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RAPID COMMUNICATION

Anxiogenic Effects of Acute and Chronic Cocaine Administration: Neurochemical and Behavioral Studies

XIAO-MIN YANG, A. LAUREL GORMAN,
ADRIAN J. DUNN[1] AND NICK E. GOEDERS

Department of Pharmacology and Therapeutics,
Louisiana State University Medical Center, Shreveport, LA 71130-3932

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YANG, X.-M., A. L. GORMAN, A. J. DUNN AND N. E. GOEDERS. *Anxiogenic effects of acute and chronic cocaine administration: Neurochemical and behavioral studies.* PHARMACOL BIOCHEM BEHAV 41(3) 643–650, 1992.– The effects of cocaine on defensive withdrawal behavior in rats and elevated plus-maze behavior in mice were investigated. Cocaine (20 mg/kg IP) injected daily for 7 or 14 days induced defensive withdrawal; that is, the latency to emerge from a small chamber in an open field and the mean time in the chamber were both significantly increased. Acute cocaine administration also induced defensive withdrawal, and this effect was prevented by prior treatment with chlordiazepoxide (5 mg/kg IP). Both acute and chronic cocaine treatments significantly increased plasma concentrations of corticosterone and reduced the ratios of 3,4-dihydroxyphenylacetic acid to dopamine and 5-hydroxyindoleacetic acid to serotonin in several brain regions. Further evidence for an acute anxiogenic effect of cocaine was obtained from mice studied in the elevated plus-maze. Acute cocaine administration decreased both the number of entries into and the time spent in the open arms of the maze. These results taken together strongly support an anxiogenic action of acute and chronic cocaine administration.

Cocaine Defensive withdrawal Anxiety Corticosterone Dopamine Serotonin Elevated plus-maze Chlordiazepoxide

ALTHOUGH initial cocaine use is often reported by humans to produce profound subjective feelings of well-being and a decrease in anxiety (17,18), chronic use or the administration of high doses of the drug can be anxiogenic (5,32). The drug has even been reported to precipitate episodes of panic attack in some individuals (1,2,40). Furthermore, some of the major symptoms associated with cocaine withdrawal often include severe anxiety, restlessness, and agitation (7,18,38). Thus anxiety may be involved in the etiology of cocaine use and withdrawal in humans.

The present study was designed to examine whether acute and/or chronic cocaine administration would induce defensive withdrawal behavior in rats. In this paradigm, when rats are familiar with the apparatus, they explore it and spend little time in a small enclosed chamber set in an open field (4,37, 44). Restraint or pretreatment with anxiogenic agents, such as inverse benzodiazepine agonists or corticotropin-releasing factor (CRF), elicits defensive withdrawal behavior (37,44), whereas anxiolytic drugs, such as chlordiazepoxide (CDP), reverse restraint-induced defensive withdrawal (44). Thus defensive withdrawal could be interpreted as a measure of anxiety. To further substantiate the potential anxiogenic nature of acute cocaine administration, mice were also tested in an elevated plus-maze, a test commonly used to measure anxiety (26).

METHODS

Animals and Materials

Adult male Sprague-Dawley rats (250–300 g) obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN) were housed individually in plastic cages in a temperature- and

This paper is dedicated to the memory of Dr. Xiao-Min Yang who died as a result of an automobile accident July 5, 1990.

[1] Requests for reprints should be addressed to Dr. Adrian J. Dunn, Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, P.O. Box 33932, Shreveport, LA 71130-3932.

light-controlled American Association for Accreditation of Laboratory Animal Care (AAALAC)-accredited facility (lights on from 6:00 a.m. to 5:30 p.m.) for 1 week before the experiment. Food and water were available continuously in the home cages. Male CD-1 mice (25-30 g) obtained from Charles River were similarly maintained. Cocaine and CDP were obtained from Sigma Chemical Co. (St. Louis, MO).

Behavioral Procedures

Defensive withdrawal. Testing was conducted in an opaque Plexiglas open field (110 × 110 × 35 cm), the floor of which was marked with 20 × 20 cm squares and illuminated by a fluorescent lamp. A galvanized steel cylinder (15 cm deep and 13 cm in diameter) closed at one end was secured to the floor of the open field next to the wall in a lengthwise direction in the center of one side of the open field (37,44).

Chronic study. In two separate experiments, 26 rats were randomly divided into saline and cocaine groups (n = 13). On day 0, rats were habituated to the apparatus for 15 min by placing them in the center of the open field. On day 1, all animals were injected IP with normal saline, and baseline behavioral responses were scored 20 min later. Thereafter, animals in the saline group were injected daily with saline (1 ml/kg IP), and those in the cocaine group with cocaine (20 mg/kg IP) for 14 days (days 2-15). The behavioral test was performed only on days 1, 2, 8, and 15. Testing commenced 20 min after the IP injections. On days 7 and 14, rats were placed in the center of the open field without the small chamber to refamiliarize them with the apparatus. On each test day, the following behavioral measures were scored during the 15-min test session: the latency to leave the chamber, defined as the placement of all four paws in the open field; the mean time in the chamber (MTIC), defined as the average

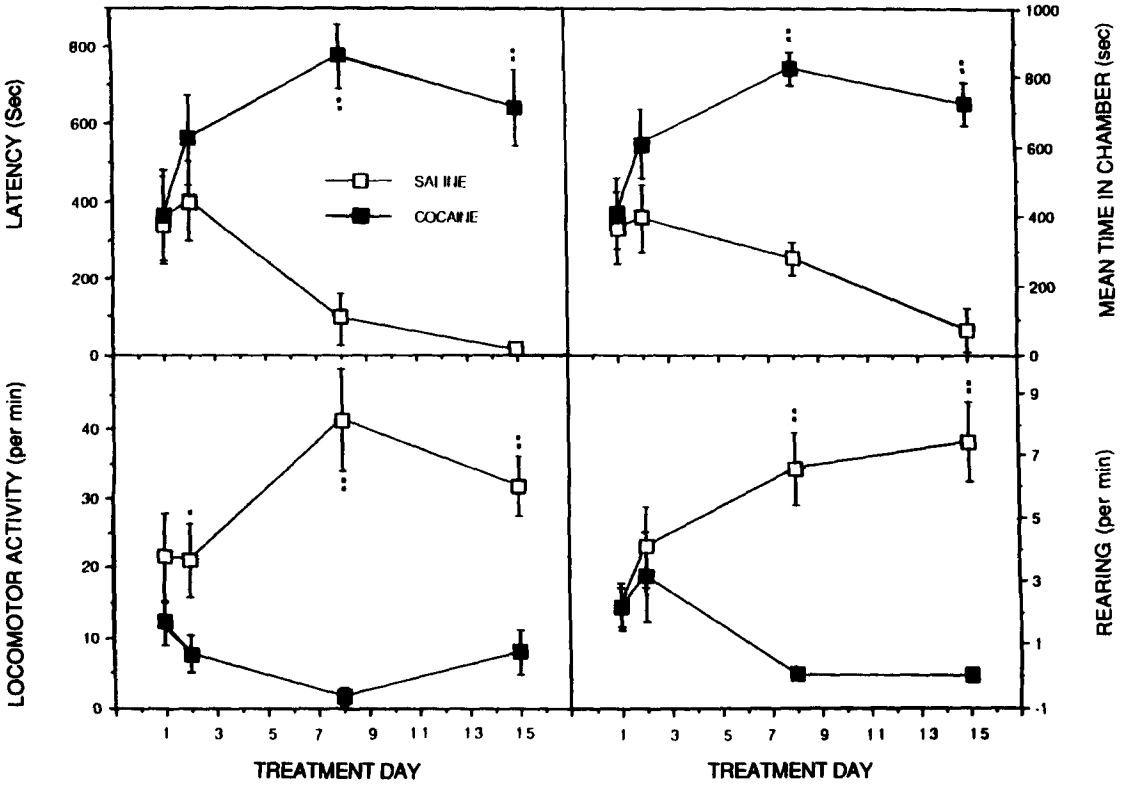

FIG. 1. Effects of daily cocaine treatment on defensive withdrawal behavior. On day 1, both groups of rats were injected IP with saline 20 min before testing. On days 2, 8, and 15, rats were injected with saline or cocaine (20 mg/kg IP) 20 min before the behavioral test. On the other days, animals received injections of saline or cocaine (20 mg/kg IP) but were not tested. (A) Latency to emerge from the small enclosed chamber; (B) Mean time spent in the small chamber (MTIC); (C) Line-crossings per minute spent outside the small chamber; (D) Rears per minute spent outside the small chamber. N = 13 per group. ANOVA indicated main effects of the cocaine treatment on the latency, $F(1,24) = 18.1$, $p < 0.001$; MTIC, $F(1,24) = 23.2$, $p < 0.0001$; line crossings/min $F(1,24) = 17.1$, $p < 0.001$; and rears/min $F(1,24) = 16.4$, $p < 0.001$. There was also a significant days effect within each treatment group [latency: saline, $F(3,36) = 6.3$, $p < 0.002$; cocaine, $F(3,36) = 3.6$, $p < 0.02$; MTIC, $F(3,36) = 5.0$, $p < 0.001$, $F(3,36) = 7.5$, $p < 0.001$; line-crossings, $F(3,36) = 6.0$, $p < 0.002$, $F(3,36) = 3.9$, $p < 0.02$; rears, $F(3,36) = 5.9$, $p < 0.01$, $F(3,36) = 4.6$, $p < 0.01$]. Each measure also had a significant treatment × days interaction [latency: $F(3,75) = 8.1$, $p < 0.0001$; MTIC, $F(3,75) = 9.4$, $p < 0.0001$; line-crossings, $F(3,75) = 8.9$, $p < 0.0001$; rears, $F(3,75) = 9.6$, $p < 0.0001$]. The cocaine treatment was significantly different from saline on days 8 and 15 for each measure, day 8: latency, $F(1,24) = 40$; MTIC, $F(1,24) = 43$; line-crossings, $F(1,24) = 29$; rears, $F(1,24) = 32$, all $p < 0.0001$; day 15: latency, $F(1,24) = 41$; MTIC, $F(1,24) = 93$; line-crossings, $F(1,24) = 20$; rears, $F(1,24) = 32$, all $p < 0.001$, and on day 2 for line-crossings/min, $F(1,24) = 5.4$, $p < 0.03$.

Significant difference from saline control (*$p < 0.05$; **$p < 0.01$).

time in the chamber per entry (including the initial placement); and measures of locomotor activity, defined as the number of lines crossed on the floor of the open field and as the number of rears. Both locomotor activity scores were expressed per minute spent outside the small chamber. After each test, the apparatus was cleaned with 1% acetic acid to prevent olfactory cues from affecting the behavior of subsequently tested rats. Rats were killed by decapitation immediately after removal from the apparatus on day 15.

Acute study. Rats were exposed to the open field without the small chamber on the first day and were then tested for defensive withdrawal for four successive days as described in the chronic experiment. On days 1, 2, and 3, all rats received saline injections 20 min before placement in the apparatus. On day 4, rats received either saline or cocaine (20 mg/kg IP) 20 min before testing. Rats were killed by decapitation immediately after removal from the apparatus on day 4. In a separate experiment, the same paradigm was used except that two additional groups of rats received CDP (5 mg/kg IP) 40 min before cocaine on day 4.

Elevated plus-maze. Mice were tested in an elevated plus-maze as described by Lister (26) 20 min after receiving a single injection of either cocaine (20 mg/kg IP) or saline. To start the test, mice were placed in the center of the Plexiglas maze facing a closed arm. Both the number and the duration of entries into each arm were recorded by an observer blind to the treatment groups. Scoring was performed using an NEC 8201A computer. After each 5-min trial, the plus-maze was cleaned with a 0.5% solution of acetic acid. The apparatus was illuminated by a 25-watt bulb, and testing was conducted in a room separate from the colony room.

Determination of Cerebral Biogenic Amines

The medial prefrontal cortex, nucleus accumbens, caudate putamen, hypothalamus, hippocampus, and brain stem were excised as rapidly as possible after decapitation and were weighed and frozen as previously described (9). The brain samples were homogenized in 0.1 M $HClO_4$ and stored frozen at $-70°$ until analysis by high-performance liquid chromatography (HPLC) with electrochemical detection as previously described (9). This system separated dopamine (DA); 3,4-dihydroxyphenylacetic acid (DOPAC); homovanillic acid; norepinephrine (NE); normetanephrine; 3-methoxy,4-hydroxyphenylethyleneglycol (MHPG); serotonin (5-HT); 5-hydroxyindoleacetic acid (5-HIAA); tyrosine; tryptophan, and uric acid. MHPG was not measurable in samples from rat brain.

Determination of Plasma Corticosterone

Trunk blood was collected following decapitation immediately after the test on day 15 in the chronic experiments and day 4 in the acute experiments. Plasma corticosterone was determined by radioimmunoassay following extraction with methylene chloride according to the procedure of Gwosdow-Cohen et al. (22).

Statistical Analysis

The data are presented as the means ± SEM. Statistical analysis of behavioral data was performed by analysis of variance (ANOVA) using the SAS program. The comparison of multiple means was accomplished by Duncan's test. Student's *t*-tests were used to analyze data from the elevated plus-maze and the plasma corticosterone and neurochemical data.

RESULTS

Chronic Cocaine Treatment and Defensive Withdrawal

The results obtained in both chronic experiments were very similar and have therefore been combined in Fig. 1. On day 1, animals in the saline- and cocaine-treated groups had similar latencies to emerge from the small enclosed chamber, similar mean times in the chamber (MTIC), as well as similar locomotor activity scores. Rats treated with saline progressively habituated to the apparatus. On day 8, they emerged very rapidly from the small enclosed chamber compared with day 1, $F(1,24) = 3.8$, $p \approx 0.05$. The MTIC decreased, but this effect was not statistically significant, $F(1,24) = 0.6$. Line crossings per minute and rears per minute outside the small chamber were both significantly increased, $F(1,24) = 4.2$, $p < 0.05$; $F(1,24) = 10$, $p < 0.01$. On day 15, the latency to emerge, $F(1,24) = 8.8$, $p < 0.01$, and the MTIC, $F(1,24) = 9.2$, $p < 0.01$, were both significantly decreased compared to day 1, and rearing frequency was increased, $F(1,24) = 12.5$, $p < 0.01$.

Rats in the cocaine-treated group displayed typical defensive withdrawal behavior. The first dose of cocaine (day 2) increased the latency and MTIC and decreased line-crossing and rearing frequencies (Fig. 1). However, compared with saline controls, only the cocaine-induced decrease in line-crossing frequency was statistically significant. In comparison with saline-treated controls, animals in the cocaine group appeared to be very alert, and urinated and defecated during the 15-min test session. On day 8, the latency to emerge from the

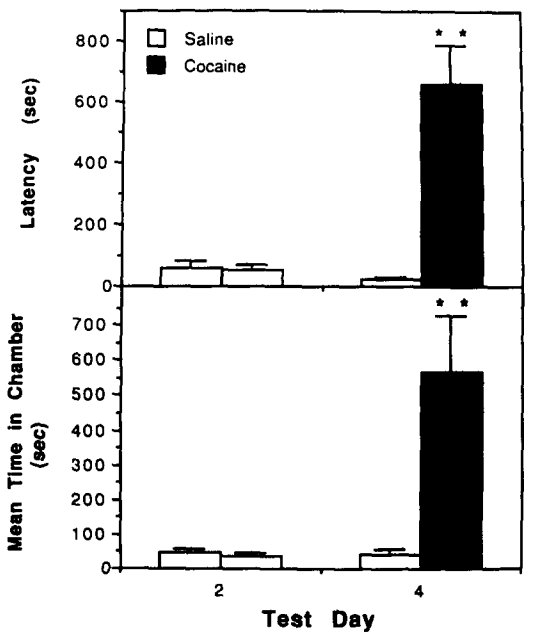

FIG. 2. Effects of acute cocaine treatment on defensive withdrawal behavior. After familiarization with the apparatus (day 0), rats were placed in the small chamber each day for 4 successive days 20 min after receiving an IP injection. On days 1-3, each rat received an injection of saline; on day 4, each rat received an injection saline (open bars) or 20 mg/kg cocaine (solid bars). The data from day 3 have not been presented because the scoring was disrupted by circumstances beyond our control. Top: latency to emerge from the small chamber; bottom: mean time in chamber. **Significantly different from saline control ($p < 0.01$).

small chamber and the MTIC in cocaine-treated rats were significantly higher than in saline controls. The rats also showed less locomotor activity. On day 15, the latency and the MTIC following chronic cocaine treatment were significantly higher than in saline controls, and locomotor activity was lower.

Acute Cocaine Treatment and Defensive Withdrawal

Because the anxiety or stress caused by being picked up and placed in the apparatus may have obscured an effect of acute cocaine administration, a second experiment was performed in which rats were habituated to the procedure for 3 days before cocaine administration. Figure 2 shows that acute cocaine treatment significantly elevated both the latency to emerge from the chamber, $F(1,11) = 21.5, p < 0.01$, and the MTIC, $F(1,11) = 15, p < 0.01$. Line-crossing frequency was not significantly altered, but the number of rears per minute outside the small chamber decreased, $F(1,11) = 11$, $p < 0.01$. Rather similar results were obtained in two replicate experiments. Figure 3 shows that pretreatment with CDP (5 mg/kg IP) 40 min before cocaine on day 4 reversed the effects of cocaine on the latency to emerge (cocaine × CDP interaction) $F(1,16) = 8.1$, $p = 0.011$, and on the MTIC (cocaine × CDP interaction) $F(1,16) = 4.2, p < 0.05$.

Plasma corticosterone was measured in the trunk blood of the rats from the experiments depicted in Figs. 1 and 2 immediately after testing on the final day. Both acute and chronic cocaine treatment significantly increased the concentrations of corticosterone in trunk blood relative to the saline-injected controls (Fig. 4). Samples were also taken for determination of plasma corticosterone in the experiment of Fig. 3, but on a fifth day of testing in which the treatments were identical to those on day 4. The data indicated once again that

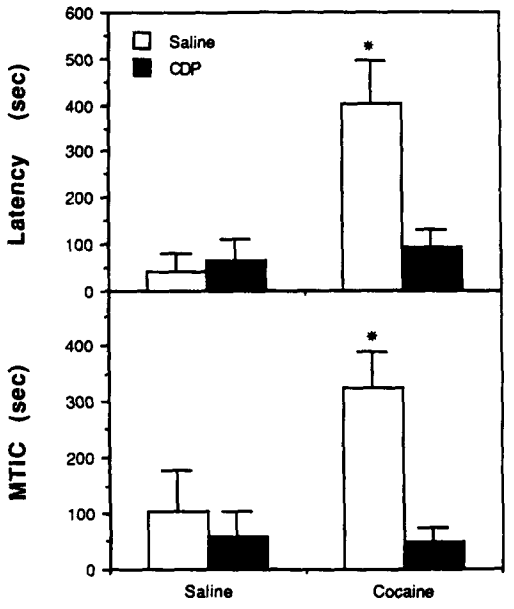

FIG. 3. Effects of acute cocaine treatment in chlordiazepoxide (CDP) pretreated rats on defensive withdrawal behavior. In an experiment similar to that of Fig. 2, rats were pretreated with CDP (5 mg/kg IP) 40 min before cocaine on Day 4 and tested in defensive withdrawal. Top: latency to emerge from the small chamber; bottom: mean time in chamber. *Significantly different from saline control ($p < 0.05$).

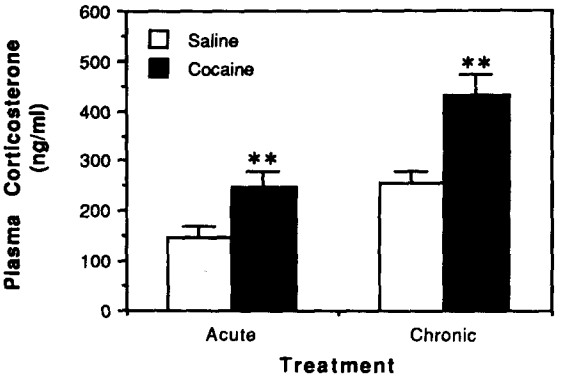

FIG. 4. Effects of acute and chronic cocaine administration on plasma corticosterone. The animals from the experiments depicted in Figs. 1 and 2 were decapitated on day 15 or 4, respectively, immediately after the final behavioral test, and trunk blood was collected for assay of plasma corticosterone. **Significantly different from saline control ($p < 0.01$).

cocaine increased plasma corticosterone (saline: 295 ± 25; cocaine: 429 ± 56; CDP: 236 ± 36; CDP + cocaine: 290 ± 51); $F(1,17) = 4.36, p < 0.05$. However, although CDP clearly attenuated the effect of cocaine in this experiment, the interaction between CDP and cocaine was not statistically significant, $F(1,17) = 0.83$.

Brain samples from the rats used in the experiments depicted in Figs. 1 and 2 were taken for neurochemical analyses. Acute cocaine treatment significantly decreased DOPAC : DA ratios in the medial prefrontal cortex, nucleus accumbens, hypothalamus, and caudate putamen (Table 1). Chronic cocaine treatment reduced DOPAC : DA ratios in the medial prefrontal cortex, hypothalamus, and brain stem (Table 2). Both acute and chronic cocaine treatments decreased 5-HIAA : 5-HT ratios in all regions studied, although this effect fell short of statistical significance in the caudate and hippocampus in the chronic study (Tables 1 and 2). Concentrations of norepinephrine were not altered in any region.

As an additional test of the hypothesis that acute cocaine administration induced anxiogenic effects, mice were tested in the elevated plus-maze. Figure 5 shows that cocaine treatment significantly decreased the number of entries into the open arms. In addition, the total time spent on the open arms was significantly reduced, and time on the closed arms significantly increased.

DISCUSSION

In the present study, both acute and chronic cocaine administration induced defensive withdrawal responses in rats familiar with the experimental apparatus, that is, the latency to emerge from the small enclosed chamber and the MTIC were both significantly increased. In the chronic study, the first administration of cocaine (day 2) had no significant effect on defensive withdrawal behavior, even though the animals urinated, defecated, and appeared to be very alert while exploring the open field. However, after 8 and 15 days of cocaine treatment, significant defensive withdrawal behavior was observed. The results of the acute study suggest that the potential effects of this acute cocaine treatment were masked in the chronic study because the rats were not yet completely familiar with the apparatus. Different stressors (novel experimental environment, restraint) and anxiogenic agents (inverse

TABLE 1

EFFECTS OF ACUTE COCAINE ADMINISTRATION ON CEREBRAL DOPAMINE, SEROTONIN AND CATABOLITES

Region	N	DA	DOPAC	DOPAC : DA	5-HT	5-HIAA	5-HIAA : 5-HT
Medial prefrontal cortex							
Saline	5	0.070 ± 0.004	0.037 ± 0.004	0.53 ± 0.04	0.057 ± 0.003	0.74 ± 0.04	13.1 ± 0.66
Cocaine	5	0.082 ± 0.009	0.030 ± 0.006	0.36 ± 0.04*	0.071 ± 0.005*	0.54 ± 0.03†	7.8 ± 0.70‡
Nucleus accumbens							
Saline	6	4.12 ± 0.34	1.18 ± 0.11	0.28 ± 0.01	1.04 ± 0.07	1.29 ± 0.08	1.24 ± 0.04
Cocaine	6	4.60 ± 0.23	1.02 ± 0.07	0.22 ± 0.01‡	1.18 ± 0.17	0.97 ± 0.15§	0.83 ± 0.03‡
Caudate putamen							
Saline	6	6.85 ± 0.55	1.09 ± 0.11	0.16 ± 0.01‡	0.60 ± 0.04	1.29 ± 0.04	2.19 ± 0.09
Cocaine	6	9.08 ± 2.06	1.05 ± 0.26	0.11 ± 0.00	0.61 ± 0.07	1.04 ± 0.13*	1.71 ± 0.08†
Hypothalamus							
Saline	5	0.17 ± 0.01	0.042 ± 0.004	0.24 ± 0.02	0.107 ± 0.01	1.18 ± 0.04	11.6 ± 1.13
Cocaine	5	0.21 ± 0.02	0.039 ± 0.003	0.19 ± 0.01†	0.142 ± 0.02§	0.92 ± 0.01‡	6.83 ± 0.76†
Hippocampus							
Saline	6	–	–	–	0.10 ± 0.01	0.61 ± 0.04	6.77 ± 1.16
Cocaine	6	–	–	–	0.13 ± 0.02	0.51 ± 0.05	4.92 ± 1.35
Brain stem							
Saline	6	0.083 ± 0.012	0.048 ± 0.006	0.59 ± 0.02	0.37 ± 0.03	0.72 ± 0.07	1.95 ± 0.07
Cocaine	6	0.081 ± 0.024	0.041 ± 0.009	0.57 ± 0.07	0.50 ± 0.12	0.74 ± 0.21	1.45 ± 0.05‡

(DA), dopamine; (DOPAC), 3,4-dihydroxyphenylacetic acid; (5-HT), serotonin; (5-HIAA), 5-hydroxyindoleacetic acid.

The rats from the experiment depicted in Fig. 2 were decapitated immediately after removal from the apparatus on day 4 and catecholamines, indoleamines, and metabolites measured by HPLC. DA, DOPAC, 5-HT, and 5-HIAA concentrations are expressed in ng/mg wet weight. The low hippocampal content of DA precluded collection of reliable data.

Significant difference from saline-injected controls (*$2p < 0.05$; †$2p < 0.01$; ‡$2p < 0.001$; §$p < 0.05$).

benzodiazepine agonists and ICV CRF) have been reported to elicit similar behavioral responses in the defensive withdrawal paradigm (4,37,44), suggesting that cocaine administration induced stress or anxiety. Insofar as the elevated plus-maze is regarded as a model for anxiety (26), acute cocaine administration can also be considered to be anxiogenic in mice. Moreover, because CDP is regarded as a prototypic anxiolytic, the ability of CDP to reverse the effects of cocaine administration on defensive withdrawal supports the interpretation that the cocaine-induced changes in defensive withdrawal reflect anxiety. Consistent with this, preliminary data have indicated that intracerebroventricular injection of 50 μg of the CRF antagonist alpha-helical CRF_{9-41} reversed the effect of acute cocaine on defensive withdrawal.

Other investigators have reported anxiogenic responses to cocaine. In drug discrimination studies, cocaine will generalize to a pentylenetetrazol discriminative stimulus, suggesting that noncontingent cocaine administration and/or withdrawal causes an anxiogenic response in rats (35,42,43) which is also blocked by diazepam, but not by haloperidol (43). Anxiogenic behavior has also been reported in mice tested in a black/white two-compartment model of anxiety (6) and in rats tested in the conditioned suppression of drinking conflict model following acute or chronic administration of cocaine (13) and during withdrawal (6,13). Ettenberg and Geist (11) recently reported that cocaine resulted in both reinforcing and anxiogenic behavior in rats trained to self-administer the drug by traversing the length of a straight alley. Following repeated testing, the latency to enter the goal box where intravenous cocaine infusions were delivered gradually increased, suggesting a pro-conflict or anxiogenic response. Diazepam pretreatment reduced the latency to enter the goal box.

Changes in plasma corticosterone are a sensitive physiological response of stress or anxiety (12). We found that both acute and chronic cocaine administration significantly elevated plasma corticosterone concentrations. These data are consistent with recent reports that acute and chronic cocaine administration activate the hypothalamic-pituitary-adrenal (HPA) axis (3,14,19,28), an effect that appears to be mediated through CRF (34). These results indicate that the cocaine-treated animals were in an anxious or stressful state. The observation that CDP pretreatment also attenuated the effects of cocaine on plasma corticosterone implicates benzodiazepine receptors as modulators of the HPA axis and strengthens the concept that cocaine had an anxiogenic effect.

The neurochemical changes observed in this study are generally consistent with those observed previously following cocaine (45). DOPCA : DA ratios were decreased in the medial prefrontal cortex, nucleus accumbens, caudate putamen, and hypothalamus by acute cocaine, but only in the hypothalamus and brain stem (and possibly the medial prefrontal cortex) by chronic cocaine. Karoum et al. (23) also observed decreases in DOPAC in the prefrontal cortex, septum, nucleus accumbens, striatum, and hypothalamus, but also noted decreases in DA in prefrontal cortex and accumbens after chronic cocaine administration. We observed decreases in 5-HIAA : 5-HT ratios in all brain regions studied following acute cocaine, but again only in the hypothalamus and brain stem following chronic administration. Friedman et al. (15) observed a decrease in the concentration of 5-HIAA in whole brain following acute injections of cocaine, whereas chronic cocaine treatment caused a decrease in both 5-HT and 5-HIAA without any change in the 5-HIAA : 5-HT ratio in the septum caudate (39). Generally, catabolites of DA and 5-HT are increased in stressed or anxious states (10). The changes observed following cocaine administration most probably reflect the drug's

TABLE 2

EFFECTS OF CHRONIC COCAINE TREATMENT ON CEREBRAL DOPAMINE, SEROTONIN AND CATABOLITES

Region	N	DA	DOPAC	DOPAC : DA	5-HT	5-HIAA	5-HIAA : 5-HT
Medial prefrontal cortex							
Saline	5	0.062 ± 0.017	0.028 ± 0.004	0.50 ± 0.06	0.21 ± 0.02	0.13 ± 0.01	0.66 ± 0.03
Cocaine	5	0.129 ± 0.029	0.036 ± 0.006	0.32 ± 0.06*	0.20 ± 0.03	0.11 ± 0.01*	0.56 ± 0.04*
Caudate putamen							
Saline	6	5.15 ± 0.23	0.68 ± 0.03	0.13 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	1.14 ± 0.03
Cocaine	6	5.36 ± 0.36	0.74 ± 0.05	0.14 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	1.12 ± 0.06
Hypothalamus							
Saline	6	0.111 ± 0.009	0.033 ± 0.003	0.30 ± 0.01	0.35 ± 0.02	0.34 ± 0.03	0.98 ± 0.03
Cocaine	6	0.099 ± 0.015	0.024 ± 0.004	0.24 ± 0.02†	0.35 ± 0.02	0.28 ± 0.03	0.81 ± 0.06†
Hippocampus							
Saline	6	–	–	–	0.17 ± 0.01	0.16 ± 0.00	0.95 ± 0.04
Cocaine	6	–	–	–	0.18 ± 0.01	0.14 ± 0.01	0.84 ± 0.06
Brain stem							
Saline	6	0.055 ± 0.002	0.030 ± 0.003	0.54 ± 0.03	0.69 ± 0.02	0.46 ± 0.01	0.68 ± 0.01
Cocaine	6	0.059 ± 0.004	0.025 ± 0.003	0.43 ± 0.02†	0.73 ± 0.03	0.40 ± 0.02†	0.56 ± 0.02‡

(DA), dopamine; (DOPAC), 3,4-dihydroxyphenylacetic acid; (5-HT), serotonin; (5-HIAA), 5-hydroxyindolacetic acid.

The rats from one of the experiments depicted in Fig. 1 were decapitated immediately after removal from the apparatus on day 15 and catecholamines, indoleamines, and metabolites measured by HPLC. DA, DOPAC, 5-HT, and 5-HIAA concentrations are expressed in ng/mg wet weight. The low hippocampal content of DA precluded collection of reliable data.

Significant difference from saline control group (*$p < 0.05$; †$2p < 0.05$; ‡$2p < 0.01$).

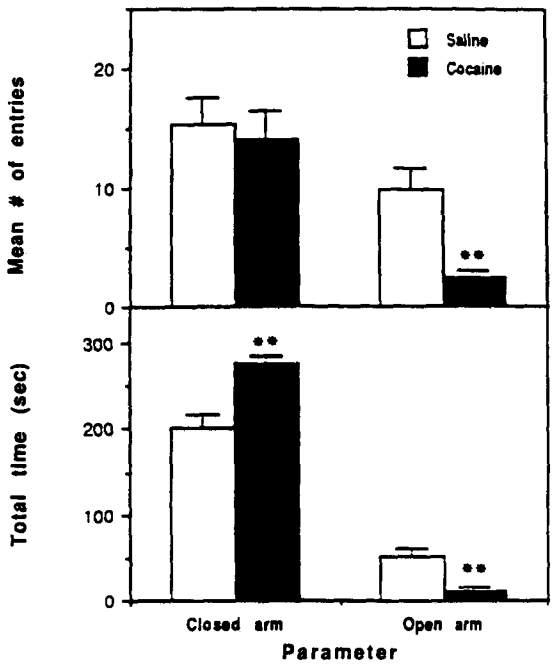

FIG. 5. Effects of acute cocaine on behavior of mice in the elevated plus-maze. Mice ($n = 8$) were injected with saline or cocaine (20 mg/kg IP) 20 min before placement in the plus-maze. Top: the mean number of entries onto the closed and open arms. Bottom: the total time spent on the closed and open arms. Student's t-test indicated statistical significance for the effect of cocaine on the number of entries into the open arms, $t(1,14) = 4.15$, $2p < 0.002$; the total time spent on the closed arms, $t(1,14) = 4.22$, $2p < 0.002$; and that on the open arms, $t(1,14) = 4.01$, $2p < 0.002$. **Significantly different from saline-injected mice ($p < 0.01$).

inhibition of DA or 5-HT re-uptake (33,41), resulting in decreased production of deaminated catabolites. We speculate that this pharmacological effect of cocaine may have masked any increases in DA and 5-HT metabolism paralleling the anxiety.

These neurochemical changes are consistent with binding data. We have recently reported a selective effect of chronic cocaine administration using a dosing schedule similar to that used in this investigation on benzodiazepine (20) and CRF (21) binding sites in brain regions associated with the mesocorticolimbic dopaminergic system. We have also reported selective changes in serotonin uptake sites (i.e., [^{3}H]imipramine binding) in the prefrontal cortex and dorsal raphe and serotonin binding (i.e., 5-HT$_{1A}$ receptors labeled with [^{3}H]8-OH-DPAT) in the central medial nucleus of the amygdala following chronic cocaine treatment (8).

In conclusion, the results of this investigation suggest that both acute and chronic cocaine administration can induce anxiety- or stress-like behavioral and endocrine changes in rats in the defensive withdrawal paradigm. These changes were reversed by benzodiazepine pretreatment, indicating the ability of benzodiazepine receptors to modulate pathways involved in anxiogenic responses. Acute cocaine administration had a similar anxiogenic effect on mice studied in the elevated plus-maze. These data may be relevant from a clinical perspective. Recently, it has been reported that vulnerability to intravenous amphetamine self-administration in rats is associated with the animal's reactivity to a novel environment (29,30), suggesting that physiological responses to stress may be predictive of individual abuse liability. Further studies demonstrated that environmental conditions (27) or even exogenous infusions of corticosterone (31) can increase the likelihood that a rat will acquire self-administration of low doses of amphetamine, suggesting that changes in activity within the hypothalamic-pituitary-adrenal axis may be involved in the abuse liability of stimulant drugs. In non-laboratory settings, social users of cocaine are often able to control their drug

intake and, therefore, do not escalate their patterns of use to levels that increase the risk of dependency and toxicity (36). These data suggest that factors in addition to cocaine's reinforcing properties may determine why some individuals can remain causal recreational users of the drug while others progress to compulsive drug use. In fact, a subpopulation of chronic cocaine users may actually be self-medicating to regulate painful feelings and psychiatric symptoms via their drug use (16,24,25). Therefore, a better understanding of the involvement of anxiety in the behavioral, neurochemical, and endocrine effects of cocaine may assist in the development of more effective and efficient treatment strategies for cocaine use and withdrawal in humans.

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