Alterations in Locomotor Activity during Chronic Cocaine Administration: Effect on Dopamine Receptors and Interaction with Opioids

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ABSTRACT

Chronic cocaine administration can produce tolerance or sensitization to locomotor activating effects, depending on the treatment paradigm. The effects of chronic, continuous cocaine were measured on locomotor activity for 1 hr daily for 7 days. Cocaine produced significant increases in locomotor activity 4 hr after osmotic minipumps were implanted, and an even higher level of activity after 24 hr. This was likely a rapid sensitization to the locomotor activating effects of cocaine, because neither brain nor plasma levels of cocaine were significantly altered over the treatment period. By day 4, activity levels diminished, but remained significantly higher than in saline-treated animals. Twenty-four hr after pump removal, there were no changes in dopamine D1 or D2 receptor binding, or in dopamine-stimulation of adenylyl cyclase activity in either caudate putamen or nucleus accumbens of cocaine-treated animals. Chronic naltrrexone produced a slight, nonsignificant decrease in locomotor activity and when combined with cocaine, produced the same pattern of activity as cocaine alone, but with slightly less stimulation on all days. Morphine produced a smaller increase in activity than cocaine that remained constant throughout the treatment week. Cocaine with morphine was additive, producing greater activity and less tolerance than cocaine alone. Thus, continuous cocaine administration produces a rapid sensitization that is lost over the course of the treatment period, yet does not produce any immediate alterations in dopamine receptors or regulation of adenylyl cyclase. The pattern of behavior is not altered by an opioid antagonist, while the sensitization period appears to be prolonged in the presence of an opioid agonist.

Chronic administration of cocaine has been shown to produce either tolerance or sensitization (also called reverse tolerance) to locomotor activating effects, depending on the paradigm by which cocaine is administered. In general, intermittent injections of cocaine produce sensitization while continuous infusion leads to tolerance (Post and Rose, 1976; Reith et al., 1987; Inada et al., 1992; King et al., 1994). Similarly, chronic cocaine treatment has produced variable results on neurochemical measures (e.g., Reith et al., 1987; Pettit et al., 1990; Pilotte et al., 1991; Zeigler et al., 1991; Baumann et al., 1993). We have previously shown that there are differential changes in dopamine transporter function in the nucleus accumbens and caudate putamen after 7 days of chronic continuous cocaine administration (Izenwasser and Cox, 1992), and that these changes are different from those produced by daily cocaine injections (Izenwasser and Cox, 1990). In those studies, we found that the curve for the inhibition of dopamine uptake by cocaine was shifted to the left after continuous administration, and to the right after intermittent injections. In neither of these conditions, however, was the density of dopamine transporters altered (Izenwasser and Cox, 1990; Izenwasser and Cox, 1992). Similarly, there are conflicting reports of changes in dopamine D1 receptors, with increases in receptor number observed immediately after 15 days of intermittent treatment, followed by decreases 14 days later (Kleven et al., 1990) and no changes seen 7 days after a 6-day treatment period (Mayfield et al., 1992) or 1 day after 8 days of cocaine injections (Peris et al., 1990). Functional studies also produced variable results, with no change in dopamine D1 receptor regulation of adenylyl cyclase activity reported in caudate putamen or nucleus accumbens after withdrawal from 6 days of injections (Mayfield et al., 1992); but an increased inhibition of cell firing by D1 agonists after 2 wk of cocaine treatment, a sensitization that persisted for at least 1 mo after cessation of treatment (Henry and White, 1991). These studies have differed from one another in the length of treatment, doses of cocaine administered, and time since the last drug administration when the neurochemical assays have been done, suggesting that these factors might play an important role in determining the behavioral and neurochemical consequences.

ABBREVIATION: [3H] SCH 23390 (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine). cAMP (Adenosine 3’,5’-cyclic phosphate)
of chronic cocaine administration. In none of these studies has the effect of continuous cocaine administration been examined on dopamine receptors.

There is evidence that opioid receptors may play a role in mediating the behavioral effects of cocaine. The reinforcing effects of cocaine, as evidenced by a lowering of threshold for brain-stimulation reward, are blocked by the opioid antagonist naloxone (Bain and Kornetsky, 1987). In addition, self-administration of cocaine is decreased following administration of opioid antagonists (Mello et al., 1990; Corrigall and Coen, 1991). Conversely, chronic cocaine treatment has been shown to produce changes in opioid receptor number (Hammer, 1989; Unterwald et al., 1992, 1994) and function (Unterwald et al., 1993; Izenwasser, 1994; Izenwasser et al., 1996). Taken together, these findings suggest that the dopaminergic and opioidergic systems may interact in the production of the behavioral effects of cocaine.

Although there have been many studies aimed at understanding the sensitizing effects of cocaine, there is not much information on the tolerance produced by chronic, continuous infusion of cocaine. Studies such as these may help us to understand which neurochemical systems are involved in the production of cocaine's behavioral effects. Additionally, an understanding of the mechanisms involved in producing tolerance to cocaine may help in the development of a treatment for cocaine addiction. If tolerance is produced to a sufficient level, it is possible that the drug would no longer be reinforcing, thus eliminating the self-administration of it. Our studies were aimed at investigating the effects of continuous cocaine on locomotor activity and dopamine D1 and D2 receptors, as well as the interactions of cocaine with the opioid agonist morphine, and the opioid antagonist naltrexone on locomotor activity. These results will provide information on 1) the behavioral effects of chronic, continuous cocaine administration, 2) the roles that opioid actions play in mediating the locomotor activating effects of chronic cocaine, 3) the behavioral effects of a combination of cocaine and an opioid agonist, a commonly used drug combination (often referred to as a speedball), as well as a potential modulation of cocaine-induced tolerance by opioid receptors and 4) the effects of chronic, continuous cocaine administration on dopamine D1 and D2 receptors.

Methods

Chemicals

Chemicals and reagents were obtained from the following sources: [3H]SCH 23390 (specific activity 81.4 Ci/mmol), [3H]sulpiride (specific activity 85.6 Ci/mmol) and [3H]AMP (ammonium salt; specific activity 31.4 Ci/mmol) from New England Nuclear (Boston, MA); adenosine triphosphate, guanosine triphosphate, cAMP, theophylline, EGTA, dopamine, and cocaine hydrochloride from Sigma Chemical Co. (St. Louis, MO); naltrexone and (+)SCH 23390 from Research Biochemicals Int. (Natick, MA) and morphine from the National Institute on Drug Abuse (Rockville, MD).

Treatments

Male Sprague-Dawley rats (200-250 g, Taconic, Germantown, NY) were kept on a 12-hr light/dark cycle (lights on at 7:00 A.M.) with food and water available ad libitum. Animals were anesthetized with halothane and Alzet osmotic minipumps model 2001 (Alza Corp., Palo Alto, CA) delivering approximately 1 µl/hr for 7 days were implanted s.c. between the scapulae. The pumps contained either saline (0.9% sodium chloride; 24 µl/day), or a concentration of drug resulting in delivery of approximately 15, 30 or 50 mg/kg/day of cocaine, expressed as free base, or 5.6 mg/kg/day naltrexone hydrochloride, a dose known to up-regulate mu-opioid receptors (Tempel et al., 1985). The highest dose of cocaine tested under these conditions has been shown to produce no observable detrimental effects to the animals (i.e., there is no evidence of necrotic skin or subcutaneous lesions) (Izenwasser and Cox, 1992; Izenwasser, 1994). To get morphine into solution for delivery of approximately 15 mg/kg/day morphine sulfate, minipumps that delivered 10 µl/hr (model 2010) were used. Comparable results were observed when saline was administered using either the small or the large pumps, and small pumps were predominantly used throughout these studies. The minipumps were filled and soaked overnight in saline at 37°C before surgery to reach a constant pumping volume before being implanted into the animals. The dose of drug delivered was determined by the pumping rate and average body weight of the animals in each individual experiment, which was comprised of at least four rats in each treatment group.

Locomotor Activity

Locomotor activity was measured for 1 hr each day starting 4 hr after pump implantation and again every 24 hr for 7 days. Acrylic chambers (16 inches by 16 inches) were placed inside Digiscan activity monitors (Omnitech Electronics, Columbus, OH) that were equipped with infrared light sensitive detectors mounted 2.5 cm apart along two perpendicular walls. Mounted along the opposing walls were infrared light beams that were directed at the detectors. One count of horizontal activity was registered each time the subject interrupted two successive beams. Repetitive interruptions of the same beam due to behaviors such as grooming or head bobbing were not counted as part of horizontal activity, but were counted as stereotypy. A two-way analysis of variance (Condition x Day) was used to determine the effects of chronic drug administration on locomotor activity. Fisher’s Protected LSD was used for post-hoc testing.

Brain and Plasma Levels of Cocaine

Pumps were implanted as above (the dose of cocaine in this study was 50 mg/kg/day) into a separate group of animals and on each of the 7 days, five rats were killed by decapitation at the same time each day as the locomotor activity testing would have taken place. Brain tissue (whole brain minus cerebellum) was homogenized in saline, and trunk blood was collected and spun down and the brain and plasma samples were assayed for cocaine and metabolites. These data were provided by American Medical Laboratories (Chantilly, VA). Data were analyzed using analysis of variance followed by Fisher’s protected LSD for post hoc testing.

Receptor Binding

Dopamine D1 receptor binding. Seven days after minipump implantation, the rats were anesthetized and the pumps were removed. Twenty-four hr later the rats were killed by decapitation, the brains were rapidly removed and the caudate putamen and/or nucleus accumbens were dissected on ice. The tissues were suspended in ice-cold buffer (50 mM Tris HCl, pH 7.4) and centrifuged at 35,000 x g for 10 min at 4°C. The pellets were resuspended in Tris buffer and recentrifuged. This was repeated, and the final pellet was resuspended in 3.75 mg/ml of binding buffer (50 mM Tris, 120 mM NaCl, 5 mM CaCl2, 1 mM MgCl2, pH 7.4 and 1 µM mianserin to block binding to serotonin receptors). This equals approximately 3 mg of tissue per assay tube.

Fresh tissue homogenate was used in all experiments. [3H]SCH 23390 (final concentration 3 nM) was used to determine binding to dopamine D1 receptors, as previously described (Shah et al., 1985). Twelve point [3H]SCH 23390 saturation curves were performed over a concentration range of 0.001 to 15 nM [3H]SCH 23390 for caudate
putamen tissue. Because of the small size of the nucleus accumbens, each curve contained only six points. Nonspecific binding was determined as binding in the presence of 1 μM SCH 23390. Duplicate samples of membranes were incubated in binding buffer for 30 min at 37°C in a final volume of 1 ml. The incubation was terminated by rapid filtration through Whatman GF/B glass fiber filter paper using a Brandel Cell Harvester (Gaithersburg, MD). The filters were washed with three additional 4-ml washes and transferred to scintillation vials. Absolute ethanol (0.5 ml) and Beckman Ready Value Scintillation Cocktail (2.75 ml) were added to the vials that were counted the next day at an efficiency of approximately 40%.

Dopamine D₂ receptor binding. Binding of [³H]sulpiride to dopamine D₂-like receptors was essentially as described above for D₁ receptors with the following differences. Binding was done in a buffer containing 50 mM Tris HCl, 10 mM NaCl and 0.01% ascorbic acid, at pH 7.5. Nonspecific binding was determined as binding in the presence of 10 μM sulpiride. Nonspecific binding to filters was reduced by pre-soaking them in 0.3% polyethyleneimine/water. The incubation was for 60 min at room temperature.

Data analysis. Saturation data were analyzed by the use of the nonlinear least squares curve-fitting computer program LIGAND (Munson and Rodbard, 1980). Data from replicate experiments were modeled together to produce a set of parameter estimates (Kᵣₑ, Bₘₐₓ values) and the associated S.E. of these estimates. Fits were compared using analysis of variance and a difference was considered significant only if the P values were less than or equal to .05. Protein values were determined using a modification of the Lowry procedure (Peterson, 1977).

Adenylyl Cyclase Assays

Seven days after minipump implantation, the pumps were removed. Twenty-four hr later the rats were killed by decapitation, the brains were rapidly removed and the caudate putamen and nucleus accumbens were dissected on ice and membranes were prepared and adenylyl cyclase activity assayed as described previously (Izenwasser and Katz, 1993). Crude membrane preparations were made by homogenizing the tissues in a teflon/glass homogenizer. The tissues were suspended in 25 ml of buffer (10 mM imidazole, 2 mM EGTA; pH 7.4) and centrifuged at 15,000 × g for 15 min at 4°C. The pellets were resuspended in 25 ml of fresh buffer and centrifuged again for 15 min. The supernatants were discarded and the pellets were homogenized in 40 volumes of ice-cold buffer containing 10% glycerol and frozen at -70°C until assay.

When assayed, tissue homogenate (10 μl) was added on ice to assay tubes (final volume 0.06 ml) containing 10 mM imidazole (pH 7.4), 10 mM theophylline, 6 mM MgSO₄, 0.6 mM EGTA, 1.5 mM ATP, 0.01 mM GTP and either the drug being tested or water. Triplicate samples of membrane suspension were incubated at 30°C for 5 min in the presence or absence of dopamine or water, as appropriate. Adenylyl cyclase activity was terminated by placing the tubes into boiling water for 2 min. The amount of cAMP formed was determined by a [³H]cAMP protein binding assay (Brown et al., 1971). Briefly, [³H]cAMP was added to each test tube followed by a binding protein prepared from bovine adrenal glands. The samples were incubated on ice for 90 min and the assay was terminated by the addition of charcoal and centrifugation to separate the free cAMP from that which was bound to the binding protein. Aliquots from the supernatant were placed into scintillation vials to which three ml of Beckman Ready Value Scintillation Cocktail were added and radioactivity was determined by liquid scintillation spectrometry. Protein values were determined using a modification of the Lowry procedure (Peterson, 1977). The amount of cAMP formed as a function of concentration of agonist was analyzed using analysis of variance and linear regression techniques (Snedecor and Cochran, 1967).

Results

Locomotor activity. Cocaine (15, 30 and 50 mg/kg/day) produced a significant increase in locomotor activity as compared to saline (F₍₁,₂₃₄₎ = 25, P ≤ .0001, fig. 1A). Post hoc analyses showed that the effect of each dose of cocaine on locomotor activity was significantly different from that of saline (P ≤ .0001). In addition, the effect of the doses across days were significantly different (F₍₁,₂₃₄₎ ≤ .0001), such that the magnitudes of the increases in behavior were dose-related, with higher doses of cocaine producing greater increases in activity than lower doses. Four hr after pumps were implanted, the animals receiving the two higher doses of co-
caine exhibited significantly greater amounts of horizontal activity than the animals receiving saline infusions (P ≤ .0001). Maximal activity with cocaine was observed 24 hr later, with decreases in this activation observed over the next several days, after which the level of activity reached a plateau that was still significantly higher than control activity (fig. 1A). During this period, post hoc analyses showed that for each dose of cocaine the activity level on each successive day was not significantly different than on the day immediately preceding it. In addition, activity on the first day of the plateau was not significantly different from that observed on day seven. The locomotor activity reached a plateau by day three in the animals treated with the two lower doses of cocaine, and by day 4 in the group receiving the highest dose (50 mg/kg/day). During this plateau phase, activity levels never decreased below the level observed on the first test day. When stereotypy was measured, a similar pattern of activity was seen, with an increase in activity on day 1, followed by an even greater effect on day 2 and a decrease to a plateau still significantly higher than control activity (fig. 1B).

Morphine (15 mg/kg/day) produced a significant increase in activity as compared to saline (F(1,303) = 96.0, P ≤ .0001) that remained constant over the entire week (fig. 2A). The combination of cocaine (50 mg/kg/day) and morphine (15 mg/kg/day) produced levels of activity significantly greater than either morphine (F(1,272) = 208, P ≤ .0001) or cocaine (F(1,308) = 43.0, P ≤ .0001) alone. Further, the amount of activity in the group receiving both cocaine and morphine remained fairly constant by the second day of treatment (i.e., the level of activity on each day was not significantly different from the day immediately preceding it), and there was no significant difference between activity levels on day 1 or 2 as compared to day seven, unlike the pattern that was seen in the animals treated with cocaine alone (fig. 2A). During this time period, the combination of the two drugs appears to produce a potentiated effect, (i.e., an effect that is greater than would be expected if there was a merely additive effect of the two drugs alone).

Naltrexone (5.6 mg/kg/day) alone did not significantly change locomotor activity as compared to saline over the seven day treatment period (P ≤ .14), although there was a slight decrease in activity on most of the days, especially during the early part of the week. In combination with cocaine (50 mg/kg/day), there was a slight but significant decrease in activity, as compared to cocaine alone (fig. 2B; F(1,303) = 5.71, P ≤ .02). It is not known whether this effect is additive (because naltrexone alone produced small decreases in activity) or whether naltrexone is in fact attenuating the locomotor activating effects of cocaine. The pattern of activity of cocaine was clearly not altered by naltrexone, and was the same as that observed with cocaine alone (i.e., a large increase between days 1 and 2, followed by a slow decrease by day 4 to a level that remained constant for the rest of the treatment period).

Brain and plasma levels of cocaine. Daily levels of cocaine and its metabolites ecgonine methylester (EME) and benzoylecgonine (BE) were determined in brain and plasma (table 1). Neither the levels of cocaine nor benzoylecgonine varied significantly over the course of the study in either the brain or the plasma during treatment with 50 mg/kg/day of cocaine. There was a significant effect of day on ecgonine methylester in the brain F(6,28) = 3.22, P ≤ .016. Post hoc analysis showed that the level of ecgonine methylester on day 1 was significantly lower than that measured on day 2 and on days 4 to 7.

Dopamine receptor binding. Binding of [3H]SCH 23390 was not significantly changed in either the nucleus accumbens or caudate putamen of cocaine-treated (50 mg/kg/day), as compared to saline-treated animals, 24 hr after the pumps were removed (table 2). Neither the Kd nor the Bmax values for binding of this ligand changed after this treatment. Similarly, no significant changes were observed in [3H]sulpiride binding to D2-like dopamine receptors in either of these brain regions (table 3).

Dopamine stimulation of adenylyl cyclase activity. There were no changes in basal adenylyl cyclase activity in either nucleus accumbens or caudate putamen after any of the treatments. Dopamine (100 μM) stimulated adenylyl cyclase activity in both the caudate putamen (fig. 3A) and
nucleus accumbens (fig. 3B) to approximately 200% of basal activity. The amount of stimulation at 100 μM dopamine was not significantly changed in either brain region after 7 days of chronic infusion of 50 mg/kg/day cocaine (fig. 3, A and B). Because there is not a true plateau at 100 μM it is not possible to definitively say that the maximal stimulation of cyclase activity by dopamine is unchanged, however, over the range of concentrations tested, there were no significant alterations as compared to tissue from saline-treated animals. Similarly, treatment with either morphine (15 mg/kg/day) or naltrexone (5.6 mg/kg/day), alone or in combination with cocaine (50 mg/kg/day), had no effect on dopamine-stimulated cyclase activity, as compared to saline treatment (data not shown).

**Discussion**

When cocaine is administered continuously via s.c. implanted osmotic minipumps, there is a significant increase in locomotor activity within 4 hr after treatment begins. Twenty-four hr later, activity levels are even higher, followed by an apparent tolerance to this elevated effect that develops over the course of several days, as animals return to their original level of activity as seen on day 1. These changes in activity levels are not due to differences in the amount of cocaine in either the plasma or the brain, because these do not change significantly over the course of the treatment period. In addition, this pattern of behavior does not appear to be dose-related, as the same pattern was observed with several different doses of the drug.

The increase in activity from day 1 to day 2 is interesting in that is not due to an increase in the brain cocaine level. By definition, an increased response to the same dose of a drug is sensitization. This suggests that there might be a fairly rapid development of sensitization to the behavioral effects of cocaine when it is chronically administered, and that this sensitization is lost over the next several days. This idea is further supported by the finding that the amount of activity in the cocaine-treated animals reaches a plateau at approximately the same level of activity that was observed on day 1 of the treatment period. It is difficult to determine whether the animals are truly tolerant to the initial effects of cocaine, or to the sensitization produced by the chronic infusion of cocaine, however, there is some evidence suggesting that the latter might be true. Previously, it has been shown that mice treated with a continuous infusion of cocaine exhibit increased locomotor activity as compared to saline-treated animals, but that tolerance to this effect develops after approximately 1 wk of drug administration (Reith et al., 1987). In addition, the same mice treated for 18 days with chronic cocaine infusions are tolerant to a challenge injection of cocaine when tested 1 wk later as compared to mice pretreated with saline. Under the current conditions, rats that received injections with cocaine 2 days after the pumps are removed do not appear to be tolerant to an injection of cocaine, as compared to animals that had been treated with continuous saline via osmotic minipump (French and Izenwasser, 1996). However, if a challenge injection of cocaine is given to rats 10 days after pump removal, tolerance to the locomotor-activating effects has developed, as compared to animals that had been previously treated with continuous saline infusions (French and Izenwasser, 1996). Thus, it does appear that the development of tolerance might be related to
an extended withdrawal from the drug, as opposed to the drug itself. This information, coupled with the finding that the brain level of cocaine did not significantly change over the course of the treatment, suggests that the large increase in behavior between day 1 and 2 of treatment is likely a sensitized response (i.e., within the first 24 hr). Further, this sensitization is what is lost over the course of the next several days as the level of activity returns to its original magnitude.

It is important to note that this does not suggest that this treatment (i.e., continuous infusion of cocaine) is fundamentally equal to an intermittent injection regimen. Sensitization occurs during the course of repeated injections and lasts for a long period of time, whether or not the injections continue to be administered, with no evidence of tolerance even after an extended withdrawal period (Post and Rose, 1976; Kalivas et al., 1988; Peris and Zahniser, 1989; Keller et al., 1992).

Neither the brain nor plasma levels of cocaine changed significantly over the 7-day treatment period, suggesting that any differences in behavior were not due to an increased or decreased bioavailability of the drug. Similarly, it has been shown that the brain level of cocaine is not altered after a challenge injection 1 wk after continuous cocaine infusion ends (Reith et al., 1987), or after repeated intraventricular administration of cocaine, a paradigm that produces behavioral sensitization (Orona et al., 1994). These findings are in contrast to other studies showing that increased cocaine levels are present in brain in response to an intraperitoneal (Pettit et al., 1990; Cass and Zahniser, 1993) but not an intraventricular (Pan et al., 1991; Pettit and Pettit, 1994) injection of cocaine that follows a regimen of repeated i.p. cocaine administration. Together these results suggest that increased cocaine is seen in brain only after repeated i.p. injections, suggesting that this may be due to alterations in the distribution of the drug from the injection site, as opposed to a greater entry into the brain. These differences do not appear to be related to the dosing paradigm (i.e., intermittent vs. continuous administration), because intermittent i.p. injections produce a different effect than i.v. injections (Pan and Mojaverian, 1991), but is more likely due to the fact that during the continuous infusion, the drug is administered s.c.

If the marked changes in locomotor activity are not due to alterations in cocaine bioavailability, this suggests that the level of sensitization is related to differences in the effect of cocaine after it enters the brain. This is not likely due to an increase or decrease in drug metabolism, because levels of two of the major metabolites of cocaine, benzoylecgonine and ecgonine methyl ester, did not vary considerably over the course of the treatment. Only the level of ecgonine methyl ester in the brain varied significantly over the course of the treatment period, such that the level on day 1 was significantly lower than that observed over most of the rest of the treatment period. However, ecgonine methyl ester has been shown to have no observable stimulatory effects (Misra et al., 1975). In fact, at extremely high levels (300-800 µg), it produced sedation (Schuelke et al., 1995). The level of this metabolite does not correlate with the behavioral effects that were observed, in that the lowest level of EME was observed on day 1, with a significant increase by day 2. Because, the level of activity observed on day 2 was significantly higher than that seen on day 1, it is unlikely that a sedative effect of ecgonine methyl ester is regulating this behavior. The level of benzoylecgonine did not vary significantly over the course of the week in either the brain or in plasma. Both cocaine and this metabolite produce increases in locomotor activity (Misra et al., 1975; Schuelke et al., 1995) but the lack of significant changes in brain levels cannot account for the variable activity levels measured over the course of the treatment period.

Unlike the pattern of behavior seen with cocaine, the stimulation of locomotor activity produced by morphine remained constant over the course of the week. Morphine, at a dose

Fig. 3. Stimulation of adenylyl cyclase activity by dopamine in (A) caudate putamen (n = 4) or (B) nucleus accumbens (n = 6) of animals treated with saline (○) or cocaine (●). Data are presented as % of basal activity (100%). Data are expressed as mean ± S.E.M. of N independent experiments, each performed in triplicate. Each independent experiment was comprised of tissue from an individual animal. There was not a significant change in the ability of dopamine to stimulate adenylyl cyclase activity in either brain region after cocaine treatment.
that produces tolerance to analgesia (Izenwasser, 1994) produced a significant increase in locomotor activity, to which neither tolerance nor sensitization occurred. When cocaine and morphine were chronically coadministered, activity levels were greater than those seen with either drug alone, and did not significantly change over the course of the week. It appears that morphine extends the period of sensitization to cocaine, whereas naltrexone has no effect on this pattern of behavior. This interaction between cocaine and morphine (i.e., a potentiated effect when the two drugs are coadministered) is similar to some reports of the reinforcing effects of stimulants and opioids. For example, administration of a stimulant with morphine produces greater reinforcement than does either drug alone, as measured using either the brain-stimulation (Hubner et al., 1987; Izenwasser and Kornetsky, 1989) or place preference (Masukawa et al., 1993) model of drug reinforcement. In rhesus monkeys, however, although a combination of heroin and cocaine is self-administered, there does not appear to be a potentiated effect at all doses tested (Mello et al., 1995). In animals trained to discriminate cocaine from saline, although opioid agonists alone do not consistently substitute for cocaine (Dykstra et al., 1992; Spealman and Bergman, 1992; Mello et al., 1995), they do appear to potentiate its discriminative stimulus effects (Spealman and Bergman, 1992; Mello et al., 1995; Suzuki et al., 1995; but see also Dykstra et al., 1992).

Although the administration of naltrexone alone did not produce a significant alteration in locomotor activity, there was a small decrease on most days, especially during the first half of the week. When naltrexone was administered with cocaine, slightly less locomotor activation was observed on each day of the treatment period as compared to cocaine alone. It is not known whether this is merely an additive effect of the two drugs, or whether in fact naltrexone did slightly attenuate this behavioral effect of cocaine. This is in contrast to an almost complete loss of activation when naltrexone is coadministered with cocaine either acutely (Houdi et al., 1989) or with repeated single daily injections (Sala et al., 1995). It is not known why these findings are so discrepant. However, one big difference in our study is that cocaine is continuously present with this treatment paradigm, whereas with intermittent administration there are extended periods of cocaine-free time between injections. Because naltrexone has a much longer half-life than cocaine, it is present during the cocaine-free periods between injections, making these two paradigms similar in respect to the opioid antagonism. Thus, it is possible that the blockade of cocaine’s effects by naltrexone occurs only if it is present in the absence of cocaine. This is supported by our previous findings (Izenwasser, 1994) that chronic coadministration of cocaine with naltrexone produces a diminished opioid receptor sensitization (suggesting a diminished action of naltrexone when cocaine is present).

Twenty-four hr after cessation of the continuous infusion of cocaine (50 mg/kg/day) there were no significant effects on dopamine D₁-like or D₂-like receptors in either the caudate putamen or nucleus accumbens. Neither the number or function of D₁ receptors was altered, and there were no changes in binding to dopamine D₂ receptors. We have previously shown that there is also no change in binding to the dopamine transporter after this continuous cocaine treatment (Izenwasser and Cox, 1992; Kunko et al., 1997). Thus, although cocaine produces marked behavioral changes during the course of this treatment, there do not appear to be significant changes in dopamine receptor binding or regulation of adenylyl cyclase activity immediately after this chronic infusion. Thus, these findings support the previously published studies suggesting that cocaine does not have marked neurotoxic effects on the dopaminergic system (Kleven et al., 1988; Yeh and DeSouza, 1991), and extend this conclusion to a continuous dosing regimen. In addition, these data suggest that the changes in behavior that are seen after chronic cocaine treatment might reflect changes in other systems, or perhaps differential interactions between systems.

In conclusion, these findings show that chronic continuous infusion of cocaine (50 mg/kg/day) for 7 days does not produce any immediate (within 24 hr) effects on dopamine D₁ or D₂ receptor binding or function in the caudate putamen or nucleus accumbens. In addition, the apparent tolerance that occurs during a chronic infusion of cocaine might actually be a rapidly developing sensitization and then loss thereof. This pattern of activity is not altered by an opioid antagonist, but is prolonged in the presence of morphine, the prototypical opioid agonist. It is not yet known whether animals treated with both cocaine and morphine will show tolerance after a withdrawal period, as do animals treated continuously with cocaine, but the findings do suggest that it is possible to alter the consequences of chronic cocaine via activation of opioid receptors. These findings further suggest that tolerance to the behavioral activating effects of cocaine may be due to changes that occur during withdrawal from a continuous treatment, as opposed to a direct effect of the drug during the treatment phase.

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References

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