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Effects of repeated injections of cocaine on D₁ and D₂ dopamine receptors in rat brain

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In order to determine if chronic administration of cocaine produced long-lasting alterations in dopamine receptor binding, rats were treated with single daily injections of cocaine (0, 10, or 20 mg/kg) for 15 consecutive days and killed either 20 min or 2 weeks after the last injection. The density of D₁ binding sites in frontal cortex was either unchanged (10 mg/kg) or slightly increased (20 mg/kg) 20 min after the last daily injection, but was decreased 2 weeks later. D₁ sites in striatum were decreased both immediately and 2 weeks after the injection regimen. Decreases in D₁ binding site density in nucleus accumbens were observed only immediately after the last injection. In contrast to these effects on D₁ binding sites, D₂ binding sites were decreased in striatum and frontal cortex and increased in the nucleus accumbens 20 min after repeated cocaine, but were unaffected 2 weeks after repeated cocaine. Computer-assisted analysis of the saturation isotherms revealed that chronic administration of cocaine did not affect the affinity (K_d) of the radioligands used to label D₁ or D₂ sites. These findings suggest that repeated administration of cocaine results in long-term decreases in D₁ binding sites in striatum and frontal cortex and transient decreases in D₂ binding sites. Furthermore, cocaine caused opposite, transient effects on D₁ and D₂ site density in nucleus accumbens.

INTRODUCTION

With the recent increase in cocaine abuse, the effects of long-term exposure have become an important public health issue. However, the behavioral and neurochemical consequences of repeated or prolonged exposure to cocaine are not well understood. Because of the prevailing view that dopamine (DA) is involved in many of the effects of cocaine^{4,10,32}, attention is now focused on neurochemical and receptor alterations in DA systems as a consequence of chronic exposure. In addition, certain similarities between cocaine and amphetamine-like compounds raise the possibility that repeated exposure to cocaine might produce long-term neurotoxic changes similar to those caused by *D*-methylamphetamine³⁴. These toxic effects include reductions in the concentration of DA and/or serotonin in several brain regions that are the result of destruction of nerve terminals containing these monoamines³⁴.

While chronic administration of cocaine does not cause long-term depletions of brain DA^{18,20}, changes in DA metabolism and/or utilization have been reported^{17,18}; both single and multiple injections of cocaine have been reported to enhance stimulated DA release for a period

of up to 2 weeks following exposure^{18,26}. In addition, several recent studies have provided functional evidence that DA receptors are altered as a consequence of chronic cocaine treatment^{21,26}. Goeders and Kuhar¹² reported that single daily injections of cocaine for 15 days caused a decrease in the density of D₂ receptor binding sites in striatum and an increase in D₂ sites in the nucleus accumbens when these regions were examined shortly after the last injection of cocaine. Taken together these studies suggest that it is possible that cocaine has long-lasting effects on DA neurotransmission in the absence of amphetamine-like neurotoxicity.

The present study was designed to examine the effects of repeated cocaine exposure on DA binding sites in brain. One purpose was to determine whether reported effects of cocaine on the density of D₂ binding sites are long-lasting. Furthermore, since the effects of repeated cocaine on D₁ DA receptors have not been fully characterized, potential changes in D₁ receptor sites were also examined. The results show that changes in the density of D₂ receptor binding sites are transient, whereas cocaine-induced decreases in the density of D₁ receptors in several brain regions lasted at least 2 weeks.

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MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Harlan, IN), weighing 200–250 g at the start of the study were used. Food (Teklad Rat and Mouse Diet, Teklad, Winfield, IA) and water were continuously available. Rats were housed individually in stainless steel cages in room with lights on a 12-h dark-light cycle (lights on from 07.00–19.00 h) and temperature maintained at approximately 24 °C.

Methods

In the first experiment, rats ($n = 6/\text{group}$) were administered either saline or cocaine (10 or 20 mg/kg, i.p.) once daily at approximately 15.00 h for 15 consecutive days and were killed either 20 min or 2 weeks after the last daily injection. The second experiment was designed to replicate the findings of the first experiment and was extended to include the examination of an additional region (nucleus accumbens). In the second experiment, rats ($n = 6/\text{group}$) were administered either saline or cocaine (10 mg/kg, i.p.) once daily for 15 consecutive days and then killed either 20 min or 2 weeks after the last injection. In both experiments, rats were killed by guillotine and their brains were dissected over ice into various regions according to a previous published procedure¹⁴. The brain sections were wrapped individually in aluminum foil and stored at -70 °C until assayed.

Radioligand binding methods for D_1 - and D_2 -DA receptor binding sites were adapted from original techniques^{13,35}. D_1 binding sites were labeled with [¹²⁵I]SCH 23982 (NEN Dupont, 2200 Ci/mmol). In the first experiment D_2 binding sites were labeled using [¹²⁵I]spiperone (NEN Dupont, 2200 Ci/mmol); in the second experiment, D_2 binding sites were labeled with [³H]spiperone (NEN Dupont, 32.4 Ci/mmol). The change in radioligands used to label D_2 binding sites was made when separate experiments demonstrated no advantage of the more expensive and labile iodinated ligand, [¹²⁵I]spiperone over [³H]spiperone.

For all binding studies, immediately prior to each assay, tissue from individual animals was slowly thawed and disrupted by sonification using intermittent 5-s bursts with a Branson sonifier in 20 vols. (w/v) of 50 mM Tris-HCl buffer (pH 7.5, ca. 25 °C, with 5 mM NaEDTA and 50 mM NaCl). This homogenate was centrifuged at $45\,000 \times g$ for 15 min at 4 °C. The resulting pellet was resuspended in 20 vols. 50 mM Tris-HCl buffer (pH 7.5, ca. 25 °C with 50 mM NaCl) and centrifuged as above. The final pellet was suspended in assay buffer.

D_1 receptor binding sites were quantified using saturation studies (6 concentrations ranging from 0.01–1.5 nM). In brief, aliquots of tissue (final concentration of 50–120 μg protein/ml) in 50 mM Tris-HCl buffer (pH 7.5, ca. 25 °C, with 100 mM NaCl) were incubated with increasing concentrations of radioligand for 60 min at 37 °C. Parallel incubations in the presence of 10 μM flupenthixol defined specific binding. Final assay volume was 200 μl .

For the D_2 assays, increasing concentrations of [¹²⁵I]spiperone (6 concentrations ranging from 0.05–4.0 nM) were incubated with aliquots of tissue (final concentration of 50–120 μg protein/ml) in 50 mM Tris-HCl buffer (pH 7.5, ca. 25 °C, with 100 mM NaCl) were incubated for 45 min at 37 °C. Parallel incubations in the presence of 10 μM (+)-butaclamol or 10 μM haloperidol defined specific binding. Ketanserin (1 μM) was included in all tubes to inhibit radioligand binding to 5-HT₂ serotonin receptor binding sites. Final assay volume was 300 μl . For the [³H]spiperone, assay buffer, incubation conditions, ligand concentrations and definition of specific binding were the same as in the [¹²⁵I]spiperone assay. Assay volume for the tritiated ligand was 500 μl .

For all assays, incubation was terminated by filtration under reduced pressure over Whatman GF/B filters using a Brandel Cell Harvester modified for radioligand binding assays. Filters were rinsed 3 times with 5.0 ml ice-cold 50 mM Tris-HCl buffer (pH 7.7, ca. 25 °C). For [³H]spiperone, filters were dried overnight and placed in disposable glass mini-vials (Research Products International). 3.0 ml of a 95% Econofluor/5% Protosol solution (New

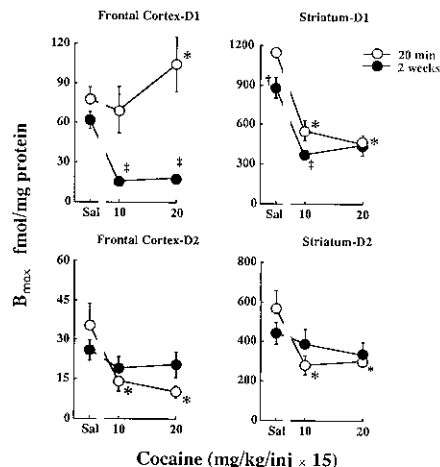


Fig. 1. Striatal and frontal cortex D_1 and D_2 binding sites in rats treated with saline or cocaine (10 or 20 mg/kg/inj) for 15 consecutive days and killed either 20 min or 2 weeks after the least daily injection. Symbols represent the mean \pm S.E.M. ($n = 5\text{--}6/\text{group}$); open symbols, 20 min; closed symbols, 2 weeks after the last daily injection. * Significantly different from saline-treated rats; † significantly different from 20 min group; ‡ significantly different from saline-treated and from 20 min group.

England Nuclear, Waltham, MA) was added and the samples counted by liquid scintillation spectrometry with an efficiency of 30–45%. For [¹²⁵I]SCH 23982, the filters were placed in vials and counted on a Micromedic gamma counter.

Raw counts from all assays were transformed using RADLIG²³ a radioligand binding analysis software package which contains EBDA and a version of LIGAND derived from the original²⁵. In all cases saturation isotherms were best fit by a single site model and final derivation of radioligand affinity (K_d) and the density of binding sites (B_{max}) was made using weighted Eadie-Hofstee plots as described by Zivin and Waud³⁹ and adapted for Lotus 1-2-3. In all cases, computer-assisted analysis of [¹²⁵I]SCH 23982, [¹²⁵I]spiperone, and [³H]spiperone binding isotherms indicated a single, saturable site of interaction. Specific binding ranged from 85–30%, varying as a function of the density of binding sites and concentration of radioligand (i.e. as the sites were saturated, specific binding decreased). As previous pharmacological characterizations of these binding sites in our laboratory and elsewhere^{13,35} have demonstrated that the [¹²⁵I]SCH 23982 binding sites are D_1 -DA receptor binding sites, and that the [¹²⁵I]spiperone and [³H]spiperone sites are D_2 receptor binding sites, they are hereafter referred to as D_1 or D_2 receptor binding sites. Protein content was determined using the commercially available Bio-Rad assay. Data were analyzed for statistical significance using 2-way ANOVA followed by post hoc comparisons using either Duncan's or, where appropriate, Dunnett's test. The criterion for significance was $P < 0.05$.

RESULTS

Experiment 1

 D_1 receptor binding sites

Repeated cocaine administration significantly decreased the density of D_1 binding sites in the frontal cortex and in the corpus striatum (Fig. 1). In the 20 mg/kg group, D_1 binding sites in frontal cortex were significantly

increased from 77.9 ± 9.2 (mean \pm S.E.M.) to 104.6 ± 20.7 fmol/mg protein when examined at 20 min, but were decreased to 18.3 ± 2.6 fmol/mg protein when examined 2 weeks following the last daily injection of cocaine (Fig. 1). In the 10 mg/kg group, no increase in D_1 sites in the frontal cortex was seen when examined at 20 min, but a significant decrease, to 16.1 ± 1.9 fmol/mg protein, was seen at the 2-week time point. In the corpus striatum, repeated daily injection of either dose of cocaine (10 or 20 mg/kg) resulted in a significant decrease in the density of D_1 receptor binding sites when examined either 20 min or 2 weeks after the last dose (Fig. 1). Surprisingly, a small but significant decrease in striatal D_1 binding sites was observed in saline-treated rats 2 weeks after the last daily injection.

In contrast to effects on B_{max} , cocaine treatment resulted in no differences in the affinity (K_d) of [125 I]SCH 23982 for D_1 receptor binding sites in either frontal cortex (0.295 ± 0.013 nM, mean \pm S.E.M. of pooled groups) or corpus striatum (0.367 ± 0.015) whether examined 20 min or 2 weeks after the last dose of cocaine.

D_2 -receptor binding sites

A significant decrease in the density of D_2 binding sites was seen in corpus striatum and frontal cortex when examined 20 min after the last injection of either the 10 or 20 mg/kg dose of cocaine (Fig. 1, bottom panels). While the density of D_2 binding sites in both regions in cocaine-treated rats 2 weeks after the regimen was lower compared to the 20-min saline-treated rats, no differences were found in the density of these sites in comparisons with the 2-week control groups.

No group differences in the affinity of [125 I]spiperone for the D_2 receptor binding sites were seen at 20 min or 2 weeks following repeated cocaine treatment in either frontal cortex (0.789 ± 0.045 nM, mean \pm S.E.M. of pooled groups) or corpus striatum (0.780 ± 0.040).

Experiment 2

D_1 -receptor binding sites

With respect to changes in B_{max} in frontal cortex and striatal DA receptor binding sites following repeated administration of cocaine (10 mg/kg), the results were qualitatively similar to those obtained in the first experiment (Table I). In frontal cortex, as with the similarly treated group in Exp. 1, D_1 receptor binding density was not significantly affected at 20 min but decreased to 38% of control 2 weeks after the last injection of cocaine (Table I). In the corpus striatum, D_1 receptor binding sites were decreased to approximately 50–60% of control at both 20 min and 2 weeks after the 10 mg/kg dose of cocaine (Table I). Furthermore, the density of D_1 binding

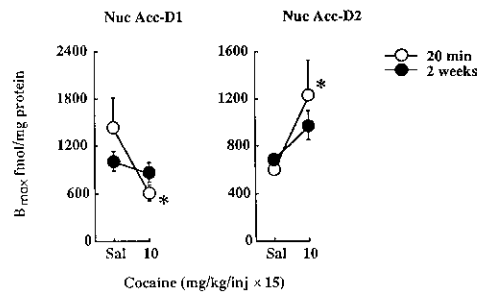


Fig. 2. D_1 and D_2 binding sites in nucleus accumbens of rats treated with saline or cocaine (10 mg/kg/inj) for 15 consecutive days and killed either 20 min or 2 weeks after the last daily injection. Symbols represent the mean \pm S.E.M. ($n = 5-6$ /group); open symbols, 20 min; closed symbols, 2 weeks after the last daily injection. * Significantly different from saline-treated rats; † significantly different from 20 min group; ‡ significantly different from saline-treated and from 20 min group.

sites in the nucleus accumbens was significantly decreased, from 1441 ± 372 to 617 ± 100 fmol/mg protein, 20 min after the last dose of the chronic regimen of cocaine, but was not different 2 weeks later (Fig. 2).

No group differences were observed in the affinity of [125 I]SCH 23982 for these sites in frontal cortex (0.518 ± 0.028 nM, mean \pm S.E.M. of pooled groups), corpus striatum (0.515 ± 0.033), or nucleus accumbens (0.419 ± 0.036).

D_2 -receptor binding sites

As observed in Exp. 1, the density of D_2 binding sites in both corpus striatum and frontal cortex was significantly decreased (to 29–34% of control) only at the 20-min time-point (Table I). No differences in the density of D_2 sites were observed at the 2-week time point. In the nucleus accumbens, the density of D_2 binding sites was

TABLE I

Average B_{max} values in corpus striatum and frontal cortex following repeated administration of saline or cocaine (10 mg/kg)

Values are means \pm S.E.M. B_{max} expressed in fmol/mg protein.

Group	[125 I]SCH 23982		[3 H]Spiperone	
	Frontal cortex	Striatum	Frontal cortex	Striatum
<i>20 min</i>				
Saline	77.3 ± 12.5	1654 ± 160	30.8 ± 6.0	982 ± 96.6
Cocaine	64.8 ± 10.6	$899 \pm 56^*$	$10.5 \pm 1.2^*$	$286 \pm 40.0^*$
% of control	84%	54%	34%	29%
<i>2 weeks</i>				
Saline	89.7 ± 10.8	1509 ± 190	$18.0 \pm 4.0^{**}$	860 ± 158
Cocaine	$34.4 \pm 3.8^{***}$	$891 \pm 105^*$	16.0 ± 2.2	$606 \pm 62.8^{**}$
% of control	38%	59%	89%	71%

* $P < 0.05$ compared with saline

** $P < 0.05$ compared with corresponding 20-min group.

significantly increased 20 min following the last dose of cocaine from 613 ± 48 to 1229 ± 295 fmol/mg protein, but was not different from control 2 weeks later (Fig. 2). Additionally, the density of D_2 binding sites in corpus striatum in cocaine-treated rats 2 weeks after the regimen was lower compared to the 20-min saline-treated rats (30.8 ± 6.0 vs 18.0 ± 4.0 fmol/mg protein, 20-min saline vs. 2-week saline, respectively).

No differences in the affinity (K_d) of [3 H]spiperone were demonstrated in frontal cortex (0.672 ± 0.034 nM, mean \pm S.E.M. of pooled groups), corpus striatum (0.673 ± 0.034), or nucleus accumbens (0.692 ± 0.037) examined 20 min or 2 weeks following repeated cocaine or saline.

DISCUSSION

The major result of the present study is the demonstration of long-term alterations in D_1 receptor binding site density in frontal cortex and corpus striatum following repeated daily administration of cocaine for 15 days. In contrast, the density of D_2 binding sites in these regions was altered 20 min but not 2 weeks after daily exposure to cocaine. In the nucleus accumbens, D_1 receptor binding sites were decreased and D_2 sites increased immediately, but not 2 weeks, after the cocaine regimen. These results suggest that repeated administration of cocaine causes both short- and long-term changes in DA receptors in the CNS. These results must, however, be interpreted with caution since radioligand binding techniques as used in the present study are able to examine only the receptor recognition site. Therefore, extrapolation to *in vivo* changes in receptor functioning must be guarded.

Previous evidence of alterations in DA receptor binding site density following repeated exposure to stimulants including amphetamine, bromocriptine, and cocaine has been equivocal, with increases, decreases or no change reported^{26,29,33}. While these results seem contradictory, true comparison of these results cannot be made as a variety of dosing regimens, survival times and radioligand binding methods were used. In general, however, short-term increases in striatal binding of the relatively D_2 selective ligand, [3 H]spiroperidol after acute or repeated cocaine dosing have been reported^{3,24,37}. On the other hand, Goeders and Kuhar¹² reported that repeated cocaine caused a decrease in the number of striatal D_2 receptor sites and an increase in nucleus accumbens D_2 receptor sites 20 min after the last injection. Reductions in the density of D_2 sites have been reported in several different studies utilizing chronic exposure to various DA agonists^{2,16}. The longevity of such changes has not been determined previously,

whereas the present results show that cocaine-induced changes in D_2 receptor binding site density are not long-lasting.

In the present study, chronic cocaine resulted in increased D_2 receptor density in nucleus accumbens and decreased D_2 receptor density in corpus striatum and frontal cortex. As noted previously, the opposite effects of cocaine on D_2 receptors in corpus striatum and nucleus accumbens may be related to differences in neurochemical effects of acute and chronic cocaine in these regions¹². A variety of other factors may be involved in the observed differential regional changes in DA receptor density: presence or absence of DA autoreceptors, interactions between D_1 and D_2 receptors, intracellular interactions between DA-mediated adenylate cyclase and calmodulin, and the interactive effects of other neurotransmitters on pre- or post-synaptic functioning which alters the intrasynaptic milieu. It has been suggested that one or more of these mechanisms is responsible for changes in sensitivity following chronic exposure to stimulants³³.

The delayed reduction in D_1 receptor binding sites in frontal cortex and the return of D_2 receptors to control numbers indicates that modification of DA receptor subtypes continues to occur in the absence of daily cocaine injections. These changes in D_1 and D_2 receptors may reflect different roles of the DA receptors in response to acute and chronic cocaine. For example, D_1 receptors play a permissive role in modulating stimulation of D_2 receptors³⁸ and may play a similar role in the behavioral effects of cocaine^{6,19}. Although it is possible that a decrease in D_1 receptor density would result in diminished D_2 -mediated effects, the functional consequences of changes in relative numbers of DA receptor subtypes cannot be predicted without more information about homeostatic changes in DA and/or other involved mechanisms. Nonetheless, the present study shows that D_1 and D_2 receptors are differentially modified, both regionally and temporally as a consequence of chronic exposure to cocaine.

The relationship between the observed changes in DA receptor binding site density and altered behavioral effects of chronic cocaine administration is not clear. It is well known that repeated administration of psychomotor stimulants results in enhanced sensitivity upon subsequent exposure^{5,9,11,15,28,30,31} suggesting that neuroadaptation results from repeated exposure to stimulants. This phenomenon is not limited to behavioral effects such as locomotor stimulation and stereotypy, since sensitization to convulsant and neurochemical effects of cocaine has also been described^{7,8,17,18,27,36}. Although the present finding that D_1 receptors remain decreased for at least 2 weeks following chronic cocaine is consistent with long-lasting changes in sensitivity to cocaine, it is difficult to

associate a decrease in presumably post-synaptic receptors with enhanced sensitivity to cocaine's indirect DA agonist effects. It is also unlikely that the increases in D₁ (frontal cortex, 20 min) and D₂ receptors (nucleus accumbens, 20 min) observed in the present study are responsible for changes in sensitivity to cocaine. These changes were transient, whereas behavioral supersensitivity lasts for several months³⁰. It seems likely that altered densities of D₁ and D₂ binding sites following chronic cocaine are homeostatic changes resulting from other neurochemical effects of cocaine administration such as increased rate of DA release^{17,18,26}.

A small decrease in DA receptor binding was observed in rats 2 weeks after the last injection of saline which reached significance in several cases: striatal D₁ and frontal cortex D₂. However, this effect was not robust across the 2 experiments. Nonetheless, in many cases the apparent difference between cocaine and saline-treated rats was reduced at the 2-week time-point. It is possible that the higher density of, in particular, D₂ receptors observed 20 min after the regimen may be due to the handling and injection procedure. Similar effects of stress have been reported previously^{1,22}. Although these find-

ings do not detract from the observed decreases in D₁ receptors 2 weeks after the cocaine regimen, since groups were compared with similarly-treated controls, the effect of interactions induced by handling and/or injections remains to be addressed.

In summary, the present study shows that chronic cocaine has differential effects on D₁ and D₂ receptor binding site density in various brain regions. Cocaine caused a longer-lasting decrease in the density of D₁ binding sites in frontal cortex and corpus striatum. Although these changes may be related to altered sensitivity to cocaine, further studies are required to examine this as well as the molecular mechanisms related to the differential regulation of DA receptor subtypes by cocaine.

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