R KO and WT mice were 92.3 (±11.0) and 83.4 (±4.8), respectively, and did not significantly differ. There were also no differences in the asymptotic performances maintained under the discrimination procedure. The percentages of responses on the cocaine-appropriate key after saline administration were uniformly low (1.464±0.334 and 2.27±0.626 for DA D_4R WT and KO mice, respectively) and did not significantly differ

Table 1 Comparisons of potency of cocaine discriminative-stimulus effects

Genotype	Stimulation of locomotor activity		Cocaine discrimination	
	ED_{50} value (95% confidence limits)	Relative potency (95% confidence limits)	ED_{50} value (95% confidence limits)	Relative potency (95% confidence limits)
DA D_4R WT	12.0 (7.35–31.2)	–	2.59 (1.28–4.49)	–
DA D_4R KO	9.33 (5.96–21.8)	0.517[b] (0.261–0.906)	0.502[a]	0.338[b] (0.0717–0.840)

[a] The value is an estimate due to a significant amount of variability
[b] The value is an estimate due to a effect of preparations

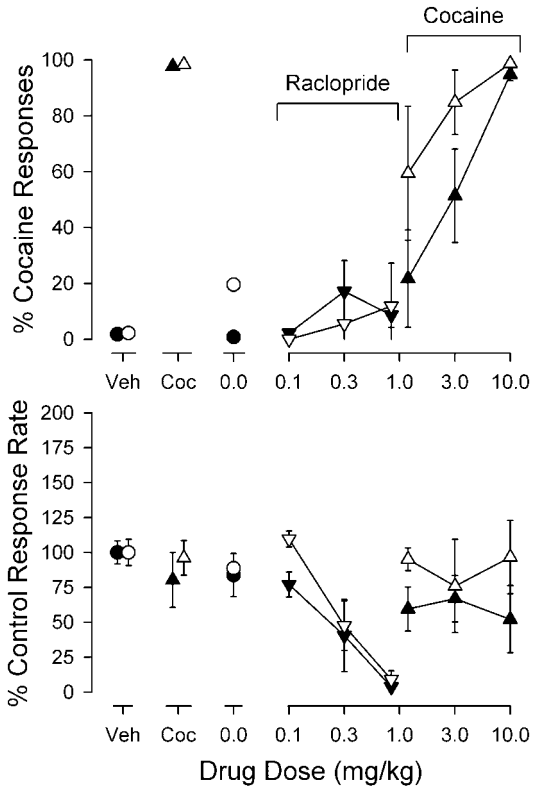

Fig. 2 Effects of cocaine and raclopride in DA D_4R WT and KO mice trained to discriminate 10 mg/kg cocaine from vehicle. All vertical bars about points indicate 1 SEM; where no bars are present the variability is encompassed by the symbol. Points above "Veh" represent values obtained during vehicle training sessions. Points above "Coc" represent values obtained during cocaine training sessions. Points above "0.0" represent values obtained during vehicle test sessions. Values are the means of six WT and four KO subjects. The *filled symbols* represent data from DA D_4R WT mice; *open symbols* represent data from DA D_4R KO mice. *Top row* shows the distribution of responses on the two levers expressed as a percentage of responding on the cocaine-appropriate lever. *Bottom row* shows the rate of responding expressed as a percentage of response rate during saline training sessions

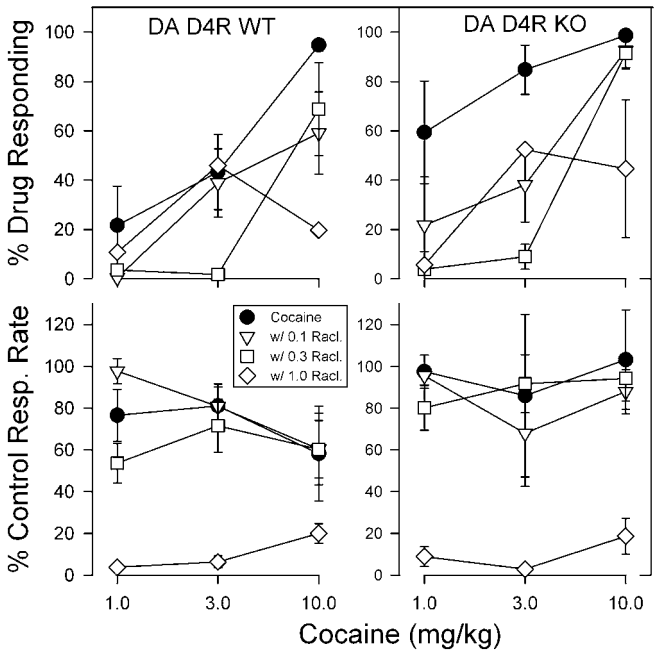

Fig. 3 Effects of cocaine alone and in combination with various doses of raclopride in DA D_4R WT and KO mice trained to discriminate cocaine from vehicle. All vertical bars about points indicate 1 SEM; where no bars are present, the variability is encompassed by the symbol. Values are the means of six WT and four KO subjects. *Filled circles* represent the effects of cocaine alone (in combination with vehicle injections). *Triangles* represent effects of cocaine in combination with 0.1 mg/kg raclopride. *Squares* represent effects of cocaine in combination with 0.3 mg/kg raclopride. *Diamonds* represent effects of cocaine in combination with 1.0 mg/kg raclopride. *Top row* shows the distribution of responses on the two levers expressed as a percentage of responding on the cocaine-appropriate lever. *Bottom row* shows the rate of responding in responses per second

with regard to genotype (see Fig. 2; top panel: points above "Veh"). The percentage of drug-appropriate responses after cocaine administration approached 100% for both genotypes (97.063±0.667 and 98.461±0.295 for DA D_4R WT and KO mice, respectively) and did not significantly differ with regard to genotype (see Fig. 2; top panel: points above "Coc").

Cocaine produced significant dose-related increases [$F(2,25)=7.851$; $P=0.002$] in the percentage of drug-appropriate responses (Fig. 2, top panel, triangles pointing up). The ED_{50} values for the DA D_4R WT and KO mice are shown in Table 1, and indicate a greater potency of cocaine in the DA D_4R KO mice compared to WT littermates. An ANOVA of these effects indicated a significant effect of genotype [$F(1,25)=4.756$; $P=0.039$]. As also shown in Fig. 2 (bottom panel, triangles pointing up), cocaine did not produce significant dose-related effects on response rates.

Table 2 Comparisons of potency of raclopride effects on response rates

Genotype	ED_{50} value (95% confidence limits)	Relative potency (95% confidence limits)
DA D_4R WT	0.290 (0.000–19.5)	–
DA D_4R KO	0.396 (0.080–2770)	1.45 (0.643–3.83)

Raclopride, when administered alone, did not produce a significant effect of dose on drug-appropriate responding (Fig. 2, top panel, triangles pointing down). In contrast, significant dose-related decreases in response rates [$F(2,21)=16.488$; $P<0.001$] were obtained with raclopride. These effects of dose did not depend on genotype; the ED_{50} values for the effects of raclopride on response rates did not significantly differ and the relative potency difference was not significant (Table 2).

Raclopride produced dose-related shifts in the discriminative effects of cocaine in both lines of mice (Fig. 3; upper panel, compare open to filled points). At the highest dose of raclopride, the antagonism was insurmountable, over the range of cocaine doses that were

Table 3 Comparisons of potency of cocaine discriminative-stimulus effects alone and in combination with raclopride

Genotype	Raclopride dose (mg/kg)	ED_{50} value (95% confidence limits)	Relative potency (95% confidence limits)
DA D_4R WT	0 (Cocaine alone)	2.59 (1.28–4.49)	–
	0.1 Raclopride	6.11 (3.29–32.2)	2.21[a] (1.03–6.50)
	0.3 Raclopride	7.13 (4.77–15.7)	3.10 (1.51–8.03)
	1.0 Raclopride	NS regr[b]	IA[c]
DA D_4R KO	0 (Cocaine alone)	0.502[d]	–
	0.1 Raclopride	3.03 (1.28–6.85)	3.51[a] (1.27–26.8)
	0.3 Raclopride	5.46 (4.70–6.34)	5.27[a,e] (2.43–20.0)
	1.0 Raclopride	NS regr	IA

[a] The value is an estimate due to a significant effect of preparations
[b] Non-significant linear regression
[c] Insurmountable antagonism over the range of doses studied
[d] The value is an estimate due to a significant amount of variability
[e] The value is an estimate due to a significant deviation from parallel

studied. The shifts in the cocaine dose-effect curve are shown quantitatively in Table 3 in terms of dose-related changes in ED_{50} values and relative potency estimates. The significance of the shift in the curve is demonstrated by 95% confidence limits for the relative potency estimates that are exclusive of the 1.0 value. The shifts in the dose-effect curve were used to calculate the Apparent K_B values for raclopride of 0.297 and 0.106 μmol/kg in DA D_4R WT and KO mice, respectively.

Discussion

Cocaine produced reliable dose-dependent increases in locomotor activity that were similar to effects reported previously in rodents (Dews 1953; Kelly and Iversen 1976; Izenwasser et al. 1994). As with previous cocaine discrimination studies (D'Mello and Stolerman 1977; Woolverton and Trost 1978), cocaine produced a reliable interoceptive stimulus effect. Saline administration produced almost exclusive responding on the vehicle-paired key, whereas cocaine produced a dose-related increase in responding on the drug-appropriate key, which was virtually exclusive at the 10 mg/kg training dose.

DA D_4R KO mice have been found to display lower levels of locomotor activity in a novel environment compared to WT mice (Rubinstein et al. 1997). In contrast, in the present study there were no differences between genotypes with regard to baseline levels of activity, probably because of the procedures employed. In the study by Rubinstein et al., the activity was determined on days 1–3 of exposure to the test environment. In the present study, subjects were repeatedly exposed to the test apparatus. Dulawa et al. (1999) showed that DA D_4R KO mice show reduced levels of novelty-induced exploration compared to DA D_4R WT mice.

The effects of cocaine on locomotor activity in the current study were generally consistent with those of Rubenstein et al. (1997). In that and the present study, cocaine increased activity more so in the DA D_4R KO mice than in their WT littermates. In the previous study, the differences between genotypes were observed at 30 mg/kg cocaine, whereas in the present study, the differences were obtained at 3, but not 10 mg/kg. It is not currently obvious whether this difference in reactivity to cocaine in the two studies was contributed to by the differences in the baselines in the previous study or to some other differences in how the two studies were conducted. Nonetheless, the differences between genotypes are even more compelling when the reliability across studies conducted in different manners is considered.

Cocaine was also more potent in DA D_4R KO compared to WT mice in producing a discriminative-stimulus effect. Moreover, the difference in potency between genotypes, while modest, was significant and similar across the two behavioral procedures. Both the differences in the discriminative-stimulus effects of cocaine and in the locomotor stimulant effects were obtained despite an absence of appreciable novelty in the testing environments, suggesting that a modified response to novelty played little or no role in the differences in sensitivity to cocaine observed in the present study.

Raclopride shifted the cocaine discriminative-stimulus effects to the right in both genotypes, with the lowest dose (0.1 mg/kg) producing significant antagonism. This dose was lower than the 1.0 mg/kg dose previously reported as the minimally effective antagonist dose in Swiss Webster mice (e.g. Chausmer and Katz 2001), suggesting important differences between these lines of mice, or these behavioral effects, in their sensitivity to antagonist effects of raclopride.

Raclopride was used as an antagonist in this study because it has negligible affinity for DA D_4 receptors (e.g. Millan et al. 1998). The comparable ED_{50} values for raclopride in decreasing response rates in DA D_4R WT and KO mice are consistent with minimal activity of raclopride at D_4 receptors. Further, the effectiveness of raclopride as an antagonist of the discriminative-stimulus effects of cocaine suggests a minimal role of DA D_4 receptors in the discriminative effects of cocaine. Costanza and Terry (1998) found that the selective D_4 dopamine receptor antagonist, L-745,870, was inactive as an antagonist of the discriminative-stimulus effects of cocaine in rats. In addition, Caine et al. (2002) found a

limited effect of L-745,870 on cocaine self administration in rats. Each of these findings is consistent with the present conclusions. Together, these results suggest that the actions of cocaine observed in the present study are due to activity at other D_2-like dopamine receptors

The present results do indicate a difference between the DA D_4R WT and KO mice with regard to their sensitivity to raclopride as an antagonist of the discriminative-stimulus effects of cocaine. The apparent affinity constants (K_B values) for raclopride antagonism of these effects were 0.297 and 0.106 μmol/kg in the DA D_4R WT and KO mice, respectively. The apparent K_B value for raclopride in WT mice was similar to that obtained in other preparations. For example, in a previous study from this laboratory, the apparent K_B value of raclopride as an antagonist of the discriminative stimulus effects of cocaine in DA D_2R WT mice was calculated (after publication of the study) as 0.315 μmol/kg. An increased sensitivity to raclopride in the DA D_2R KO mice compared to WT mice suggests a greater role of the DA D_2R in the discriminative-stimulus effects of cocaine in these subjects. This may have unfolded as a result of some compensatory mechanism secondary to D_4R deletion.

Rubinstein et al. (1997) examined D_2-like receptor binding in striatum of DA D_4R WT and KO mice. In that study, there were no appreciable differences in affinity or receptor number, which is in apparent contrast with the suggestion of an enhanced function of the DA D_2R as a result of DA D_4R deletion. Thus, changes in D_2R function may be localized in areas other than striatum, or may be of small magnitude, either of which would preclude their detection in the previous study.

An alternative explanation of the present findings is that in WT subjects, the D_4R has an inhibitory role in the expression of DA D_2R activity. The removal of this inhibitory effect in the DA D_4R KO mice would underlie the enhanced sensitivity to stimulant drugs in DA D_4R KO compared to WT mice (Rubinstein et al 1997; Kruzich et al. 2002; Suchland et al. 2002). An interaction such as this would influence an *apparent* affinity constant derived from a complex whole-animal system. The effects of raclopride on response rates are relevant to whether the sensitivity to cocaine in the DA D_4R KO mice is the result of an enhanced effect of the D_2R, or the elimination of an inhibitory function of the D_4R. Similar ED_{50} values for this effect in the two genotypes would not be predicted from enhanced sensitivity of the DA D_2R, but are not inconsistent with an inhibitory effect of the DA D_4R. Thus, the current data are more consistent with an inhibitory role of the DA D_4R on D_2R function than on a compensatory effect of D_4R deletion on D2R function.

As delineated above, a linkage of DA D_4R variants and novelty seeking has been suggested as playing a role in substance abuse disorders. In addition, manipulation of DA D_4R receptor number through genetic alterations has been reported to influence the effect of novelty on behavior in mice (Dulawa et al. 1999). The present and previous findings with mutant mice together suggest that while D_4R deletion *decreases* the effects of novelty on behavior, this is accompanied by *enhanced* psychomotor stimulant and subjective effects of cocaine. Thus, deletion of the DA D_4 gene produces opposite effects on cocaine- and novelty-induced activities. Therefore, a simple explanation of the effects of cocaine, and likely its abuse, in terms of genetic predispositions towards responsiveness to novelty should reconcile these diametrically opposed effects of genetically manipulating the DA D_4R.

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