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ORIGINAL INVESTIGATION

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Cocaine-induced locomotor activity and cocaine discrimination in dopamine D2 receptor mutant mice

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Abstract *Rationale:* Dopamine (DA) D2-like antagonists block several effects of cocaine, including its locomotor stimulant and interoceptive discriminative-stimulus effects. Because these compounds generally lack selectivity among the D2-like DA receptors, the specific roles of the subtypes remain unclear. *Objectives:* DA D2 receptor knockout (DA D2R KO), heterozygous (HET), and wild-type (WT) mice were used to study the role of D2 DA receptors in the effects of cocaine. Some effects of the relatively selective DA D2-like antagonist raclopride were also studied to further assess the role of D2 receptors. *Methods:* DA D2R KO, HET, and WT mice were treated with cocaine (1–10 mg/kg) or vehicle, and their horizontal locomotor activity was assessed. The mice were also trained to discriminate i.p. injections of saline from cocaine (10 mg/kg) using a two-response key, fixed-ratio-20 response, food-reinforcement procedure. A range of doses of cocaine (1.0–17 mg/kg) was administered before 15-min test sessions. *Results:* Both DA D2R KO and HET mice showed reduced levels of horizontal activity relative to WT mice. Cocaine dose dependently stimulated activity in each genotype, with the highest level of activity induced in the DA D2R WT mice. All three genotypes acquired the discrimination of 10 mg/kg cocaine; tested doses of 1.0–10.0 mg/kg produced dose-related increases in the number of cocaine-appropriate responses. Raclopride, at inactive to fully active doses (0.1–1.0 mg/kg), did not fully substitute for cocaine. Raclopride dose dependently shifted the cocaine dose–effect curve to the right in DA D2R WT and HET mice. However, in DA D2R KO mice, raclopride was inactive as an antagonist. *Conclusions:* The present data indicate an involvement of D2 DA receptors in the locomotor-stimulating effects and the interoceptive discriminative-stimulus effects of cocaine in WT subjects. However, the D2 receptor is not necessary for the effects, suggesting redundant dopaminergic mechanisms for the discriminative-stimulus interoceptive effects of cocaine.

Keywords Dopamine · D2 · Knockout · Cocaine · Raclopride · Locomotor activity · Drug discrimination

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Introduction

D2-like dopamine (DA) receptors (D2, D3, D4) have been implicated in the locomotor stimulant and discriminative-stimulus effects of cocaine. For example, several studies have indicated that D2-like agonists stimulate locomotor activity (Arnt et al. 1988), and antagonists at those receptors block the stimulation produced by cocaine (Bhattacharyya et al. 1979; Chausmer and Katz 2001). Similarly, D2-like agonists will substitute for cocaine in rats and primates trained to discriminate injections of cocaine from vehicle (Barrett and Appel 1989; Kleven et al. 1990; Witkin et al. 1991), and D2-like antagonists decrease the discriminative-stimulus effects of cocaine

(Barrett and Appel 1989; Witkin et al. 1991), reflected in a rightward shift in the cocaine dose–effect curve (Spealman et al. 1991).

Although "selective" ligands have provided useful information about the function of D1-like and D2-like receptors, their utility is somewhat limited due to an inability to distinguish between the members within the D1-like or D2-like subclasses of DA receptors. Spiperone, for example, is a D2-like receptor antagonist with negligible binding at D1 receptors (Seeman and Ulpian 1988), but its selectivity for D2 over D3 receptors has been reported to be from 70-fold to none (for review see Levant 1997). Thus, in general, in vivo studies of cocaine action have not been able to distinguish the contributions of D2-like receptors from the members of that class. In one exception, Costanza and Terry (1998) examined the effects of the putative selective D4 DA receptor antagonist L-745,870 on the discriminative-stimulus effects of cocaine. In that study, the D4 antagonist was inactive in modifying the effects of cocaine, whereas the selective D1-like antagonist SCH 39166 produced a dose-dependent shift to the right in the cocaine dose–effect curve. Despite this initial study, the relative involvement of D2, D3, and D4 receptors in the discriminative stimulus properties of cocaine remains unclear.

Receptor gene knock-out (KO) technology provides a powerful approach to study the roles of individual receptors in vivo. This technique is especially useful in light of the paucity of pharmacological tools with in vivo selectivity for the various subtypes of DA receptors. As opposed to currently available pharmacological approaches, the gene KO approach can result in the complete elimination of a single gene product with only small alterations in related products. For example, in mice with a homozygous mutation that disrupts the DA D2 receptor gene (DA D2R KO mice), functional DA D2 receptors are eliminated with no change in the affinity of D1-like receptors, and only a 20% decrease (Kelly et al. 1998) or no change (Baik et al. 1995) in D1-like receptor number (B_{max} values). Further, Baik et al. reported no change in expression of D3 or D4 receptor mRNA in their D2R KO mice. Thus, the DA D2R KO mice can be compared with heterozygous (HET) and/or wild-type (WT) mice allowing some insight into the potential roles of the DA D2 receptor in vivo.

The DA D2R KO phenotype is characterized by distinctive behavioral effects (Baik et al. 1995). For example, compared with WT mice, KO mice that lack D2 receptors show reduced horizontal locomotor activity in an open field (Kelly et al. 1998). Though the effects of deleting the DA D2 receptor on horizontal locomotor activity are less than those obtained by means of pharmacological blockade of D2 DA receptors (Kelly et al. 1998), there is evidence that behavioral effects of stimulants are blunted in these subjects (Ralph et al. 1999). Interestingly, the DA D2R KO mice have been reported to self administer cocaine with a potency similar to WT littermates, though there were differences in their pattern of intake of high doses of cocaine (Caine et al. 2002). Because the effects of high doses of cocaine in the self-administration procedure may be due to behaviorally disruptive effects of accumulating cocaine (Katz 1989), the current study examined the discriminative-stimulus effects of cocaine. Using this procedure, the interoceptive effects of the drug are distinguishable from the effects on response output. We also further examined the specific role of D2 receptors in cocaine-induced locomotor activity and used the D2-like antagonist raclopride to further characterize the role of D2 DA receptors in the discriminative-stimulus effects of cocaine.

Methods

Subjects

DA D2 receptor KO, HET and WT mice weighing on average 26±0.6, 33±1.9, and 25±1.7 g, respectively, were singly housed under a 12-h/12-h light/dark cycle (lights on at 0700 hours). The subjects averaged 24 weeks of age when experiments started and were predominately male; however, there were 2, 1, and 2 females among the 8 KO, 12 HET and 8 WT subjects, respectively. The homologous recombination techniques and genealogy were described in detail in previous reports (Kelly et al. 1997, 1998). Briefly, the targeting vector was engineered to contain a portion of the mouse D2 receptor gene minus all of exon 7 and the 5' half of exon 8. The murine D2 receptor gene fragment used in constructing the vector was cloned from a genomic phage isolated from a library made from a 129 SvEv mouse. After homologous recombination, transcripts from the rearranged locus lacked D2 receptor-coding sequences beginning in the middle of the putative third intracellular domain and extending through the carboxy terminus of the receptor protein. A neomycin resistance cassette was included in the construct as a way of selecting for the recombinant D3 (129S2/SvPas) ES cells. Following confirmation that accurate targeting had occurred, recombinant D3 ES cells were then injected into day-3.5 C57BL/6 J blastocysts. Founder mice were identified and then mated with inbred, WT C57BL/6 J mice purchased from Jackson Labs. The HET offspring from these pairings were then bred with WT C57BL/6 J mice. Each new generation was genotyped and heterozygotes were identified and bred with WT C57BL/6 J mice, whose gender alternated with each generation. The (incipient) congenic mice used in the studies reported here were the product of ten generations of mating HET mice with WT C57BL/6 J mice. The specific details of the D2R targeting methodology and characterization of these mutant mice have been reported elsewhere (Kelly et al. 1997, 1998).

The mice were maintained at 85% of their unrestricted-feeding weights. All testing was performed between 1300 hours and 1600 hours. Mice were fed daily rations at least 1 h after behavioral testing. The same animals were used in locomotor activity tests and drug discrimination tests and were maintained in an AAALAC International accredited facility in accordance with NIH Policy Manual 3040-2, *Animal Care and Use in the Intramural Program* (released 1 November 1999).

Drugs

(–)-Cocaine HCl (Sigma Chemical Company) and (*S*)-raclopride tartrate (Research Biochemicals, Inc.) were dissolved in 0.9% physiological saline and administered i.p. in a volume of 1.0 ml/100 g.

Procedure

Locomotor activity

Ambulatory activity was studied using clear acrylic chambers (40×40×40 cm) that were placed inside monitors (Omnitech Electronics, Columbus, Ohio.) equipped with light-sensitive detectors spaced 2.5 cm apart along the two perpendicular walls. Mounted on 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. From that information the apparatus computed distance traveled in centimeters. Mice were pretreated with cocaine (1–10 mg/kg) or vehicle just prior to being placed in the apparatus for 60 min, with horizontal activity counts collected every 10 min. Each dose was studied in DA D2R WT (n=7), KO (n=8), and HET (n=8) mice. At least 48 h separated each test session.

Drug discrimination

Mice were placed in operant conditioning chambers (Med Associates, modified Model ENV 307A, St. Albans, Vt.; inside dimensions: 15.9×14×12.7 cm; length×width×height) which contained two response keys (levers requiring a force of about 2 g to activate) and a dispenser for the delivery of food reinforcement (one 20-mg Precision food pellet, BioServe, Frenchtown N.J.). Above the keys were stimulus lights (LEDs) and, at the top of the wall, a single 28-V d.c. lamp which provided general illumination of the chamber. The chamber was enclosed in a sound-attenuating outer chamber to isolate the subject during experimental sessions. Sessions were conducted daily, 5 days per week, in which subjects were trained to press the key with food reinforcement under a fixed-ratio (FR) 20 response schedule in which the 20th response produced a food pellet. During sessions preceded by a vehicle injection, responses on one of the keys were reinforced; during sessions preceded by a cocaine injection (10 mg/kg), responses on the alternate key were reinforced. Cocaine and vehicle keys were counterbalanced across subjects, and sessions were sequenced according to a double alternation schedule (e.g., vehicle–drug–drug–vehicle). During the first 5 min of the session, all lights were off and responses had no scheduled consequences. After 5 min, a 15-min session began with the house light and LEDs illuminated and the appropriate response key active. After delivery of food, the lights were turned off for a 20-s time-out period during which neither key was active. The session ended either at the end of 15 min or after 20 food presentations, which ever occurred first. Testing began after the following criteria were met for four consecutive daily training trials: at least 80% injection-appropriate overall responding during the entire session; 80% injection-appropriate responding during the first FR 20 only; and response rates of at least 0.02 responses per second, which correspond to the completion of one FR 20. After these criteria were met, animals received test doses of cocaine (0, 1, 3, and 10 mg/kg), raclopride (0.1–1.0 mg/kg), or vehicle prior to individual test sessions. At least two training sessions separated successive test sessions. Each subject was on average tested on 14.6, 14, and 17 occasions, with an average inter-test interval during testing of 15.0, 10.8, and 9.8 days, for DA D2R WT, HET, and KO genotypes, respectively. Testing criteria had to be met during the two sessions prior to every test session or training continued until the criteria were met. Raclopride (or vehicle) injections were given 15 min prior to cocaine (or vehicle) injections, which were given just before the subjects were placed in the chamber (5 min before behavioral testing). Each dose was studied in DA D2R KO (n=5–7), HET (n=6–11), and WT (n=5–8) mice.

Statistical analysis

For locomotor activity, data were segregated into the first and second 30 min of the 1-h observation period and analyzed separately. For clarity, only data from the first 30 min are presented, though data from the second 30 min generally confirm conclusions from the first 30 min. One- or two-way analyses of variance (ANOVAs) were performed such that the effects of cocaine dose, genotype, and their interaction were assessed. Due to unequal variances in the raw data, $\log_{10}$ transforms were conducted for all analyses. Bonferoni post-hoc tests provided pair-wise comparison information. The criterion for significance was α=0.05.

For the drug discrimination data, rates of responding under the FR schedule and the percentage of responses on the cocaine-appropriate key (excluding time-out periods) were used for dose–effect functions, which were analyzed using ANOVA and linear regression techniques to determine significance of the effects of dose, regression, and deviation from the regression (Snedecor and Cochran 1967). From these analyses, ED_{50} values and their 95% confidence limits were derived from data using the linear portion of the dose–effect curves. In order to further analyze the results, relative potencies were calculated using standard parallel-line bioassay techniques, as described by Finney (1964). This analysis involves a one-way ANOVA, which determines whether the slopes of the two dose–effect curves are different from parallel and fits a common slope to the two dose–effect curves. It then compares the ratio of doses for a given effect (in this case 50% cocaine-appropriate responses) to provide a value for relative potency. This value represents the dose of the standard equal to 1 mg/kg of the comparison. A significant relative potency difference is indicated when the 95% confidence limits for that ratio do not include 1.0. Relative potencies of cocaine were calculated for each of the DA receptor mutants relative to their WT littermates as the standard, and within each genotype, for each dose of raclopride with cocaine relative to cocaine with raclopride vehicle.

Due to the potential for a lack of reliability, the percentage of responses on the cocaine-appropriate key for an individual subject were not included in the average if response rates fell below 0.02 responses per second. In addition, if half of the subjects tested at a particular dose did not meet this response rate criterion, the average percentage of responses on the cocaine-appropriate key was omitted from the analysis and graphs. Response rate data, however, were always included.

Results

Locomotor activity

There was a significant difference among genotypes in locomotor activity after saline injection with each genotype different from the others (Fig. 1, unconnected points at left; $F_{2,20}$=124.48, P<0.001). Overall ANOVA indicated significant effects of genotype ($F_{2,80}$=129.191, P<0.001) and cocaine dose ($F_{3,80}$=83.806, P<0.001). Post-hoc analysis indicated that cocaine significantly stimulated activity at the highest dose in DA D2R WT and HET mice, and significantly increased locomotor activity at all doses of cocaine in the DA D2R KO mice (Fig. 1, right panel).

Drug discrimination

Each genotype acquired the discrimination of 10 mg/kg cocaine from saline. The number of sessions to criterion (±SD) for DA D2R WT, HET, and KO mice were 65.1±9.0, 53.3±5.7, and 55.5±8.1, respectively, and did not significantly differ ($F_{2,23}$=0.692, P=0.51). Similarly, there were no differences in the asymptotic performances

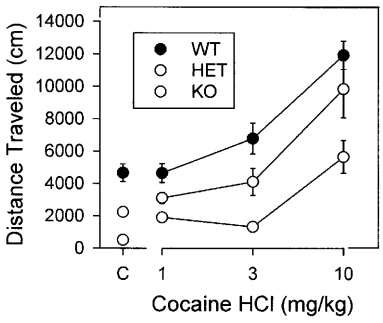

Fig. 1 Locomotor responses of dopamine (DA) D2 receptor (D2R) wild-type (WT), heterozygous (HET), and knockout (KO) littermates after vehicle injection (*points* above *C*) and after administration of various doses of cocaine. The locomotor activity is expressed as average distance traveled (cm). Values are the arithmetic means of from seven (WT) to eight (KO and HET) subjects. The *black*, *gray*, and *white symbols* represent data from DA D2R WT, HET, and KO mice, respectively. *Vertical bars* around points represent ±SEM (where no *bars* are present, the variability is encompassed by the *symbol*)

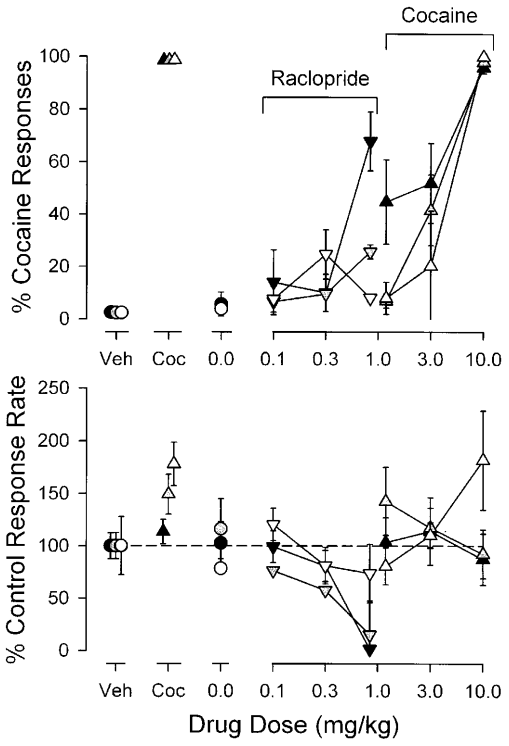

Fig. 2 Effects of cocaine and raclopride in dopamine (DA) D2 receptor (D2R) wild-type (WT), heterozygous (HET), and knockout (KO) mice trained to discriminate 10 mg/kg cocaine from vehicle. All *vertical bars* about *points* indicate ±SEM; where no *bars* are present, the variability is encompassed by 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. The *black*, *gray*, and *white* symbols represent data from DA D2R WT, HET, and KO mice, respectively. *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

maintained under the cocaine discrimination procedure. As can be seen in Fig. 2 (top panel), the percentage of responses on the cocaine-appropriate key after administration of saline (points above "Veh") were similarly low across genotypes, and less than 5%. There were no significant differences among genotypes with regard to these values. The percentage of cocaine-appropriate responses after cocaine (Fig. 2, points above "Coc") approached 100% and did not significantly differ with genotype. Absolute response rate (data not shown) was significantly affected by genotype as observed during sessions with saline ($F_{2,296}$=35.963, P<0.001) and cocaine ($F_{2,296}$=50.974, P<0.001). There were no significant differences between response rates in DA D2R HET and KO mice.

Cocaine produced dose-related increases in the percentage of responses on the cocaine-appropriate key for each genotype (Fig. 2, top panel, triangles). The WT mice exhibited a greater percentage of cocaine-appropriate responses at the lowest dose tested, which resulted in a significantly increased potency of cocaine in these subjects relative to the HET mice, and a trend toward a greater potency than in the DA D2R KO mice (Table 1; note the exclusion of the value 1.0 in the 95% confidence limits for the relative potency in HET mice). Cocaine did not have significant effects on response rates at any dose tested and in any of the genotypes (Fig. 2, bottom panel, triangles).

None of the doses of raclopride that were tested, from those that had no effect to those that virtually eliminated responding, fully substituted for cocaine (Fig. 2, top panel, inverted triangles). There was a partial substitution for cocaine at the highest dose examined in WT mice that was significantly greater than that obtained after vehicle injection. Response rates of WT mice were decreased in a dose-related manner by raclopride (Fig. 2, bottom panel, black inverted triangles), and the dose that produced a substitution greater than vehicle was also a dose that virtually eliminated response rates. The decreases in

Table 1 Comparisons of potency of cocaine discriminative-stimulus effects

Genotype	ED_{50} (95% confidence limits)	Relative potency (95% confidence limits)
DA D2R WT	1.62 (0.24–3.19)	
DA D2R HET	3.21 (2.59–4.01)	1.68 [a, b] (1.03–2.94)
DA D2R KO	3.68[c] (2.62–5.56)	2.12[a] (1.07–5.28)

[a] The value is an estimate due to a significant effect of preparations
[b] The value is an estimate due to a significant deviation from parallel
[c] The value is an estimate only due to a significant deviation from linearity

Table 2 Comparisons of potency of raclopride effects on response rates

Genotype	ED_{50} (95% confidence limits)	Relative potency (95% confidence limits)
DA D2R WT	0.38 (0.22–0.84)	
DA D2R HET	0.24 (0.11–0.41)	0.66 (0.26–1.41)
DA D2R KO	n.s. regression[a]	Not calculated[b]

[a] The value was not calculated due to a non-significant linear regression
[b] The relative potency was not calculated because there was no significant effect of raclopride dose in the KO mice

response rates were significant at 1.0 mg/kg in the WT mice but were not significant at any dose in the DA D2R KO mutant littermates. A comparison of potencies (Table 2) shows that raclopride decreased response rates equipotently in DA D2R HET and WT mice.

Raclopride produced shifts to the right in the cocaine dose–effect curve in DA D2R HET and WT mice (Fig. 3, top panels). These shifts, though modest, were significant; 95% confidence limits for the relative potency estimates excluded the value 1.0 in WT mice at all raclopride doses, and in the DA D2R HET mice at the highest dose of raclopride (Table 3). In addition, the shifts in the cocaine dose–effect curve were related to raclopride dose, with dose-related increases in the relative potency estimate in WT mice and a significant shift at only the highest raclopride dose in the DA D2R HET mice. In addition, at the highest dose of raclopride, there was an indication that the antagonism was not completely surmountable. In contrast, raclopride failed to shift the cocaine dose–effect curve in the DA D2R KO mice (Fig. 3), which was also reflected in the relative potency estimates (Table 3) which were uniformly not statistically different from 1.0.

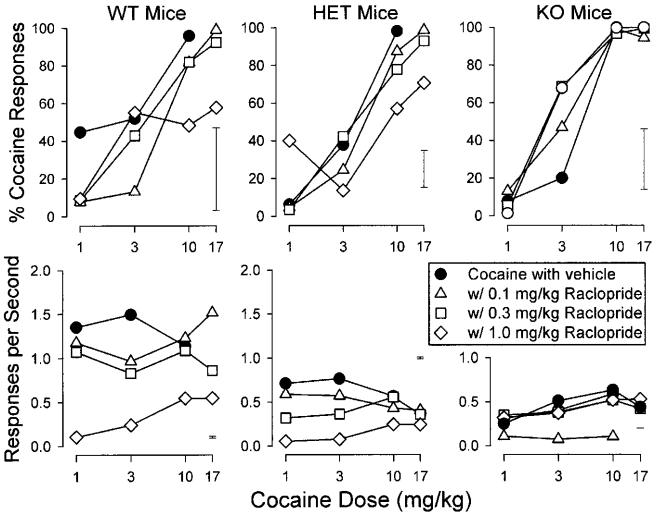

Fig. 3 Effects of cocaine alone and in combination with various doses of raclopride in dopamine (DA) D2 receptor (D2R) wild-type (WT), heterozygous (HET), and knockout (KO) mice trained to discriminate cocaine from vehicle. *Top row* shows the distribution of responses on the two levers expressed as a percentage of responding on the cocaine-appropriate lever. *Bottom row* shows the rate of responding in responses per second. Unconnected *bars* within each set of coordinates represent the critical difference bar (α=0.05) calculated as an average critical difference due to unequal numbers of subjects. The critical difference was converted to the outcome scale and represents an average difference to exceed for significance of pairwise comparisons. Specific pairwise comparisons were correctly adjusted for the number of observations within each comparison using the Tukey-Kramer method (Tukey 1953; Kramer 1956)

Discussion

Cocaine produced reliable dose-dependent increases in locomotor activity in mice that were similar to effects reported previously (Dews 1953; Kelly and Iversen 1976; Izenwasser et al. 1994). Cocaine also produced a discriminable interoceptive stimulus effect as indicated

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

Genotype	Raclopride dose (mg/kg)	ED_{50} (95% confidence limits)	Relative potency (95% confidence limits)
DA D2R WT	0 (Cocaine alone)	1.62 (0.24–3.19)	
	0.1 Raclopride	4.75[a] (3.74–6.08)	2.68[c] (1.46–4.97)
	0.3 Raclopride	3.85 (2.53–5.59)	2.08 (1.07–4.30)
	1.0 Raclopride	n.s. regression[d]	5.23 (1.42–87.7)
DA D2R HET	0 (Cocaine alone)	3.21 (2.59–4.01)	
	0.1 Raclopride	4.27 (3.42–5.39)	1.32 (0.97–1.79)
	0.3 Raclopride	4.18 (2.84–5.99)	1.30 (0.88–1.91)
	1.0 Raclopride	8.35[a] (4.19–44.12)	1.99[a,c] (1.23–3.31)
DA D2R KO	0 (Cocaine alone)	3.68[a] (2.62–5.56)	
	0.1 Raclopride	2.63 (1.96–3.54)	0.76 (0.46–1.22)
	0.3 Raclopride	2.53 (1.38–3.82)	0.88[b] (0.47–1.53)
	1.0 Raclopride	2.73 (1.60–3.91)	0.79[b] (0.41–1.36)

[a] The value is an estimate only due to a significant deviation from linearity
[b] The value is an estimate due to a significant effect of preparations
[c] The value is an estimate due to a significant deviation from parallel
[d] Could not calculate a value due to a non-significant linear regression

by the differential responding on the two levers as a function of whether saline or cocaine had been administered before the session. This performance was characterized by virtually exclusive responding on the cocaine-appropriate key after cocaine injection and virtually no responding on the cocaine-appropriate key after administration of vehicle. Results similar to these have been reported previously in several species of laboratory animals (D'Mello and Stolerman 1977; Woolverton and Trost 1978; Witkin et al. 1991).

Genetically altering expression of D2 receptors resulted in changes in horizontal locomotor activity. The DA D2R KO and HET mice showed significantly lower baseline levels of horizontal locomotor activity than WT mice, which has been previously documented (Baik et al. 1995; Kelly et al. 1998). A similar pattern was also found when evaluating response rates in operant behavior, with the response rates of WT > HET > KO mice. Interestingly, these effects do not appear to be the result of a global motor deficit because several other motor behaviors, such as grooming and sniffing, are comparable in WT and DA D2R KO mice (Drago et al. 1999; Clifford et al. 2001).

Cocaine stimulated horizontal locomotor activity in all three genotypes, with the level of activity greatest in the DA D2R WT mice followed by HET and KO mice. Of course with these effects as well as others using genetically engineered subjects, the potential exists that some compensatory mechanism may account for the differences in the effects obtained. A comparison of the effects of gene "dosage" and the reported effects of D2 DA antagonists on the cocaine dose–effect curve (Chausmer and Katz 2001) reveals certain similarities, in particular, the maximal stimulant effects of cocaine and the baseline (non-drugged) activity levels. The convergence of effects from the pharmacological and genetic manipulations suggest that the presently obtained differences between genotypes are due to the effects of gene deletion rather than due to some compensatory mechanism.

The D2-like antagonist raclopride when administered alone produced dose-related and significant decreases in response rates in DA D2R WT and HET mice, but not the KO mutants. Receptor binding studies have indicated that raclopride is equipotent at D2 and D3 DA receptors (Levant 1997). The lack of significant effects of raclopride on response rates in the KO mice is consistent with this compound producing its behavioral effects primarily through the D2 subclass of DA receptors.

Each genotype acquired the discrimination of 10 mg/kg cocaine, and there were no differences between genotypes with regard to the acquisition of this behavior. Once trained, there was a marginally greater potency of cocaine in the WT mice that was due do a difference from DA D2R HET and KO mice only at the lowest dose studied. In general, the dose–effect curves for the discriminative-stimulus effects of cocaine were remarkably similar across genotypes. These results contrast with results obtained in studies of the effects of D2-like antagonists. The D2-like antagonists produce decreases in the discriminative-stimulus effects of cocaine (Barrett and Appel 1989; Witkin et al. 1991), which is reflected in a dose-related rightward shift in the cocaine dose–effect curve (Spealman et al. 1991). Thus, there appear to be some significant differences between the alteration in the effects of cocaine due to decreases in D2 DA receptor availability by genetic or pharmacological manipulation.

As noted above, the response rate data suggest that raclopride was acting (at least to alter behavioral output) through D2 receptors. The antagonism by raclopride of the discriminative effects of cocaine in DA D2R HET and WT, but not KO, mice suggests further that raclopride blocked the discriminative effects of cocaine in WT and HET mice through its antagonist actions at D2 receptors. Thus, the antagonism data suggest a significant role of D2 DA receptors in the discriminative-stimulus effects of cocaine in the intact WT and HET mice.

A comparison across the three genotypes showed a number of "gene dosage" effects. For example as shown previously (Kelly et al. 1998), the control levels of horizontal locomotor activity were directly related to numbers of D2 receptors, with activity levels for the DA D2R HET mice between those of WT and KO mice. Most important is that the antagonism by raclopride was related to gene dosage. In the WT mice a significant shift in the cocaine dose–effect curve was obtained at the lowest dose of raclopride, a tenfold higher dose was necessary to comparably shift the cocaine dose–effect curve in DA D2R HET mice, and raclopride was not active in the KO mice.

The conclusion of a role for D2 DA receptors in the discriminative-stimulus effects of cocaine may be reconciled with the similar potency of cocaine in DA D2R WT and KO mice by drawing an analogy to exteroceptive stimuli. Training WT or KO subjects on a discrimination of cocaine could be (for simplicity of argument) considered analogous to using an array of five (WT) or four (KO) lights, as discriminative stimuli, with the lights corresponding to the five subtypes of DA receptors. Increasing the color saturation of the five (WT) or four (KO) lights may be considered analogous to changes in receptor occupancy resulting from graded changes in dose. The "generalization" gradients for each of the performances controlled by exteroceptive stimuli would be similar, as the saturation of the relevant four or five colored lights changes concomitantly, just as the dose–effect curves for cocaine across genotypes were largely similar. When one of the lights is turned off, which is analogous to administering a sufficient dose of a specific antagonist for an existing receptor, the stimulus complex provided is qualitatively different from the one to which the subject is trained. Thus, it is only with an antagonist treatment that differences between genotypes emerge.

It may very well be that D2 receptor-mediated effects, and those mediated by other DA receptors, are all involved in the subjective, discriminative-stimulus effects of cocaine in WT subjects. Several previous studies suggest that at least a subset of DA receptors are

mechanistically redundant for discriminative effects. In particular, the limitations of DA receptor antagonists to produce more than a nominal rightward shift of the cocaine dose–effect curve, suggests a redundancy among DA receptor mechanisms for the discriminative effects of cocaine (Katz et al. 1999). Extending the above model, the dose–effect curve for an agonist with relative affinities for the subtypes of DA receptors that differ from the training drug could also exhibit differences in its substitution profile (generalization gradient) in the different genotypes.

The present findings may shed some light on studies of the reinforcing effects of cocaine in D2 DA receptor mutant mice (Caine et al. 2002). In those studies, as in most studies of cocaine self administration, the complete dose–effect curve was characterized by an "inverted-U" shape. The ascending limbs of the curves (low to moderate doses per injection) were virtually identical across genotypes, with differences between DA D2R WT, HET, and KO mice exclusively on the descending limbs of the curves (moderate to high doses per injection). The lowest rates of responding at these relatively high doses were obtained in the WT mice. In contrast, when a D2 antagonist is administered before a self-administration procedure, there are parallel shifts to the right in the entire dose–effect curve (Risner and Cone 1986; Bergman et al. 1990; Caine et al. 2002). Extending the hypothesis above regarding the discriminative effects, it may very well be that D2 receptor-mediated effects, and those mediated by other DA receptors, are involved in the reinforcing as well as the discriminative-stimulus effects of cocaine. As such, at least a subset of DA receptors (including D2) are mechanistically redundant and summate for reinforcing and interoceptive stimulus effects. Thus, the dose effects are comparable for the different genotypes over the entire range of discriminable doses, and at least on the ascending limb of the self-administration dose–effect curve. The differences between genotypes on the descending limb of the self-administration dose–effect curve may be related to the genotype-dependent differences obtained in the present study for the locomotor-stimulant effects of cocaine. Effects such as locomotor stimulation may interfere with continued responding at high unit doses under the self-administration procedure. Therefore, a relatively reduced locomotor stimulation at the higher unit doses in the DA D2R KO mice would be expected to disrupt self-administration behavior less than in the other genotypes.

Thus, the present findings suggest that the discriminative and reinforcing effects of cocaine have much of their multiple pharmacological mechanisms in common. However, there may be some important differences that are primarily manifest at the highest doses and are related to the disruption of behavior. This interpretation suggests that the descending limb of the dose–effect curve in cocaine self administration reflects the behavioral disruptive effects of cocaine. These direct behavioral effects do not appear to influence the interoceptive discriminative-stimulus effects of cocaine across the entire dose range and may only disrupt the expression of a reinforcing effect exhibited through an active response. If the reinforced response was not incompatible with the effects elicited at high doses of cocaine, differences among genotypes might not be obtained. Possibly most important, the present study suggests that the interoceptive discriminative-stimulus effects, and likely the reinforcing effects of cocaine, appear to be multiply determined, with redundant contributions from several DA receptor subtypes.

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