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Cocaine Produces Low Dose Locomotor Depressant Effects in NBR and F344 Rats

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GEORGE, F. R. *Cocaine produces low dose locomotor depressant effects in NBR and F344 rats.* PHARMACOL BIOCHEM BEHAV **37**(4) 795–798, 1990.—Recently, cocaine has been shown to produce a significant locomotor depressant effect in mice at doses of 0.1–3.0 mg/kg. These low doses are below those associated with the well-described locomotor stimulant effects of cocaine, and represent a highly potent effect of this drug. It was postulated that these low doses of cocaine which depress locomotor activity do so via inhibition of serotonin uptake, resulting in potentiation of serotonergic activity. One important means of validating and extending novel findings is to determine the species generality of an effect. Thus the present study examined the effects of low doses of cocaine on locomotor activity in two rat strains, the NBR and F344. Cocaine produced low dose locomotor depressant effects in both rat strains. However, NBR rats showed a three-fold greater sensitivity to the depressant effects of cocaine relative to F344 rats, with ED_{50} values being 0.73 and 2.2 mg/kg for the two strains, respectively. As the dose of cocaine increased, activity for rats of both strains returned to baseline, but at the highest doses, large increases in locomotor activity were found only in the NBR rats. These results extend the conditions over which low doses of cocaine have been shown to depress locomotor activity to an additional mammalian species, namely rats, and confirm that significant genetic differences exist in the extent and expression of this effect.

Locomotor activity CNS depression Cocaine NBR rats F344 rats Behavior genetics

COCAINE has been commonly classified as a local anesthetic, due to its ability to block nerve conduction following local application (24). However, when administered systemically by any of several routes, cocaine produces numerous central and peripheral effects, primary among them being central nervous system (CNS) stimulation (21, 24, 34). In laboratory animals, the CNS effects of moderate cocaine doses are commonly observed as increases in ambulatory or locomotor activity, while further increases in cocaine dose can produce stereotypy, disruption of schedule-controlled behavior, seizures and, eventually, death (5, 6, 9, 16, 28, 30, 32).

The CNS effects of cocaine are believed to result from inhibition of monoamine uptake (2, 12, 14, 25–27). Evidence suggests that the locomotor stimulant effects of this drug are mediated primarily by inhibition of dopamine uptake, with a resultant increase in dopaminergic transmission. For example, the locomotor stimulant effects of cocaine can be reduced by pretreatment with dopamine receptor antagonists (29).

While the locomotor stimulant effect of cocaine may be mediated dopaminergically, cocaine has been reported to have high affinity for the serotonin-related transporter with a reported K_i some five-fold lower than that at the dopamine transporter, and greater than an order of magnitude lower than its affinity at the norepinephrine transporter (25). Augmentation of serotonergic activity has been associated with decreases in behavioral activity (3,18), and there is evidence that increased serotonin activity decreases the reinforcing potential of drugs (10, 11, 15, 25, 26, 31, 35).

In previous studies reporting behavioral depressant effects of cocaine, high doses were employed which resulted in stereotypy and disruption of behavior (8, 9, 32). However, Ruth et al. (28) showed that cocaine at low doses produced a decrease in rearing activity, and a low dose depressant effect of cocaine on locomotor ambulatory activity has recently been reported (4). In this latter study, locomotor depressant effects of cocaine were found in the range of 0.1–1.0 mg/kg in mice. Previous cocaine-related locomotor activity studies in rodents have used cocaine doses of 2.5 mg/kg or greater, and most studies have used cocaine doses in excess of 5 mg/kg [cf. (8, 9, 16, 30, 32)]. Metabolic and dispositional studies indicate that these doses should result in brain cocaine concentrations which would be maximally inhibiting reuptake at all three monoamine transporter sites. Thus it is possible that a behavioral depressant effect of cocaine specifically related to the affinity of this drug at a single high affinity site, such as the serotonin transporter, may have been masked in previous studies by effects at the other monoamine sites.

The primary purpose of this study was to replicate and extend the findings that cocaine produces a low dose locomotor depressant effect in mice by examining in two rat strains the locomotor effects of cocaine at doses below those which typically produce locomotor stimulation. If a significant depressant effect was found, a second purpose was to verify that cocaine produces a locomotor stimulant effect at doses higher than those which resulted in locomotor depression. A third purpose was to determine if these effects were genotype dependent.

METHOD

Animals

Experimentally naive male F344/CRlBR and NBR/NIH rats,

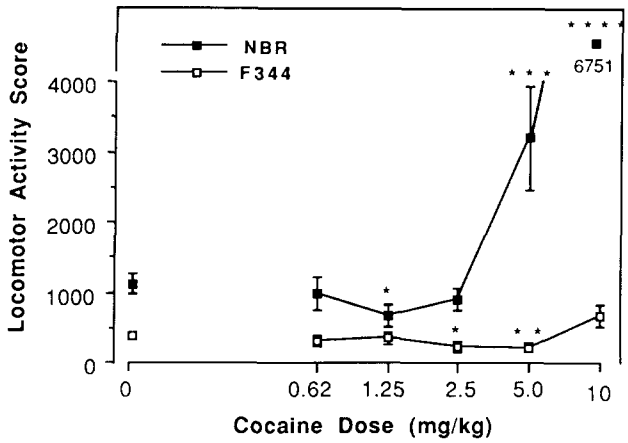

FIG. 1. Locomotor activity scores as a function of cocaine dose in NBR and F344 male rats. Data are presented as mean total horizontal ambulatory activity scores for 60 min ± SEM. Symbols represent scores significantly different from control: * = <0.05, ** = <0.02, *** = <0.01, **** = <0.0005.

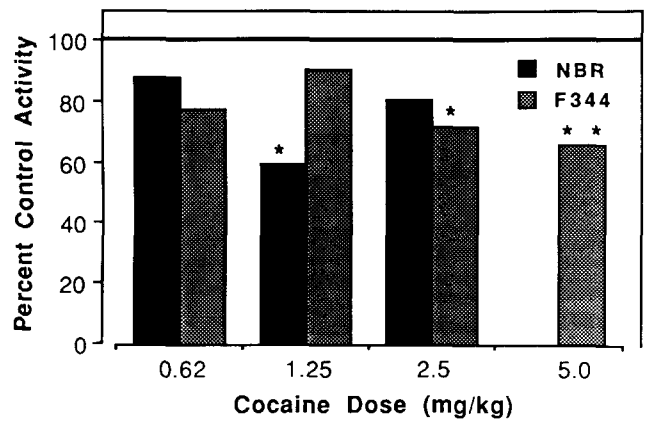

FIG. 2. Data for mean 60-min locomotor activity scores are presented as percent of control activity within genotype for those doses where activity was depressed relative to controls. Symbols represent scores significantly different from control: * = <0.05, ** = <0.02.

14–16 weeks old and weighing 220–320 g, were used. These rats were bred and raised at the NIDA Addiction Research Center, and were first generation offspring of breeders obtained from Charles River (Kingston, NY) or the NIH (Frederick, MD). The animals were housed in sexually segregated groups of 2–4 with ad lib access to Purina chow and tap water. All rats were maintained in a temperature-controlled room (26°C) with a 12-h light-dark cycle (0700–1900 lights on). All animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and the studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the NIH and adopted by the NIDA.

Procedure

The rats were randomly assigned into treatment groups of N = 6–8 per group. Within each group, rats were injected IP with one of the following doses of l-cocaine-HCl expressed as free base in 0.9% sterile saline: 0 (vehicle), 0.625, 1.25, 2.5, 5.0 or 10.0 mg/kg. All cocaine doses were administered in a volume of 3.0 ml/kg. All rats were tested between 0900 and 1300 h under white fluorescent lighting. Prior to injection, rats were placed in a Digiscan (Digiscan Instruments, Columbus, OH) activity monitor, and baseline activity was measured during a 20-min acclimation period. Immediately following injection, the rats were again placed in the Digiscan activity monitor and determinations of horizontal ambulatory activity as measured by the interruption of photocell light beams were accumulated electronically and summed every 10 min for 60 min.

Data Analysis

All analyses were conducted using SYSTAT for Macintosh (33). Group designs using nonrepeated measures analysis of variance (ANOVA) were used.

RESULTS

Cocaine produced significant overall dose-related changes in locomotor activity, $F(\text{Dose})(5,83) = 32.04$, $p<0.0001$ (Fig. 1). A significant strain difference in response to cocaine was also found, $F(\text{Strain})(1,83) = 120.73$, $p<0.0001$, with NBR rats being substantially more affected overall by cocaine than F344 rats. This significant strain difference was also evident across cocaine doses, $F(\text{Strain} \times \text{Dose})(5,83) = 27.04$, $p<0.0001$. A significant difference between the two strains in baseline activity was also shown, with NBR rats showing greater locomotor activity relative to F344 rats ($p<0.05$).

For NBR rats, cocaine produced an overall significant effect on locomotor activity, $F(\text{Dose})(5,41) = 29.13$, $p<0.0001$ (Fig. 1). Injection of cocaine resulted in a significant decrease in locomotor activity at 1.25 mg/kg, $t(14) = 2.29$, $p<0.05$ (Fig. 2). Cocaine administration produced significant increases in activity at 5 and 10 mg/kg [$t = 2.96$ and 7.66, $df = 14$ and 13, $p<0.01$ and <0.0001, respectively] (Fig. 1).

For F344 rats, an overall but less robust effect of cocaine on locomotor activity was seen, $F(\text{Dose})(5,42) = 2.40$, $p<0.05$ (Fig. 1). Injection of cocaine resulted in a significant decrease in locomotor activity at 2.5 and 5 mg/kg [$t = 2.28$ and 2.98, $df = 13$ and 13, $p<0.05$ and <0.02, respectively] (Fig. 2). Cocaine administration did not produce any locomotor stimulant effect in the F344 rats even at the 10 mg/kg dose (Fig. 1).

There were no apparent differences between strains in the time course of cocaine's effects on activity. At all effective doses of cocaine, both depressant and activating, the effect was most pronounced between twenty and forty minutes postinjection, followed by a gradual return to baseline.

DISCUSSION

The results of this study indicate that cocaine produces significant effects on locomotor activity, but that the nature and extent of these effects are both dose and genotype dependent. In NBR and F344 rats, cocaine produced locomotor depressant effects at doses below those associated with the stimulant and stereotypic effects of this drug. In addition, significant genetic differences were found in that NBR rats showed a three-fold greater sensitivity to this effect, while F344 rats were depressed across a wider dose range. One interesting finding was that, at the 5 mg/kg cocaine dose, NBR rats were significantly activated, while F344 rats were depressed, representing a substantial difference in the dose response curves for both the activating and depressant effects of cocaine. Overall, the results obtained in this study from the lowest doses of cocaine provide additional data showing that cocaine produces decreases in locomotor activity, while the re-

sults from the higher doses substantiate previous findings that cocaine produces significant increases in locomotor activity at these doses (16, 21, 30). These results substantiate other recent reports showing low dose depressant effects of cocaine on behavioral activity (4,28). Interestingly, the decreases in activity seen in this study were not associated with changes in stereotypy or rearing behavior, and little, if any, stereotypy was observed at the low doses of cocaine associated with decreased ambulatory activity.

The differences in response to cocaine between the NBR and F344 rats indicate the importance of genetic factors in these studies. F344 rats showed a significant but not robust decrease in activity, and showed no increase in locomotor activity even at 10 mg/kg, while NBR rats showed a sharp but limited decrease in activity at low doses, and showed several-fold increases in locomotor activity at higher doses. These findings agree with other recent data, where it has been shown that NBR rats are highly sensitive and highly responsive to the locomotor stimulant effects of cocaine, and where F344 rats only showed significant increases in activity when doses of at least 20 mg/kg were used (6).

Rates of cocaine metabolism were not obtained in this study. However, the large differences seen between the two strains, and the fact that the general patterns of drug-related effects over time were similar for the two strains, makes it unlikely that the differences seen between strains are due solely to pharmacokinetic factors. It is more likely that differences in neuronal mechanisms are primarily responsible for the differential responses of these strains, although a pharmacokinetic contribution with regards to either the depressant or stimulant effects of cocaine in this study cannot be ruled out.

Brain cocaine content was not directly measured in the present study. Therefore, actual receptor occupancy by cocaine cannot be precisely determined. However, receptor occupancy can be estimated based upon standard compartmentalization assumptions (17) and cocaine distribution studies (19). Estimations based upon the present data suggest that the doses of cocaine which produced significant locomotor depressant effects are in the range of 1.5 μM or less. These estimations are consistent with the findings of Ruth et al. (28) in which 5 mg/kg [^{3}H]-cocaine injected systemically resulted in a brain concentration of approximately 1.5 μM at 15 minutes postinjection in C57 and DBA mice. These estimations are also consistent with the work of Benuck et al. (1), who showed that IP injections of 10 mg/kg cocaine resulted in brain cocaine concentrations of approximately 4 μM at 15 minutes postinjection.

While it has been shown that cocaine has similar potencies for inhibiting reuptake of the three monoamines (13), several other studies support a possible serotonergic involvement in this low dose depressant effect of cocaine. The estimated brain concentrations of cocaine derived from the present findings are similar to the potency of cocaine to inhibit [^{3}H]-paroxetine binding to the serotonin transporter (25). These estimated brain concentrations are also similar to K_D values reported by Reith et al. (23) for sodium-independent [^{3}H]-cocaine binding at cortical serotonin transporters. Both reports suggest that cocaine has a greater affinity for the serotonin transporter relative to the dopamine transporter. Pitts and Marwah (20) have shown that serotonergic dorsal raphe neurons are more sensitive to cocaine than are ventral tegmental or zona compacta dopaminergic neurons. The results from studies showing that serotonin agonists depress behavioral activity (3,18) further suggest involvement of a serotonin-related mechanism in the low dose depressant effect of cocaine. Finally, Reith and Lajtha (22) showed that the locomotor depression caused by norcocaine does not involve alpha$_2$-adrenergic or presynaptic dopamine receptors, and it has recently been shown (6) that NBR and F344 rats do not differ in ligand affinity or receptor density of dopamine transporters and dopaminergic D1 and D2 receptors from striatal tissue, providing additional indirect support for a serotonin hypothesis.

The findings presented suggest several questions concerning mediation of this low dose depressant response to cocaine and its possible relationship to other cocaine effects such as self-administration. It will be important to determine the degree of monoamine uptake site and 5HT receptor system specificity for this effect. It would be useful to know if blockers of serotonin action or lesions of serotonin pathways would modulate this depressant effect. It would also be important to know if this depressant effect of cocaine is positively or negatively related to the reinforcing effects of this drug. An additional question is whether the genetic variation in the locomotor depressant response to cocaine shown in the present study can be utilized to determine the mode of transmission and molecular basis of this trait. Answers to these and other related questions will contribute to our understanding of the specific nature of cocaine's actions as well as to the more general phenomenon of substance abuse.

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