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Modifications of dopamine D_1 receptor complex in rats self-administering cocaine

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Abstract

Cocaine is spontaneously and experimentally self-administered and, when given repeatedly, it induces a stable form of sensitization to a previously assessed minimum active dose. In the present study, triads of rats chronically implanted with a jugular catheter were treated as follows: one animal was trained to self-inject cocaine, while the other two passively received either cocaine or saline whenever the self-administering rat completed the response requirement. After 30 days of stable responding, the animals were sacrificed and dopamine D_1 receptor density and adenylyl cyclase activity were measured in different brain areas. Animals receiving cocaine (both self-administering and yoked) showed a down-regulation of dopamine D_1 receptor number and of dopamine stimulated adenylyl cyclase activity in the nucleus accumbens, as compared to saline rats. In the olfactory tubercle, dopamine stimulated adenylyl cyclase activity appeared selectively and significantly down-regulated in self-administering animals.

Keywords: Adenylyl cyclase; Dopamine D_1 receptor; Cocaine; Self-administration

1. Introduction

Cocaine is among the most widely abused central stimulants although its pharmacological profile is characterized by a variety of other relevant actions. It binds with high affinity to the dopamine transporter located at the dopaminergic nerve terminals and competitively inhibits the monoamine reuptake (Heikkila et al., 1975; Taylor and Ho, 1977). Thus, the synaptic concentration of dopamine increases (Hurd and Ungerstedt, 1989; Pettit and Justice, 1989) and the enhanced transmission in the dopaminergic neuronal system which follows mediates both the behavioral (Kelley and Iversen, 1975; Kalivas et al., 1988) and the reinforcing (Koob and Bloom, 1988) effects of the compound. Cocaine, like all other central stimulants, increases locomotor activity, induces stereotyped movements, excites the reward system, affects hormone release, and all these effects are thought to be mediated by dopamine receptor stimulation (Johanson and Schuster, 1995). When administered repeatedly it also induces sensitization, which is a rather stable potentiation of the response to a previously assessed minimum active dose (Post and Rose, 1976; Shuster et al., 1977). Which type of dopamine receptor selectively mediates each of these effects is currently not known. Five distinct dopamine receptors have been cloned which have been pharmacologically divided into the D_1 (D_1, D_5) and the D_2 (D_2, D_3, D_4) classes, distinguishable by their opposite modulation of adenylyl cyclase activity and discrete brain localization (Stoof and Kebabian, 1981; Onali et al., 1985; Wamsley et al., 1989). However, while several behavioral and electrophysiological studies clearly document the phenomena of development and expression of sensitization (Ellinwood et al., 1973; Post and Weiss, 1988; Lieberman et al., 1990; Henry and White, 1991), different studies aimed at characterizing specific modifications of dopamine receptors associated with sensitization yielded quite discordant results (Taylor et al., 1979; Goeders and Kuhar, 1987; Trulson and Ulissey, 1987; Kleven et al., 1990; Unterwald et al., 1994). The observed discrepancies could be due to the use

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of different animal species or strains, different treatment protocols, and different brain areas analyzed.

In the present study the issue of measurable modifications produced by long-term exposure to central stimulants on the dopamine D_1 receptor system was addressed in rats by comparing the effects of self-administered cocaine vs. those of a non-contingent administration of the drug. Yoked animals passively receiving the same volume of saline were used as controls. Dopamine D_1 receptor binding and the kinetics of dopamine stimulated adenylyl cyclase were detected in discrete brain areas 18 h after the last session of self-administration. The results obtained are consistent with a desensitization of the dopamine D_1 receptor system in selective brain areas of rats receiving cocaine either in a contingent or non-contingent manner.

2. Materials and methods

2.1. Animals and surgical procedures

Male Fischer strain F-344 90–150-day old rats were used in groups of three littermates. Littermates were housed together in group cages with food and water available ad libitum except during experimental session. Rats were kept in a temperature controlled environment on a reversed 12-h light/dark cycle with lights on at 17:00 h. Experiments took place during the dark phase of the cycle.

Rats were implanted with external jugular venous catheters under anesthesia induced by pentobarbital (50 mg kg^{-1}, i.p.) and atropine (10 mg kg^{-1}, i.p.) according to Weeks (1962). Briefly the catheter was inserted into the right jugular vein until it terminated just outside the right atrium and anchored to muscle in the area of the vein. The other end of the catheter was guided s.c. to the back to exit between the scapulae where it continued through a shoulder harness. The catheter was threaded through a spring leash and a counterbalanced single channel fluid swivel (Brown et al., 1976) and connected to an infusion pump (Razel, Stanford, CT). Rats were administered penicillin G procaine (17 000 U, i.m.) and allowed 7 to 10 days to recover from surgery before training was initiated. Catheter patency was checked periodically with methohexital (10 mg kg^{-1}, i.v.) for loss of conscious within 5 s.

2.2. Cocaine self-administration

Triads of rats with chronic jugular catheters were exposed to three different treatment conditions in standard operant chambers (Coulborn Instruments, Allentown, PA) containing a house light, a three-cue stimulus light, response lever, a tone source, a glass food pellet tube dispensing 20 pellets day^{-1} after the session and a drinking tube providing unlimited access to water. One rat was trained to respond on the lever with i.v. cocaine reinforcement (0.33 mg/inf delivered over 5.8 s) while a littermate received simultaneous response independent infusions of cocaine and a third littermate received simultaneous infusions of vehicle (both yoked to the self-administering rat). Six-hour sessions were conducted 7 days $week^{-1}$. The session started at 09:00 h at which time a green light was illuminated, a lever was extended into the chamber and cocaine was made available until 15:00 h at which time the lever was retracted. The rats that were allowed to self-administer were trained on a fixed ratio 1 schedule of cocaine presentation (i.e., the rat had to press the lever once in order to receive the cocaine injection) that terminated after 6 h. When responding was stable under the fixed ratio 1 requirement, the ratio was raised to the terminal value of fixed ratio 2. Although not all rats started responding immediately when the lever was made available, once responding was initiated consistent interinjection intervals were maintained. Over the total course of the experiment the triads were exposed to an average of 30 sessions before the neurochemical evaluations began and received an average of 47 infusions during the daily 6-h sessions.

2.3. Tissue preparation

Rats were decapitated 18 h after the last session, the brains were removed and five different regions were dissected (olfactory tubercle, prefrontal cortex nucleus accumbens, striatum, hippocampus) and immediately used for the adenylyl cyclase assay. An aliquot of the homogenate was frozen at $-80°C$ for [^{3}H] SCH 23390 ((*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-phenyl-1*H*-3-benzazepin-7-ol hemimaleate) binding for all regions except nucleus accumbens. Due to the limited amount of tissue it was necessary to pool the accumbens of two animals per group in order to measure [^{3}H] SCH 23390 binding and adenylyl cyclase activity.

2.4. Adenylyl cyclase assay

Tissue was homogenized (1:10, w:v) in 5 mM Tris–HCl buffer (pH 7.4) containing 1 mM dithiothreitol, 1mM EGTA (TDE buffer) and 10% sucrose with a motor driven Teflon–glass tissue grinder (clearance: 0.25 mm, 400 rpm). The homogenate was then centrifuged at $900 \times g$ for 10 min and the supernatant collected and centrifuged at 9000 $\times g$ for 20 min. The P_2 fraction was washed once, recentrifuged, then resuspended and immediately used for the adenylyl cyclase activity assay. The reaction mixture contained the following reagents: 75 mM Tris–HCl, pH 7.4, 0.33 mM EDTA, 2 mM $MgCl_2$, 0.5 mM Isobutylmethylxantine (IBMX), 1 mM cAMP, 0.1 mM α-^{32}P ATP (spec. act. 30 Ci $mmol^{-1}$, New England Nuclear), 5 mM phosphocreatine, 5 U ml^{-1} of creatine phosphokinase, 10 μM GTP, 50 μg per sample bovine serum albumin, 0.33 mM dithiothreitol, 1–100 μM dopamine or 5 μM forskolin in a final volume of 150 μl. The reaction was started by adding

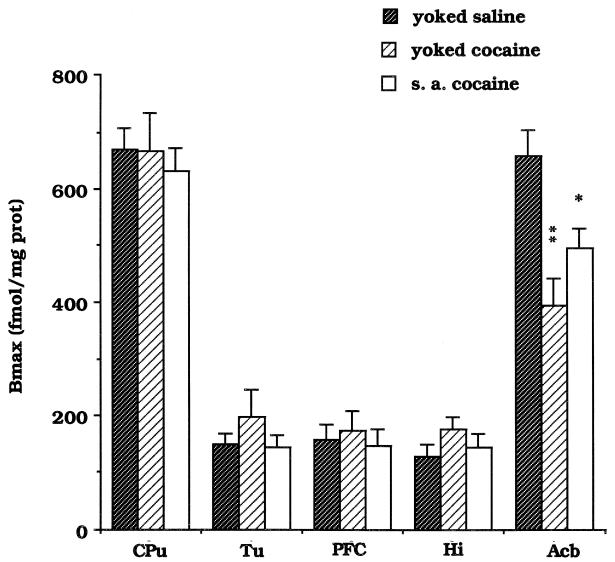

Fig. 1. Dopamine D_1 receptor density in cocaine self-administering, yoked cocaine and yoked saline rats. Five brain regions were dissected as described in Section 2. Values are expressed as mean ± S.E.M. of four experiments. ∗Significantly different from yoked saline animals ($P < 0.05$). ∗∗Significantly different from yoked saline animals ($P < 0.01$).

50 μl of membrane preparation and continued for 6 min at 37°C. The incubation was stopped by adding 200 μl of a solution containing 2% sodium dodecylsulphate (w:v) 45 mM ATP and 1.3 mM cyclic AMP pH 7.5. After the addition of ^{3}H-cAMP (spec. act. 33 Ci mmol^{-1}, New England Nuclear) to monitor cyclic AMP recovery, the samples were placed in a boiling water bath for 3 min and cyclic AMP was then isolated according to Salomon et al. (1974). The concentration of dopamine required to induce a half maximal activation of adenylyl cyclase activity (ED_{50}) and the maximal increment obtained by the stimulation (V_{max}) was calculated by linear regression analysis of Eadie–Hofstee plots obtained from concentration–response curves (Eadie, 1952; Hofstee, 1952). Protein content was determined with the method of Lowry et al. (1951).

2.5. [^{3}H] SCH 23390 binding

The homogenate was diluted in 100 volumes of ice-cold 50 mM Tris–HCl buffer, pH 7.4. and centrifuged (48 000 × g, 10 min). The resultant pellet was resuspended and washed two times in the initial volume of the same buffer and recentrifuged. The final pellet was resuspended in 50 mM Tris–HCl buffer pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Binding assay was performed according to Billard et al. (1984) with minor modifications. Briefly, 400 μl aliquots of the membrane preparations were incubated with different concentrations (0.03 to 10 nM) of [^{3}H] SCH 23390 (spec. act. 80 Ci mmol^{-1}, New England Nuclear) in a final volume of 0.5 ml. After 30 min incubation at 37°C the reaction was stopped by adding 4 ml of ice-cold 50 mM Tris–HCl buffer, pH 7.4 and the membranes were filtered through Whatman GF/B filters and rinsed with two 4-ml portions of the same ice-cold buffer using a Brandel Cell Harvester. Non-specific binding was determined in the presence of 10 mM cisflupentixol. The maximum number of binding sites (B_{max}) and the apparent dissociation constant (K_d) were calculated by linear regression analysis of Scatchard plots corresponding to the saturation curves of specific [^{3}H] SCH 23390 binding.

2.6. Drugs

Cocaine–HCl was provided by the National Institute on Drug Abuse. Pentobarbital and methohexital were provided by the pharmacy of the Wake Forest University, School of Medicine. All other chemicals were obtained from Sigma.

2.7. Data analysis

Data are expressed as mean ± S.E.M. Statistical comparisons were made by analysis of variance (ANOVA) followed by 'post-hoc' Bonferroni's test. A value of $P < 0.05$ was considered significant. Data from the self-

Table 1
^{3}H SCH 23390 binding in different areas of rat brain

Brain area	Yoked saline		Yoked cocaine		Cocaine self-administered	
	B_{max} (fmol (mg P)$^{-1}$)	K_d (nM)	B_{max} (fmol (mg P)$^{-1}$)	K_d (nM)	B_{max} (fmol (mg P)$^{-1}$)	K_d (nM)
CPu	669.89 ± 37.1	0.74 ± 0.1	666.10 ± 66.4	0.83 ± 0.2	632.34 ± 39.0	0.65 ± 0.1
Tu	150.52 ± 16.3	1.17 ± 0.1	197.17 ± 48.6	1.96 ± 0.4	144.76 ± 19.6	1.30 ± 0.7
PFC	157.36 ± 28.9	2.12 ± 0.6	173.53 ± 33.4	2.46 ± 0.4	146.89 ± 27.9	1.82 ± 0.2
Hi	129.33 ± 19.8	2.19 ± 0.2	174.75 ± 22.0	3.01 ± 0.3	143.32 ± 23.8	2.77 ± 0.2
Acb	659.66 ± 43.5	1.65 ± 0.6	395.47 ± 48.4∗∗	1.28 ± 0.7	495.63 ± 36.3∗	1.32 ± 0.3

Data are expressed as mean ± S.E.M. of six experiments.
Abbreviations: CPu: caudate putamen; Tu: olfactory tubercle; PFC: prefrontal cortex; Hi: hippocampus; Acb: nucleus accumbens.
∗ $P < 0.05$ as compared to yoked saline values.
∗∗ $P < 0.01$ as compared to yoked saline values.

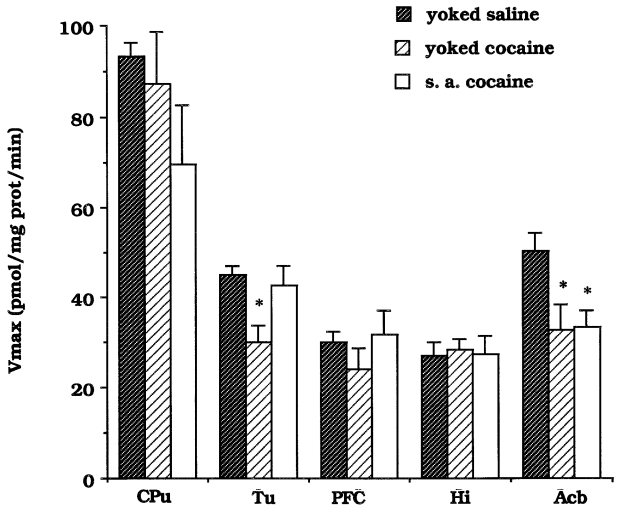

Fig. 2. Dopamine stimulated adenylyl cyclase in cocaine self-administering, yoked cocaine and yoked saline rats. Five brain regions were dissected as described in Section 2. V_{max} values are expressed as mean ± S.E.M. of at least six experiments. Basal values were not statistically different between groups in each region (caudate putamen (CPu): ys: 102.32 ± 10.35; yc: 104.47 ± 9.18; sa: 97.39 ± 9.00; olfactory tubercle (Tu): ys: 70.82 ± 9.22; yc: 71.76 ± 12.72; sa: 81.23 ± 10.67; prefrontal cortex (PFC): ys: 52. ± 57 4.82; yc: 58.77 ± 6.80; sa: 57.99 ± 5.82; hippocampus (Hi): ys: 56.20 ± 5.14; yc: 48.78 ± 4.66; sa: 51.01 ± 5.45; nucleus accumbens (ACb): ys: 72.68 ± 9.87; yc: 77.26 ± 11.89; sa: 71.00 ± 497.32; 71.76 ± 12.72; sa: 81.23 ± 10.67). * Significantly different from yoked saline and cocaine self-administering animals ($P < 0.05$).

administered rats were compared with the yoked cocaine-infused rats to determine if there was an effect of contingent drug delivery and the yoked cocaine group was compared with the yoked saline group to determine if there was an effect of the drug itself.

3. Results

The present study compared the effects of cocaine self-administration with non-contingent treatment on the activity of dopamine D_1 receptors in different brain areas of rats. The experiments were performed in triads of animals, one of which was trained to self-inject cocaine, while the other two received either the same doses of cocaine or the same volume of saline, respectively, yoked to the self-administering animal. After the training period, the average daily dose of cocaine, administered during a 6-h session to either self-injecting or yoked animals, was 40 mg kg^{-1}.

3.1. Dopamine D_1 receptor number

Rats of both groups receiving cocaine, when compared to those yoked with saline, presented a significant decrease in D_1 dopamine receptor number confined to the nucleus accumbens, with no change in the apparent K_d. As shown in Fig. 1, the decrease in the B_{max} of [^{3}H] SCH 23390 binding was more marked in yoked (−40%, $P < 0.01$) than in self-administering animals (−25%, $P < 0.05$), although the difference between the two groups did not reach significance. In the other brain areas examined cocaine administration, independently of the protocol used, did not modify either dopamine D_1 receptor density or the affinity of [^{3}H] SCH 23390 for its specific binding sites, as indicated by the calculated values of the apparent K_d (Table 1).

3.2. Adenylyl cyclase activity

Cocaine induced a pattern of change in adenylyl cyclase activity analogous to that of dopamine D_1 receptors. Thus, the enzyme response to D_1 dopamine receptor stimulation was significantly reduced (−35%) in the nucleus accumbens of both self-administering and yoked cocaine-infused rats, when compared to saline-infused animals. However, in the olfactory tubercle the V_{max} of the enzyme appeared unmodified in self-administering animals, while it was significantly decreased (−34%) in yoked cocaine compared to yoked saline rats (Fig. 2). The ED_{50} values of dopamine stimulatory activity did not appear modified in any of the areas evaluated for all three treatment conditions (Table 2).

Table 2
Dopamine stimulated adenylyl cyclase in different areas of rat brain

Brain area	Yoked saline		Yoked cocaine		Cocaine self-administered	
	V_{max} (pmol (mg P)$^{-1}$ min^{-1})	ED_{50} (μM)	V_{max} (pmol (mg P)$^{-1}$ min^{-1})	ED_{50} (μM)	V_{max} (pmol (mg P)$^{-1}$ min^{-1})	ED_{50} (μM)
CPu	93.17 ± 3.2	5.84 ± 1.2	87.17 ± 11.4	13.37 ± 4.9	69.80 ± 13.0	3.70 ± 0.9
Tu	44.99 ± 1.9	7.28 ± 2.0	29.86 ± 3.8*	19.51 ± 8.5	42.75 ± 4.3	25.76 ± 10.5
PFC	29.86 ± 2.4	12.59 ± 1.2	23.97 ± 4.5	19.35 ± 4.6	31.63 ± 5.5	10.98 ± 3.4
Hi	27.09 ± 2.9	15.06 ± 4.0	28.42 ± 2.2	16.27 ± 4.7	27.23 ± 4.0	14.26 ± 1.9
Acb	50.37 ± 4.1	4.29 ± 1.0	32.80 ± 5.6*	5.25 ± 1.5	33.27 ± 3.6*	3.26 ± 0.8

Data are expressed as mean ± S.E.M. of six experiments.
Abbreviations: CPu: caudate putamen; Tu: olfactory tubercle; PFC: prefrontal cortex; Hi: hippocampus; Acb: nucleus accumbens.
* $P < 0.05$ as compared to yoked saline values.

Table 3
Forskolin-stimulated adenylyl cyclase in different brain areas

Brain area	Yoked saline (pmol (mg P)$^{-1}$ min^{-1})	Yoked cocaine (pmol (mg P)$^{-1}$ min^{-1})	Cocaine self-administered (pmol (mg P)$^{-1}$ min^{-1})
CPu	727.47 ± 104.9	759.35 ± 54.0	711.72 ± 80;0
Tu	170.54 ± 13.6	178.32 ± 32.2	200.29 ± 18.9
PFC	184.45 ± 18.8	144.79 ± 9.8	216.36 ± 13.7
Hi	133.42 ± 5.3	140.64 ± 3.5	122.94 ± 9.2
Acb	449.09 ± 59.6	395.36 ± 56.5	374.83 ± 9.1

Data are expressed as mean ± S.E.M. of three experiments.
Abbreviations: CPu: caudate putamen; Tu: olfactory tubercle; PFC: prefrontal cortex; Hi: hippocampus; Acb: nucleus accumbens.
Basal values were not significantly different between groups in each area.
Forskolin was used at the concentration of 5 μM.

The sensitivity of the enzyme to forskolin stimulation appeared decreased in the nucleus accumbens (∼ −20%) of both animal groups receiving cocaine and in the prefrontal cortex (∼ −30%) of yoked rats, although it did not reach significance (Table 3).

4. Discussion

The effect of cocaine on dopamine D_1 receptor number and on dopamine stimulated adenylyl cyclase activity was analyzed in five discrete brain areas of rats trained to self-inject cocaine on a fixed ratio 2 schedule. Triads of animals chronically implanted with a jugular catheter were exposed to three different treatment protocols: one rat self-administered cocaine; the second and the third passively received the same cocaine dose or equal saline volumes, respectively, whenever the first animal correctly pressed the lever. Values obtained from self-injecting animals were compared to those of yoked cocaine or saline rats. In the olfactory tubercle the density of dopamine D_1 receptors was similar in the three groups of rats, while dopamine stimulated adenylyl cyclase was selectively reduced in cocaine-yoked-infused animals. Moreover, cocaine produced a significant down-regulation of the number of [^{3}H] SCH 23390 binding sites in the nucleus accumbens independently of how the drug had been delivered. Cocaine also reduced the V_{max} of dopamine stimulated adenylyl cyclase in the nucleus accumbens, while the response to forskolin stimulation appeared not to be significantly decreased. Forskolin activates adenylyl cyclase by directly stimulating the Gs protein and/or the catalytic moiety. Thus, it was not surprising to observe in the homogenate of a brain area containing different adenylyl cyclase pools connected with different receptor systems, a variation of enzyme responsiveness restricted to a specific receptor, while the total enzyme activity appeared unmodified. It is more difficult to find an explanation for the different effect observed in the olfactory tubercle of yoked rats receiving cocaine compared to those which were self-administering cocaine or passively receiving saline. It may be relevant to consider that rats non-contingently receiving cocaine are also more sensitive to its lethal effect as compared to their self-injecting companions (Dworkin et al., 1995b). Limbic nuclei seem to have a different adaptive sensitivity to long-term cocaine; thus, the olfactory tubercle neurons seem to modify their responsiveness to dopamine on the basis of the method of cocaine administration, while the nucleus accumbens neurons desensitized independently of how cocaine was administered. Finally, the lack of effect on the density of dopamine D_1 receptors in the olfactory tubercle of yoked cocaine animals was not surprising since an uncoupling of receptors from their G protein has been already described in a report on the effects of prenatal exposure to cocaine (Hoau-Yan et al., 1995).

Results similar to ours were reported in Long–Evans rats trained to self-inject cocaine daily during a 60-min session (Laurier et al., 1994). [^{3}H] SCH 23390 binding was found significantly decreased in a pool of brain areas encompassing the nucleus accumbens, the striatum and the olfactory tubercle, with complete recovery of normal values within a week. Dopamine receptor gene expression examined by Northern blot analysis showed increased dopamine D_1 receptor messenger RNA levels in the forebrain. Thus suggesting that cocaine increases the turnover rate of dopamine D_1 receptors which, at equilibrium, appear decreased in number. Such a conclusion is supported by the finding (Tella et al., 1996) that Sprague–Dawley rats exposed to a 2-h regimen of cocaine self-administration show a marked increase of the dopamine transporter which, during the withdrawal phase, results in a decreased extraneuronal dopamine concentration and a significantly increased [^{3}H] SCH 23390 binding in the nucleus accumbens. In our experimental conditions, the 6-h daily cocaine binges (i.e., the condition of pharmacologically enhanced dopamine transmission which increases dopamine D_1 receptor turnover rate) resulted in a significant down-regulation not only of the dopamine D_1 receptor number, but also of the associated adenylyl cyclase activity. In the present study, we did not measure the activity of dopamine D_2 dopamine receptors. However, a recent re-

port (Laurier et al., 1994) demonstrates that no modifications of dopamine D_2 receptors occur in cocaine self-administering animals.

The self-administration paradigm was chosen because it closely reproduces the binge pattern of substance consummation typical of most cocaine addicts. Thus, by pairing rats where an animal self-injects cocaine while the other two non-contingently receive either cocaine or saline, we can compare the possible responses to the actively or passively administered drug. Some of these differences have already been reported (Smith et al., 1982, 1984; Dworkin et al., 1995a). Self-administered heroin does not increase extraneuronal dopamine in the nucleus accumbens, as measured by microdialysis technique, while both heroin and morphine are known to produce this effect in passively treated rats (Di Chiara and Imperato, 1988; Hemby et al., 1995). The effect of cocaine on extraneuronal dopamine concentration in the nucleus accumbens is significantly more pronounced in self-administering rats compared to their yoked companions (Hemby et al., 1997). Moreover, yoked animals are more sensitive to the toxic effect of cocaine and most of them die on a high dose regimen which is, however, safe for self-injecting rats (Dworkin et al., 1995b). Analogously, yoked cocaine rats showed a significantly decreased V_{max} of dopamine stimulated adenylyl cyclase in the olfactory tubercle, while the same dose of cocaine showed no effect in self-administering animals. These differences between self-administration and passive infusion of cocaine emphasizes the importance of environmental variables in the neurochemical consequences of drug administration.

It was recently reported that a dopamine D_2 receptor agonist completely substitutes for cocaine in reinstating cocaine self-administration after saline induced extinction, and that a dopamine D_1 receptor agonist has no priming activity and indeed antagonizes the increased lever pressing frequency induced by both cocaine and dopamine D_2 receptor agonists (Self et al., 1996). This finding strongly suggests that dopamine D_2 receptor activation mediates the internal state which starts the operant behavior, while dopamine D_1 receptor stimulation determines the condition of temporary satiation which follows cocaine administration. Considering that repeated cocaine administrations seem to desensitize dopamine D_1 receptors in the nucleus accumbens and that such receptors may be crucial in mediating cocaine reinforcing activity, it may be concluded that dopamine D_1 receptor desensitization may play an important role in determining the compulsive lever pressing maintained by cocaine self-administration.

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