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Early Withdrawal From Repeated Cocaine Administration Upregulates Muscarinic and Dopaminergic D_2-Like Receptors in Rat Neostriatum

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SOUSA, F. C. F., P. B. GOMES, D. S. MACÊDO, M. M. F. MARINHO AND G. S. B. VIANA. *Early withdrawal from repeated cocaine administration upregulates muscarinic and dopaminergic D_2-like receptors in rat neostriatum.* PHARMACOL BIOCHEM BEHAV **62**(1) 15–20, 1999.—The present results show an increase in locomotor activity 24 h following repeated cocaine administration only with the higher dose (10 mg/kg, IP, daily for 1 week) compared to controls (administered with saline). Binding assays were done and the ligands used were [^{3}H]N-methylscopolamine ([^{3}H]-NMS), [^{3}H]-SCH 23390, and [^{3}H]-spiroperidol to determine muscarinic (M_1- and M_2-like), D_1 and D_2 receptors, respectively. Scatchard analyses revealed alterations in B_{max} not only for muscarinic, but also for D_2-like receptors that were significantly increased. On the other hand, no alterations were detected on D_1-like receptors densities and dissociation constant values. However, the K_d value was significantly increased for D_2 receptors. The changes in muscarinic receptors were observed predominantly on M_2-like, which presented an increase of 84% with the 10 mg/kg, IP, dose only. On D_2-like receptors, increases of 63 and 54% were demonstrated with the doses of 5 and 10 mg/kg, IP. The preferential effects of cocaine on muscarinic and D_2-like receptors were also demonstrated in vitro where decreases in [^{3}H]-NMS and [^{3}H]-spiroperidol binding were observed. The results indicate that the effects of cocaine on muscarinic and dopaminergic postsynaptic receptors are functions of dose, duration of treatment, and time of drug withdrawal. © 1998 Elsevier Science Inc.

Cocaine Muscarinic and dopaminergic receptors Rat neostriatum

ALTHOUGH it is largely accepted that dopamine (DA) plays a major role in the behavioral actions of cocaine (23,39,63), additional work has provided more definitive evidence of DA role in mediating the reinforcing effects of cocaine and other psychomotor stimulant drugs (11). With repeated administration, the behavioral effects of cocaine become progressively greater, a phenomenon known as sensitization or reverse tolerance (18,21,22,30,31,34,51,52,64). Both locomotor activity and stereotyped behaviors become sensitized in response to repeated cocaine administration, and are thought to be mediated primarily via the mesolimbic and nigrostriatal dopaminergic pathways, respectively (8). Possible changes that could contribute to sensitization of behaviors mediated by dopaminergic systems include changes in the synaptic DA transporter, DA release, and/or DA receptors (49).

Besides dopamine, other neurotransmitter systems are implicated in the action of cocaine, and the most recent involves the *N*-methyl-d-aspartate (NMDA) receptor–nitric oxide (NO) system. Activation of NMDA receptors triggers formation of NO by activating NO synthase (46). Recently, it was shown (6) that chronic treatment with cocaine alters NO synthase activity in brain regions and the spinal cord. Pap and Bradberry (47) showed that the ability of cocaine to elevate extracellular dopamine is dependent in part upon an excitatory amino acids (EAA) neurotransmitter system, and EAA was also important to the cocaine-induced behavioral activation.

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There is a significant positive correlation of the potencies of cocaine and some related compounds as DA uptake blockers with their ability to serve as reinforcers in self-administration studies in the rhesus monkey (54). These data strongly suggest that the blockade of DA reuptake is an essential step in the mediation of the reinforcing effects of cocaine.The role of D_1 and D_2 receptors in the mediation of the reinforcing effects of cocaine has also been studied. Much of the earlier self-administration work (23) using DA agonists and antagonists pointed to a prominent role for postsynaptic D_2 receptors, although the specificity of this role has been questioned (66).

However, it has been reported that SCH 23390, a relatively specific D_1 antagonist, also blocks cocaine reinforcing effects in rats (38,42), while other works (65) failed to show that SCH 23390 affected cocaine self-administration in the rhesus monkey. Furthermore, studies in rats have concluded that both D_1 and D_2 antagonists specifically decrease the reinforcing effects of cocaine, suggesting that both receptor subtypes are important to that action (20,55). There is also a recent study (62) demonstrating that the D_1 agonist SKF 81297 has reinforcement effects in rhesus monkeys. Thus, at the present time, interactions among the various dopaminergic receptors that mediate cocaine-reinforcing effects have not been completely delineated.

On the other hand, there is a well-characterized interaction between dopamine and the cholinergic system in the brain (5), and it is largely accepted that the function of cholinergic neurons is controlled by DA, which inhibits ACh function directly through D_2 receptors located on striatal neurons (4,7,10). It has also been shown (61) that the activation of D_2 receptors with the D_2 agonist, quinpirole, or of muscarinic receptors with carbachol induces an inhibition of [^{3}H]-ACh release in the striatum.

Synthesis and release of DA have been shown to be regulated by DA receptors, particularly of D_2 subtype (13,27). There is some evidence demonstrating an increased DA release (29,50) after chronic cocaine administration that has been shown to require DA receptor activation (48). Repeated exposure to cocaine appears to enhance basal release (1,28,29), and to modify the autoregulatory functions responsible for the reduced effects of cocaine challenge. Alterations induced by continuous cocaine administration could be associated with changes in D_2 receptor functioning. Thus, behavioral sensitization could be mediated by presynaptic D_2 autoreceptor subsensitivity, and result in decreased feedback inhibition of DA release and enhanced extracellular DA levels after a cocaine challenge. The result of such subsensitivity would be an augmented behavioral response to cocaine. Conversely, the decrease in extracellular DA levels with a cocaine challenge after the continuous administration of cocaine would occur via D_2 autoreceptor supersensitivity (35). However, evidences for changes in D_2 autoreceptor sensitivity after chronic drug treatment are not conclusive. Some researchers reported autoreceptor subsensitivity (19,67), whereas others have reported no changes (15) or even supersensitivity (14,49). The observed discrepancies may be due to differences in administration schedules or procedures used to measure receptor sensitivity. Undoubtedly, dopaminergic receptors and possibly others as well (9,39,44,53,56), including muscarinic receptors (58), play some role in cocaine reinforcement and discrimination effects.

The objectives of the present work were to study the effects of repeated cocaine administration on rat locomotor activity, and also on brain muscarinic and dopaminergic receptors, to further elucidate the actions of cocaine on these two neurotransmitter systems.

METHOD

Animals and Experimental Protocol

Female Wistar rats (150–200 g weight) were treated daily for 7 days with cocaine (5 and 10 mg/kg, IP). Controls received the same volume of saline. Animals were decapitated 24 h after the last injection and immediately had their brains dissected on ice. The neostriatum was then used for the preparation of total homogenates. The experiments in vitro were performed, adding cocaine at several concentrations to homogenates from nontreated rats.

Muscarinic and Dopaminergic Receptor Binding Assays

Muscarinic receptors were measured using [^{3}H]-*N*-methylscopolamine, ([^{3}H]-NMS, 85 Ci/mmol, New England Nuclear, Boston, MA), according to the method presented by Dombrowski et al. (12). This nonselective ligand binds to all subtypes of muscarinic receptors. The M_1-like receptors assay was performed with [^{3}H]-NMS in the presence of 100 μM carbachol for blocking M_2 sites, while M_2-like receptors were labeled with [^{3}H]-NMS in the presence of 40 μM pirenzepine for blocking M_1 sites (26). Total homogenates corresponding to 140–170 μg protein were prepared in a 150 mM sodium phosphate buffer, pH 7.4, containing 2.35 nM [^{3}H]-NMS for single-point experiments or 0.0031 to 5.95 nM [^{3}H]-NMS for saturation experiments in a final volume of 0.2 ml. After incubation at 37°C for 30 min, time to reach equilibrium, the reaction was terminated by filtering the incubation mixture through Whatman GF/B filters in a cell harvester apparatus from Brandel, USA. The filters were then washed five times with 4 ml of ice-cold saline, dried for at least 2 h in the oven at 60°C, and placed in vials with 3 ml of toluene-based scintillation fluid. The radioactivity was measured with a Beckman scintillation counter, model 6500, USA, at a counting efficiency of 67%. Specific binding was calculated as total minus nonspecific binding performed in the presence of atropine (12.5 μM), and results were reported as femtomoles per milligram of protein. Protein was determined using bovine serum albumin as standard (41).

The densities of D_1 and D_2-like receptors were determined according to methods presented by Kessler et al. (33) and Meltzer et al. (45). In the case of D_1 receptors, the specific ligand [^{3}H]-SCH 23390 (87 Ci/mmol, from New England Nuclear) was used. Total homogenates were incubated in a 50 mM Tris-HCl buffer, pH 7.4, with the following composition (mM): NaCl [120], $CaCl_2$, $MgCl_2$, EDTA , and ascorbic acid. The buffer contained 5.75 nM of [^{3}H]-SCH 23390 for single-point experiments or 0.06 to 8.8 nM for saturation experiments in a final volume of 0.2 ml. Specific binding was defined as total binding minus nonspecific binding carried out in the presence of 5 μM dopamine. After incubation at 37°C, 60 min, the experiment proceeded as described above.

For the determination of D_2 receptors, the specific ligand, [^{3}H]-spiroperidol (114 Ci/mmol, from New England Nuclear) was utilized. Total homogenates were incubated in a 50 mM Tris-HCl buffer, pH 7.4, containing 5 μM mianserin for blocking serotonergic receptors and 17.3 nM [^{3}H]-spiroperidol for single-point experiments or 0.43 to 21.6 nM for saturation experiments in a final volume of 0.2 ml. Specific binding was defined as total minus nonspecific binding carried out in the presence of 5 μM dopamine. After incubation at 37°C, 60 min, the experiments proceeded as described above for the muscarinic binding.

In the case of in vitro assays, ligands ([^{3}H]-NMS, 2.35 nM; [^{3}H]-spiroperidol, 17.3 nM, and [^{3}H]-SCH 23390, 5.75 nM)

were incubated for 30 min (muscarinic receptors) or for 60 min (dopaminergic receptors) in the presence of different concentrations of cocaine, and assays were carried out in duplicates as described above.

Assessment of Locomotor Activity

As described by Jorgensen et al. (25), locomotor activity was observed by placing the rat in a square open-field area (50 × 50 cm), illuminated by red light. The floor was divided into four quadrants. After 1 min habituation, the horizontal activity for the following 3-min period was recorded as the number of crossings from one quadrant to another. The rats received cocaine (5 or 10 mg/kg, IP) or saline (controls), once daily for 7 days. In the last day of treatment, 60 min after drug injection, each animal was placed in the centre of the open-field area, and the number of squares crossed was recorded by means of hand operated counters. The observers were aware of the group treatments. A square was considered crossed only when the animal entered it with the four limbs. Control experiments were carried out simultaneously with the drug experiments. The floor of the open-field area was thoroughly cleaned before the next testing by removing any clues left by previous animals and avoiding possible bias effects.

Drugs

The drugs used were cocaine hydrochloride (obtained from the Federal Police at the State of Ceará, Brazil) and radioactive isotopes ([^{3}H]-NMS, [^{3}H]-SCH 23390, and [^{3}H]-spiroperidol) purchased from Amersham Life Science, USA. All other drugs and reagents were of analytical grade.

Statistical Analysis

For statistical analyses, the *t*-test was used for comparison of two means, ANOVA for multiple comparisons, and the Fisher test as a post hoc one for differences that were considered statistically significant at $p < 0.05$.

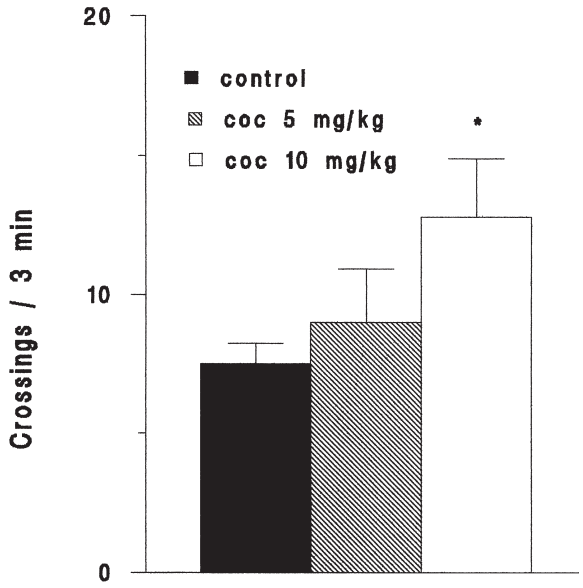

FIG. 1. The locomotor activity in rats was measured as the number of crossings from one quadrant to another. Injections of saline (controls) or cocaine were given intraperitoneally, 1 h before the test. *$p < 0.05$ (ANOVA and the Fisher test as a post hoc one).

TABLE 1

EFFECTS OF COCAINE ON THE [^{3}H]-NMS BINDING IN THE RAT NEOSTRIATUM

Cocaine (mg/kg, IP)	[^{3}H]-NMS Binding (fmol/mg Protein)	
	M_1-Like Receptors	M_2-Like Receptors
0	288.5 ± 17.45 (7)	35.5 ± 1.26 (6)
5	366.9 ± 40.52 (6)*	46.0 ± 2.64 (6)
10	407.9 ± 19.40 (6)*	65.3 ± 5.87 (12)*

Animals were treated daily for 1 week with cocaine 5 and 10 mg/kg, IP, and measurements were done 24 h after the last injection. Data are reported as means ± SEM for the number of experiments shown in parentheses. For statistical analyses, ANOVA and the Fisher test as a post hoc one were used.

*$p < 0.05$ compared to controls.

RESULTS

There was an increase (71%) in rat locomotor activity after cocaine treatment with the higher dose (10 mg/kg, IP) compared to controls. No significant change was observed with the lower dose of 5 mg/kg, IP (Fig. 1).

Smaller, but significant increases of 27 and 41% were observed on M_1-like receptors after 5 and 10 mg/kg, IP, cocaine treatment, respectively. The repeated administration of cocaine (5 and 10 mg/kg, IP) produced dose-dependent increases of 30 and 84% on M_2-like receptors, respectively, compared to controls (Table 1).

The effects of repeated cocaine administration on D_1- and D_2-like receptors are presented in Table 2. While no significant changes were seen on D_1-like receptors, increases of 63 and 54% were demonstrated with the doses of 5 and 10 mg/kg, IP, respectively, on D_2-like receptors.

Table 3 shows the effects of the incubation of untreated rat homogenates with cocaine in vitro on [^{3}H]-NMS, [^{3}H]-SCH 23390, and [^{3}H]-spiroperidol bindings. The decreases in [^{3}H]-NMS binding reached values around 70% with concentrations of 12.5, 25, and 100 μM. Although no significant effects were

TABLE 2

EFFECTS OF COCAINE ON THE [^{3}H]-SCH23390 (D_1-LIKE RECEPTORS) AND [^{3}H]-SPIROPERIDOL (D_2-LIKE RECEPTORS) BINDINGS IN THE RAT NEOSTRIATUM

Cocaine (mg/kg, IP)	Specific Binding (fmol/mg Protein)	
	D_1-Like Receptors	D_2-Like Receptors
0	168.7 ± 16.69 (13)	241.9 ± 10.03 (6)
5	145.1 ± 15.90 (9)	393.5 ± 23.96 (10)*
10	221.3 ± 24.36 (14)	372.5 ± 8.15 (6)*

Assays were carried out as described in Table 1. Data are reported as means ± SEM for the number of experiments shown in parentheses. For statistical analyses, ANOVA, and the Fisher test as a post hoc one were used.

*$p < 0.05$ compared to control.

TABLE 3

BINDINGS OF [3H]-NMS, [3H]-SCH23390 AND [3H]-SPIROPERIDOL IN RAT NEOSTRIATUM IN THE PRESENCE OF COCAINE IN VITRO

Cocaine (μM)	Specific binding (fmol/mg protein)		
	[3H]-NMS	[3H]-SCH 23390	[3H]-Spiroperidol
0	456.5 ± 47.10 (10)	147.6 ± 14.60 (4)	253.3 ± 26.63 (4)
12.5	153.7 ± 14.45 (5)*	—	—
25	183.2 ± 23.40 (7)*	—	—
100	130.2 ± 16.60 (6)*	126.8 ± 10.34 (4)	118.2 ± 18.70 (6)*

Homogenates from untreated rats were incubated for 30 min with cocaine (12.5, 25, and 100 μM), in the presence of either [3H]-NMS, [3H]-SCH 23390, or [3H]-spiroperidol, specific liands for muscarinic, D1- and D2-like dopaminergic receptors, respectively. Assays proceeded as described in Table 1. For statistical anlyses, ANOVA and the Fisher test as a post hoc one were used.

*$p < 0.05$ compared to controls.

noticed on [3H]-SCH 23390 binding after incubation of the homogenates with 100 μM cocaine, a 70% inhibition of [3H]-spiroperidol binding was demonstrated with the same dose.

Table 4 presents the B_{max} and K_d values for M_1- plus M_2-like receptors and for D_1- and D_2-like receptors from cocaine-treated rats and controls. In the case of muscarinic receptors, there was an increase of 52% in the B_{max} compared to controls, with 5 mg/kg, IP, cocaine, and no significant differences in K_d values. On the other hand, while no alterations were detected on D_1-like receptor density or on the dissociation constant, significant increases were observed in these parameters on D_2-like receptors.

DISCUSSION

The present work showed significant and dose-dependent increases of muscarinic M_1- and M_2-like receptors measured 24 h after repeated cocaine administration, in the rat neostriatum. Although many of the effects of cocaine have been associated primarily with dopamine uptake inhibition in the brain (54), it has been shown that cocaine also exhibits affinity for muscarinic receptors (57).

Thus, Sharkey et al. (57) showed that cocaine inhibits muscarinic receptors in rat heart and brain with a higher affinity at M_2 than at M_1 receptors. More recent work (40) demonstrated decreases in muscarinic receptor binding in striatum and hipocampus, 21 days after a 5-day cocaine exposure. However, 12 h after the 5-day cocaine administration, there was a significant increase in [3H]-QNB binding in the striatum. Similar results were also seen in the hippocampus. Previous works (32,57) showed that cocaine can directly antagonize cholinergic receptors in a dose-dependent manner in rat cerebral cortex in vitro. Our results also showed that cocaine exhibited affinity not only for muscarinic, but also for D_2 receptors. No effects were seen on D_1 receptors.

Effects of cocaine administration on muscarinic and dopaminergic receptor densities are still a matter of controversy. While several in vivo studies can be found in the literature (37,49,69), only a few in vitro studies are available (16). Ziegler et al. (69) showed in cocaine-treated animals (daily, 5 days, followed by a 30-day drug-free recovery period) an elevated [3H]-spiperone binding in caudate and substantia nigra, and a decreased [3H]-QNB binding in the hippocampus and amygdala, while no significant changes were observed with [3H]-SCH 23390 binding. Mayfield et al. (43) demonstrated that 6 days of cocaine administration followed by 7 days of discontinuation yielded no differences in striatal or nucleus accumbens D_1 receptors as assayed by [3H]-SCH 23390 autoradiography.

In the present work, all measurements were performed 24 h after the last cocaine injection. Only negligible effects were shown on D_1 receptors after repeated cocaine administration, and the predominant alteration on the dopaminergic system was observed on D_2 receptors, which presented an upregulation similar to that seen with muscarinic receptors. However, although no significant change was observed in the dissociation constant for muscarinic receptors, a drastic increase in this parameter was detected in D_2 receptors.

Our results did not agree with those presented by Alburges et al. (2) and Alburges and Wamsley (3), who showed significant increases in rat cortical and striatal D_1 receptor bindings and no changes in the D_2 receptor population after cocaine (15 mg/kg, IP) administration up to 21 days. In this case, measurements were performed 21 days after the last injection and, according to the authors, the increase in brain D_1 receptors indicates that the system was possibly desensitized in the presence of cocaine.

Like our data, other works (69) also showed an increase in [3H]-spiperone binding and no significant changes in [3H]-SCH 23390 bindings in the caudate and substantia nigra from rats treated with daily injections of cocaine (20 mg/kg, SC) and then submitted to a 30-day drug-free period. Similarly, Peris et al. (49) measured D_2 and D_1 receptors 1 day after an 8-day cocaine treatment, and verified that the nucleus accumbens was the only area presenting a significant increase in D_2 receptors. D_1 receptors were unaltered.

TABLE 4

EFFECTS OF REPEATED COCAINE ADMINISTRATION ON MUSCARINIC, D_1 AND D_2 DOPAMINERGIC RECEPTORS ON B_{max} AND K_d FROM RAT NEOSTRIATUM

Cocaine (mg/kg, IP)	M_1+M_2		D_1		D_2	
	B_{max}	K_d	B_{max}	K_d	B_{max}	K_d
0	511.4 ± 32.67 (4)	0.76 ± 0.24 (4)	216.0 ± 21.68 (5)	2.40 ± 0.39 (5)	246.2 ± 43.72 (4)	1.8 ± 0.51 (4)
5	776.9 ± 76.30 (4)*	1.67 ± 0.62 (4)	146.6 ± 25.81 (3)	1.78 ± 0.57 (4)	363.3 ± 43.94 (4)*	8.5 ± 1.46 (4)*

The assays were carried out as described in Table 1. Data are reported as means ± SEM for the number of experiments shown in parentheses. B_{max} and K_d values are expressed in fmol/mg protein and nM, respectively. For statistical analyses, the paired *t*-test was used.

*$p < 0.05$.

According to Zahniser et al. (68), at least two mechanisms could contribute to a transient increase in the responsiveness of the nucleus accumbens after repeated cocaine exposure, for example, the increased density of D_2 receptors and the enhanced sensitivity to agonists of D_2 postsynaptic receptors in this area. Goeders and Kuhar (17) reported that single injections of cocaine for 15 days caused a decrease in the density of D_2 receptor sites in the striatum and an increase in D_2 sites in the nucleus accumbens. Other works (36) showed that, in this area, D_1 binding sites were decreased and D_2 sites increased immediately, but not 2 weeks after repeated cocaine injection.

Unterwald et al. (59) showed that the daily administration of cocaine-produced locomotor activation. This effect was accompanied by an increase in the density of D_1 receptors in the nucleus accumbens, olfactory tubercle, ventral pallidum, and substantia nigra after 14 days. In contrast, D_2 receptors were elevated in the olfactory tubercle, nucleus accumbens, and caudate-putamen after 7 days, but returned to control levels by 14 days of treatment.

Our data also showed a significant increase in locomotor activity only after a high dose of cocaine. With repeated exposure, the locomotor and stereotypic behavior of cocaine have been shown to increase in magnitude, a phenomenon known as sensitization, which seems to be mediated by the dopaminergic system (24).

It has been demonstrated that rats exposed to repeated cocaine injections, and after a 30-day drug-free recovery period, presented hyperactivity (69). Recent work (60) showed that cocaine increased locomotor activity and stereotypic behavior in rats in a dose-dependent manner. The treatments with D_1 and D_2 receptor antagonists significantly reduced the effects of cocaine in locomotor activity, indicating that its stimulatory effect involves the activation of both receptors.

In summary, evidences so far indicated that, besides the increase in locomotor activity, cocaine causes both short- and long-term changes in DA receptors in the striatum. In addition, it presents effects on muscarinic and dopaminergic postsynaptic receptors, which are functions of dose, duration of treatment, and time of drug withdrawal. Further studies are needed to clarify the differential regulation of receptor subtypes by cocaine.

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