

D2R DNA Transfer Into the Nucleus Accumbens Attenuates Cocaine Self-Administration in Rats

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ABSTRACT Dopamine (DA) D2 receptor (D2R) agonists and antagonists can modulate self-administration behavior, conditioned place preference, and locomotor responses to cocaine. Low levels of D2R have also been observed in cocaine addicted subjects and in non human primates after chronic cocaine exposures. Prior studies had shown that D2R upregulation in the nucleus accumbens (NAc) in rodents trained to self-administer alcohol markedly attenuated alcohol preference and intake. Here we assess the effects of D2R upregulation in the NAc on cocaine intake in rats trained to self-administer cocaine. Following 2 weeks of i.v. cocaine self-administration (CSA), rats were stereotaxically treated with an adenovirus that carried the D2R gene to upregulate D2R in the NAc. D2R vector treatment resulted in a significant decrease (75%) in cocaine infusions and lever presses (70%) for cocaine. This effect lasted 6 days before cocaine consumption returned to baseline levels, which corresponds roughly to the time it takes D2R to return to baseline levels. These findings show that CSA and D2R in the NAc are negatively correlated and suggest that cocaine intake is modulated in part by D2R levels in NAc. Thus strategies aimed at increasing D2R expression in NAc may be beneficial in treating cocaine abuse and addiction. **Synapse 62:481–486, 2008.** Published 2008 Wiley-Liss, Inc.[†]

INTRODUCTION

The involvement of the mesolimbic DA pathway in the initiation and maintenance of substance abuse including cocaine, has been generally established (Steketee, 2005). Specifically, among the structures in the mesolimbic reward circuit the nucleus accumbens (NAc) is believed to play a critical role in cocaine's reinforcing effects (Carelli et al., 1999; Chang et al., 1994; Laurier et al., 1994; Nicola and Deadwyler, 2000).

Cocaine's primary mechanism of action on reward is believed to be its ability to block DA transporters [dopamine transporter (DAT)], thus rapidly increasing the availability of DA in the synaptic cleft (Ritz et al., 1987). Increased levels of striatal DAT occupancy by cocaine correlate with subjective reports of "highs" in human subjects (Fowler et al., 2001), and microdialysis studies show that both active and passive administration of cocaine lead to elevated levels of extracellular DA in the NAc of rats and mice (Rouge-Pont

et al., 2002). In addition, DA receptor agonists and antagonists can modulate or disrupt self-administration behavior (Corrigall and Coen, 1991; Hubner and Moreton, 1991; Koob et al., 1987b), conditioned place preference (Baker et al., 1998; Sora et al., 1998; Vorel et al., 2002), and locomotor responses to cocaine (Baker et al., 1998; Chausmer and Katz, 2001).

The D2R subtype in particular has been suggested as an important component of the reinforcing effects of cocaine and other drugs (Rouge-Pont et al., 2002). Low D2R levels in ventral striatum (where the NAc is located) have been found in the brains of cocaine

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addicted humans, including cocaine abusers (Volkow et al., 1996), and in strains of rats bred to self-administer large quantities of ethanol (McBride et al., 1993; Thanos et al., 2004), whereas in these same rats the density of D1R and D3R receptor subtypes does not appear to differ from levels found in wild type animals (McBride et al., 1997). It has also recently been found that mutant mouse strains which do not express the D2R self-administer cocaine at a far greater rate when compared to wild-type animals (Caine et al., 2002). These lines of evidence suggest that the D2R plays a significant role in the reinforcing effects of cocaine and other abused substances, and that D2R levels may be one of the neurobiological variables that modulate the vulnerability of individuals to drug abuse.

In humans, the A1 allele of the D2R gene is associated with alcoholism, cocaine abuse, smoking, methamphetamine abuse, opioid abuse, gambling, obesity, schizophrenia, and depression [for review see (Noble, 2003)] and lower D2R in key structures in mesolimbic reward pathways such as the NAc and amygdala (Pohjalainen et al., 1998). However, some have failed to document such associations (Gelernter et al., 1993; Sery et al., 2001) and therefore the involvement of the A1 allele of the D2R gene remains controversial.

Previous studies have demonstrated that D2R gene-transfer into the NAc of the brain significantly attenuated alcohol intake and preference in Sprague-Dawley rats (Thanos et al., 2001, 2004); inbred alcohol preferring P rats (Thanos et al., 2004) and in mice (Thanos et al., 2005). Inasmuch as ethanol and cocaine show overlap in the circuits involved in their rewarding effects (Di Chiara et al., 1992; Koob et al., 1987a), we hypothesized that upregulation of D2R in the NAc of rats self-administering cocaine would reduce their cocaine intake.

MATERIALS AND METHODS

Animals

Sixteen adult male Sprague-Dawley rats (300–400 g) were individually housed in a controlled environment ($22 \pm 2^\circ\text{C}$, $50 \pm 10\%$ relative humidity) and subjected to a 24 h reverse 12 h light/12 h dark cycle (lights off at 0800 h) to maintain activity during the daytime. Weights were obtained daily and food and water was provided ad libitum. Experiments were conducted in conformity with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NAS and NRC, 1996) and Brookhaven National Laboratory Institutional Animal Care and Use Committee protocols.

Drugs

Cocaine Hydrochloride (0.3 mg/kg; Sigma, St. Louis, MO) was dissolved in saline (0.9% NaCl).

Procedures

Rats were trained to lever press for 45 mg food pellets (Bio-Serv, Frenchtown NJ) in operant chambers (Coulbourn Instruments, Allentown PA) on a 2 h fixed ratio 1 (FR-1) schedule daily. Data retrieval and processing was completed using Graphic State software (Coulbourn Instruments, PA). After reaching a criterion of at least 50 pellets/session for three consecutive days, rats were operated on and jugular catheterization was performed as previously described (Thanos et al., 2007). While rats were anesthetized [ketamine and xylazine (100 mg/kg, 10 mg/kg)] for jugular catheter implantation, they were also placed in a Kopf stereotaxic apparatus and were implanted unilaterally with a 22-gauge guide cannula (Plastics One, Roanoke, VA) into the NAc shell (+1.2 mm AP, ± 1.4 mm ML, -6.8 mm DV) (Paxinos and Watson, 1986). Laterality of cannula placement was randomly assigned so that half the rats received left NAc implants while the other half received implants into the right NAc. The guide cannula was then secured to the skull with four small stainless steel screws and dental cement.

Following a 1 week recovery from surgery, the rats were reintroduced to the operant-conditioning apparatus and the food FR1 protocol for 3–4 days before being started on the cocaine protocol. In the cocaine protocol, lever responses were followed by i.v. cocaine infusions. Each daily cocaine self-administration (CSA) session lasted 2 h and was conducted at approximately the same time of day (1000–1200 h). Approximately 100 μl of cocaine solution (0.3 mg/kg) was delivered i.v. following a single press of the reinforced (active) lever, followed by a 30 s timeout period during which presses of the active lever were recorded but not reinforced. A second or “dummy” lever was also provided in the conditioning box, and responses on this lever were recorded but had no programmed consequences.

After 7 days of CSA, all animals were treated with a microinfusion into the NAc of the control, replication-deficient adenovirus or Null Vector (AdCMV.Null), as previously described (Thanos et al., 2001, 2004), and then returned to the daily CSA sessions for 1-week. Subsequently, all animals were similarly microinfused once into the NAc with the D2R vector (AdCMV.DRD2), [see previous studies for details (Ikari et al., 1995; Ingram et al., 1998; Thanos et al., 2001, 2004; Umegaki et al., 1997)] and then returned to the CSA for 14 days.

Microinfusion of the vectors was carried out using an automated syringe pump (Razel, Stamford, CT) and a 26-gauge 5 μl Hamilton microsyringe connected to a 28-gauge internal cannula. Each microinfusion administered unilaterally into the NAc shell 2 μl of vector [adenoviral vector containing the cDNA for the D2R receptor (AdCMV.D2R) (10^{10} pfu/ml)] over 10 min so as to reduce the risk of procedure-induced

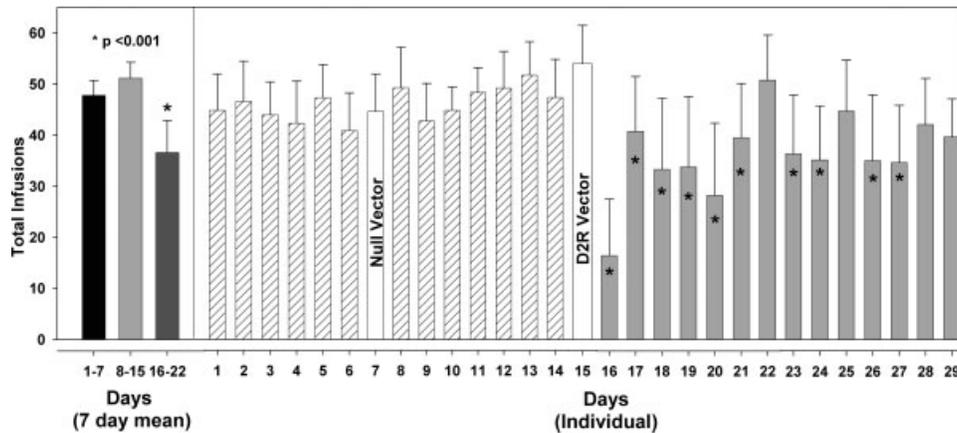


Fig. 1. Mean (+SEM) infusions of 0.3 mg/kg cocaine before and after treatment with the D2R vector. Mean Days: (*) The mean number of infusions on days 16–22 (after D2R vector treatment) was significantly lower ($P < 0.001$) than mean infusions on all other days prior to D2R vector treatment (Table 1). Individual Days: (*) Infusions on days 16–21, 23, 24, 26, and 27 were significantly lower compared to infusions on days 13, 14 or 15 ($P < 0.001$).

lesions. During the control treatment with the Null vector, the same procedure was followed, except that the solution infused was 0.2 M phosphate buffered saline.

Following completion of the behavioral experiments, brains were harvested from all rats for histological confirmation of the cannula placements. Briefly, each animal was deeply anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and the brain was rapidly removed and frozen in an isopentane/dry ice bath, and stored in a freezer at -80°C . Next, 20 μm thick coronal sections were cut on a cryostat (Leica CM3050S, Leica Microsystems, Nussloch, Germany). Sections were stained with 1% cresyl violet, coverslipped with Permount and allowed to air dry before verification of cannula placement in the NAc under light microscopy.

RESULTS

Rats did not show any signs of malaise or weight loss after treatment with the vector and no alterations in general behavior were noted. This was consistent with previous studies (Thanos et al., 2001, 2004). Treatment with the D2R vector produced a significant albeit transient reduction in the number of cocaine infusions and R lever responses in cocaine-experienced rats. In both the infusion and reinforced lever response data, by post-treatment day 11, most rats had returned to baseline levels of CSA.

Null and D2R vector treatment: infusions and lever responses

Two statistical analyses were conducted. (1) A one-way ANOVA compared the number of infusions and active lever presses during the three time phases (a)

days 1–7 (pre Null Vector treatment); (b) days 8–15 (post Null vector treatment); and (c) days 16–22 (post D2R vector treatment). (2) The second analysis, utilized a one-way repeated measures ANOVA to compare infusions and lever presses on individual days.

One-way ANOVA

Infusions

The one-way ANOVA showed a significant main effect between the three phases of CSA ($F(2, 21) = 20.776$; $P < 0.001$; Fig. 1). Multiple comparisons (Holm-Sidak) showed that the average number of infusions was not statistically different between pre and post null vector treatment (days 1–7 vs. days 8–15; $t = 1.072$; $P > 0.05$; Fig. 1). On the other hand, the average number of infusions post D2R vector (on days 16–22) was significantly lower to both pre Null vector treatment; (days 1–7; $t = 4.889$; $P < 0.001$) and post Null vector (days 8–15; $t = 6.122$; $P < 0.001$; Fig. 1).

Lever presses

Similarly with respect to active lever presses, a one-way ANOVA showed a significant main effect between the three groups ($F(2, 21) = 21.600$; $P < 0.001$). Multiple comparisons (Holm-Sidak) showed that the average number of lever presses prior to D2R vector treatment (days 1–7 vs. days 8–15) did not statistically differ ($t = 0.802$; $P > 0.05$). On the other hand, the average number of lever presses post D2R vector (days 16–22) was significantly lower as compared to both pre Null vector (days 1–7; $t = 5.172$; $P < 0.001$) and post Null vector (days 8–15; $t = 6.144$; $P < 0.001$) (Fig. 2).

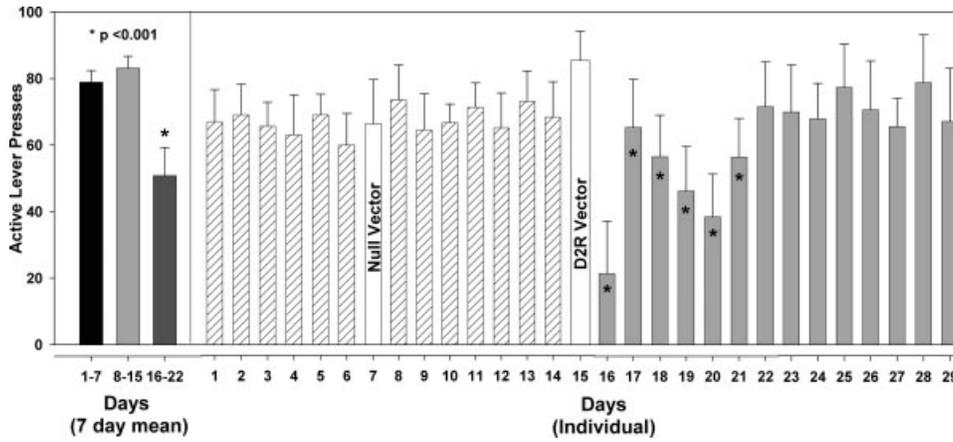


Fig. 2. Mean (+SEM) reinforced (active) lever responses to 0.3 mg/kg cocaine before and after treatment with the D2R vector. Mean Days: (*) The mean number of active lever presses on days 16–22 (after D2R vector treatment) was significantly lower ($P < 0.001$) than mean infusions on all other days prior to D2R vector treatment (Table II). Individual Days: (*) Lever Presses on days 16–21 were significantly lower compared to infusions on day 15 ($P < 0.001$).

TABLE I. Infusions: One-way repeated measures analysis of variance – pairwise comparisons ($\alpha = 0.05$)

Comparison (Days)	T	P
13.000 vs. 16.000	4.856	2.72E-06
14.000 vs. 16.000	4.292	2.99E-05
15.000 vs. 16.000	4.155	5.17E-05
13.000 vs. 20.000	3.557	0.000488
14.000 vs. 20.000	2.992	0.00319
13.000 vs. 18.000	2.992	0.00319
13.000 vs. 19.000	2.941	0.00373
15.000 vs. 20.000	2.856	0.00484
14.000 vs. 18.000	2.428	0.0162
14.000 vs. 19.000	2.377	0.0186
13.000 vs. 21.000	2.308	0.0222
15.000 vs. 18.000	2.291	0.0232
15.000 vs. 19.000	2.24	0.0264
13.000 vs. 27.000	2.206	0.0288
13.000 vs. 17.000	2.172	0.0313
13.000 vs. 26.000	2.154	0.0326
13.000 vs. 24.000	2.137	0.034
13.000 vs. 23.000	1.984	0.0489

TABLE II. Active lever responses: One-way repeated measures ANOVA – pairwise comparisons ($\alpha = 0.05$)

Comparison (Days)	T	P
15.000 vs. 16.000	7.623	1.73E-12
13.000 vs. 16.000	6.82	1.57E-10
14.000 vs. 16.000	6.488	9.36E-10
15.000 vs. 20.000	5.248	4.6E-07
13.000 vs. 20.000	4.444	0.000016
15.000 vs. 18.000	4.16	5.06E-05
14.000 vs. 20.000	4.112	6.12E-05
15.000 vs. 19.000	4.003	9.38E-05
13.000 vs. 18.000	3.357	0.000976
13.000 vs. 19.000	3.199	0.00165
14.000 vs. 18.000	3.025	0.00288
14.000 vs. 19.000	2.867	0.00467
15.000 vs. 21.000	2.632	0.00929
15.000 vs. 17.000	2.143	0.0335

One-way repeated measures ANOVA

Infusions

A one-way repeated measures ANOVA showed a significant main effect over time for cocaine infusions ($F(28, 202) = 2.418; P < 0.001$). Multiple pairwise comparisons (Holm–Sidak method) showed that treatment with the D2R vector significantly attenuated cocaine infusions (Fig. 1). We did not find any statistically significant difference in the number of cocaine infusions prior to D2R vector (days 1–15; Fig. 1). Significant pairwise comparisons are illustrated in Table I for the number of infusions during the last three days before D2R vector treatment and all the days following D2R vector treatment.

Lever presses

A one-way repeated measures ANOVA showed a significant main effect over time for active lever

responses ($F(28, 202) = 2.387; P < 0.001$). Multiple pairwise comparisons (Holm–Sidak method) showed that treatment with the D2R vector significantly attenuated active lever responses to cocaine (Fig. 2). Since again we did not find any statistical significance for any day prior to D2R vector (days 1–15); only the significant pairwise comparisons are illustrated in Table 2 for the number of lever presses during the last three days before D2R vector treatment and all the days following D2R vector treatment.

DISCUSSION

D2R adenoviral upregulation caused a significant decrease in CSA (infusions and lever responses) for up to six days post-treatment. More specifically, the D2R vector had a robust (75% decrease) effect in the number of cocaine infusions. This effect was of short duration and was observed for the first 6 days following D2R vector treatment and then returned to baseline levels with the exception of a few sporadic days during the next six days. The attenuation of cocaine

consumption by D2R upregulation was consistent with previous studies on ethanol consumption, where alcohol consumption was significantly attenuated. In previous alcohol studies, D2R upregulation attenuated ethanol intake for eight days in Sprague Dawley rats (Thanos et al., 2001, 2004) and 20 days for the inbred alcohol preferring P rats (Thanos et al., 2001, 2004). It has been previously shown that this D2R vector produced significant D2R upregulation for eight days before returning to baseline by day 10 (Ingram et al., 1998; Ogawa et al., 2000; Thanos et al., 2001, 2004).

Several factors may be involved in explaining the difference in the duration of this effect of D2R upregulation in cocaine versus alcohol administration. To start with, the role of D2R is different in the mechanisms of cocaine and alcohol reward. Previous work has suggested that ethanol-induced DA release in the NAc is insensitive to DA autoreceptor (D2R and/or D3R) regulation (Adam-Vizi, 1992; Levi and Raiteri, 1993; Yan, 2003) whereas, cocaine-induced DA release in the same region is thought to depend on regulation by D2R and D3R (Yan, 2003).

Furthermore, it has been shown that D2R knockout mice can still be trained to self-administer cocaine, and do so often at higher rates than wild type mice (Caine and Ralph-Williams, 2002), suggesting that D2R did not represent the only route through which the pharmacologically rewarding effects of cocaine took place. It is not clear however what specific route may be involved in CSA in these mice since like all transgenic animals, D2R knockout mice may show compensatory changes in other receptors and neurotransmitter systems (Bolon, 2004) involved in CSA. In contrast, it is much harder to compensate for the inhibition of the rewarding effects of alcohol on D2R KO mice (Risinger et al., 2000; Thanos et al., 2005).

The findings from this study are consistent with our work and that of others proposing that D2R play an important role in modulating drug self-administration behavior. By increasing the absolute number of D2R in the NAc, we have successfully, if transiently, disrupted CSA. Since the main effect of upregulating D2R in the NAc is likely to be increased transmission along the included DA pathways, analogies related to the other determinants of synaptic DA transmission in the NAc can be made. For example, studies showing that CSA may be triggered by low levels of accumbal DA in cocaine-experienced rats (Gerrits et al., 2002) suggest one way that our treatment might be decreasing the desire to self-administer cocaine. D2R upregulation could increase the probability of an interaction between transient DA increases and receptor activation, which would increase the sensitivity to the DA enhancing effects of drugs of abuse. As a result the amount of drug required to activate reward pathways would be lowered explain-

ing the decrease in the total amount of cocaine ingested. Alternatively D2R upregulation may increase the number of constitutively active D2R receptors. However, the mechanism(s) underlying how D2R upregulation decreases CSA requires further investigation.

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