Pharmacology Biochemistry & Behavior, Vol. 10, pp. 267–271. Printed in the U.S.A.

Metabolism Correlates of Cocaine-Induced Stereotypy in Rats[1]

VICENTE S. ESTEVEZ, BENG T. HO AND LEO F. ENGLERT

Texas Research Institute of Mental Sciences and
The University of Texas Health Science Center at Houston
Houston, TX 77030

(Received 22 May 1978)

ESTEVEZ, V. S., B. T. HO AND L. F. ENGLERT. *Metabolism correlates of cocaine-induced stereotypy in rats.* PHARMAC. BIOCHEM. BEHAV. 10(2) 267–271, 1979.—An increase in stereotyped behavior was observed in rats injected daily with cocaine (40 mg/kg, IP), as compared with the first day. This increase persisted 14 days after discontinuation of the drug treatment, and corresponded to increased levels of ^{3}H-cocaine, norcocaine and benzoylecgonine in brain. Pretreatment of the animals with SKF-525A, an inhibitor of cocaine demethylation, produced a decrease in stereotypy rating and concomitantly a lower level of ^{3}H-norcocaine in the brain. The role of this metabolite in the production of cocaine-induced stereotyped behavior is discussed.

Cocaine Stereotyped behavior Metabolism

REPEATED administration of cocaine can result in an enhancement of its behavioral effects under certain conditions [1, 9, 15]. Reports of increase in motor activity [4, 5, 12] and stereotypy during repeated administration [10, 11, 14] have demonstrated the cocaine-induced hypersensitivity in laboratory animals. Furthermore, this increased sensitivity was found to persist after the termination of chronic drug administration [7, 13, 14].

The metabolism of cocaine has been extensively studied and one of its main metabolites, norcocaine, is a product of the microsomal enzyme system in the liver. After administration of ^{3}H-cocaine, norcocaine accounts for 10 to 15% of the total concentration of the drug and metabolites in the brain [4,17]. When administered alone, it is behaviorally active [6,18], implying it is an active metabolite of cocaine.

In a previous report, we were able to demonstrate an increase in locomotor activity in chronically drug-treated rats, as compared to that in chronic saline animals receiving a single dose of cocaine. This coincided with an increase in the levels of norcocaine and a concomitant decrease of cocaine concentration in the brains of the chronic animals at the time of measurement, indicating a faster metabolism resulting from the chronic administration [4]. In addition, the administration of SKF-525A, a microsomal enzyme inhibitor, produced an elevation of unchanged cocaine in the rat brain without altering brain norcocaine concentrations. The locomotor activities of rats after receiving cocaine were not significantly different in animals with or without pretreatment of SKF-525A [3].

The purpose of the present work was to correlate the changes on cocaine-induced stereotyped behavior in rats with drug metabolism, after acute and repeated daily injections.

METHOD

Behavioral Studies

Three groups of eight female Sprague-Dawley rats (180-200 g) were used for this experiment. The rats were single caged and received food and water ad lib. The first group of animals received daily cocaine hydrochloride (40 mg/kg IP) in saline and the stereotyped behavior was evaluated following a procedure similar to that reported by Kilbey and Ellinwood [7], i.e., on Days 1 and 14 of daily drug injection. On Day 14 following discontinuation of treatment, the rats received a final injection of cocaine and were again scored for stereotyped behavior. Behavior ratings on the 8-point scale of Ellinwood and Balster [2] were made before the animals received cocaine injections, and at varying intervals up to 90 min after receiving the drug. At each time interval, the rats were observed for 20 sec, and a score assigned. Data were calculated for significance using a Mann-Whitney U test.

The other two groups of animals were used to study the effects of SKF-525A, a microsomal enzyme inhibitor, on cocaine induced behavior. All animals received cocaine·HCl (40 mg/kg IP) daily for 14 days. On Day 14, half the rats were given SKF-525A (12.5 mg/kg IP) in saline 30 min prior to the

[1]This work was supported in part by NIH Grant Number DA-00795 from the National Institute of Drug Abuse, U.S.A.

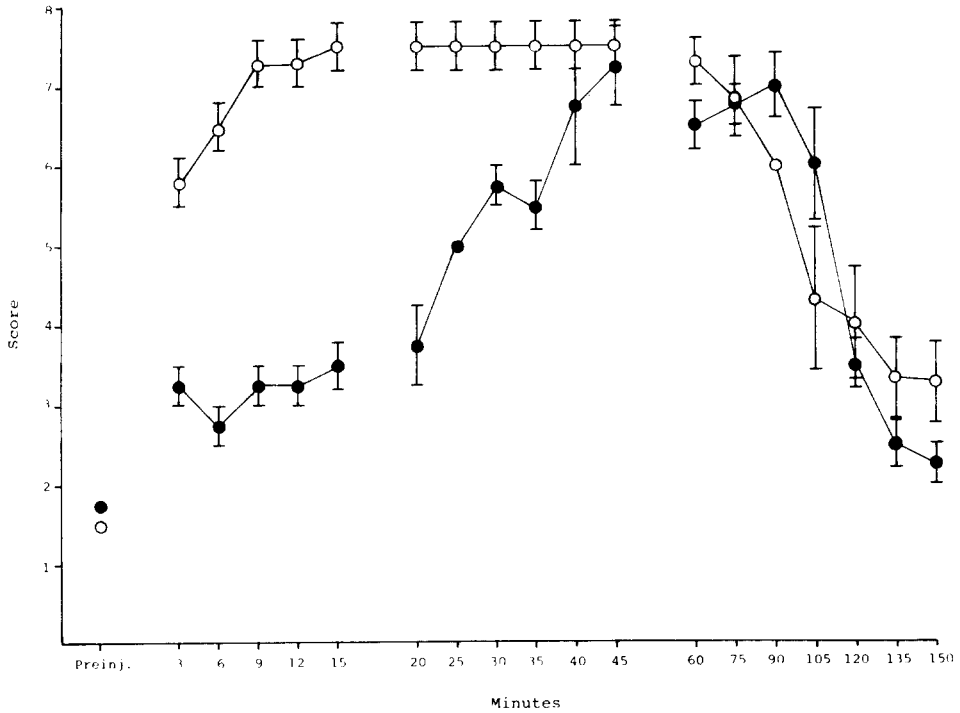

FIG. 1. Enhancement of stereotyped behavior in rats injected daily for 14 days with cocaine HCl (40 mg/kg, IP). Each value represents the mean score ± SEM of 5 animals. ●——●, the group receiving an acute dose of cocaine; ○——○, the chronically treated group.

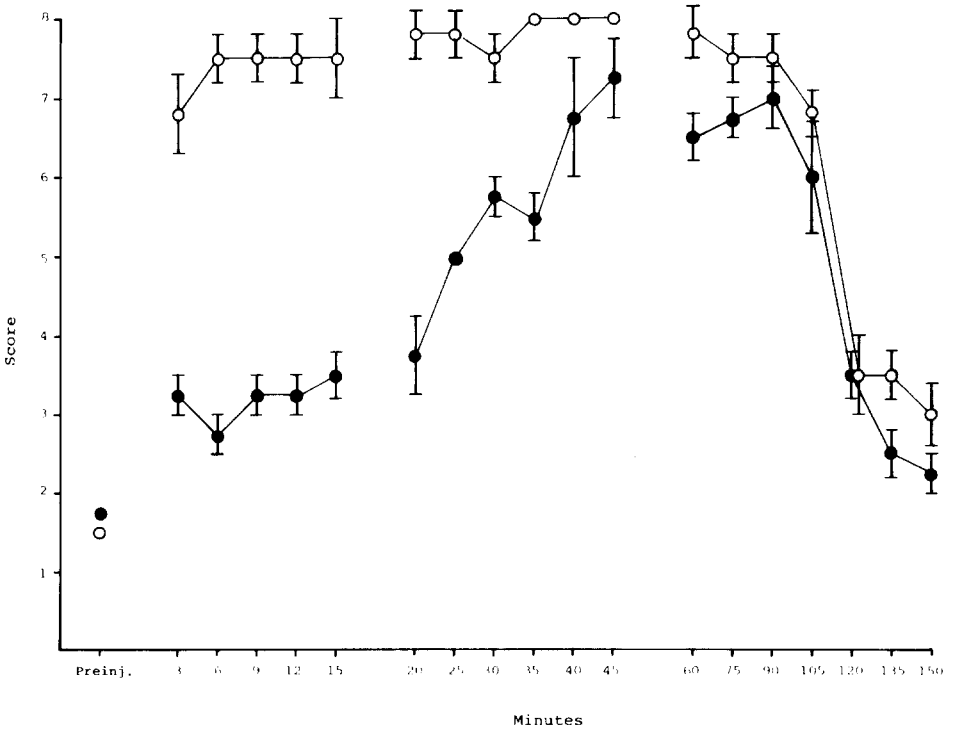

FIG. 2. Persistence of enhancement of stereotyped behavior at 7 days after discontinuation of chronic cocaine injections. Each value represents the mean score ± SEM of 5 animals of the two groups shown in Fig. 1.

TABLE 1

EFFECTS OF CHRONIC ADMINISTRATION ON THE LEVELS OF ^{3}H-COCAINE AND METABOLITES IN THE RAT BRAIN

	nmole/g		
	Cocaine	Norcocaine	Benzoylecgonine
Day 1 (40 mg/kg)	11.8 ± 0.27	3.86 ± 0.06	2.3 ± 0.16
Day 14 (40 mg/kg)	16.3 ± 0.43* (38% ↑)‡	4.72 ± 0.03* (22% ↑)‡	3.78 ± 0.05* (64% ↑)‡
Day 14 Posttreatment (40 mg/kg)	21.6 ± 2.4† (83% ↑)‡	4.82 ± 0.03* (25% ↑)‡	3.21 ± 0.05* (40% ↑)‡

Each value represents the mean ± SEM of duplicate samples from 6 rats.
*$p<0.001$, to the Day 1 group.
†$p<0.01$, compared to the Day 1 group.
Percent changes: to the Day 1 group‡.

TABLE 2

EFFECTS OF CHRONIC ADMINISTRATION ON THE LEVELS OF ^{3}H-COCAINE AND METABOLITES IN THE RAT PLASMA

	nmole/ml		
	Cocaine	Norcocaine	Benzoylecgonine
Day 1 (40 mg/kg)	3.5 ± 0.08	1.9 ± 0.02	19.9 ± 0.47
Day 14 (40 mg/kg)	6.7 ± 0.05* (90% ↑)‡	3.2 ± 0.03* (68% ↑)‡	17.5 ± 0.56† (12% ↓)‡
Day 14 Posttreatment (40 mg/kg)	5.0 ± 0.06* (43% ↑)‡	2.2 ± 0.03* (16% ↑)‡	27.2 ± 0.80* (37% ↑)‡

Each value represents the mean ± SEM of duplicate samples from 6 rats.
*$p<0.001$, compared to the Day 1 group.
†$p<0.02$, compared to the Day 1 group.
Percent changes: to the Day 1 group ‡.

drug; the other half received saline. Ratings were made as described above.

Metabolic Studies

Three groups of 6 female Sprague-Dawley rats (180–200 g) were used for the studies. The first group received intraperitoneally 40 mg/kg of ^{3}H-labeled cocaine (4 μC/mg) in saline and was sacrificed 45 min later, under ether anesthesia. The second group received nonlabeled drug (40 mg/kg IP) daily for 13 days, followed by one administration of the labeled compound on the 14th day; the animals were sacrificed 45 min postinjection. The third group was injected with cocaine·HCl daily for 14 days. The drug treatment was discontinued for 13 days and a dose of the labeled compound was given to the rats on Day 14. Sacrificing was performed in the same manner as above.

Blood was collected in heparinized tubes, and the brain removed from each rat. Plasma was separated by centrifugation at 10,000 x g for 20 min at 4°C. Aliquots of plasma and brain homogenates, 20% in water, were assayed for radioactivity. Unchanged cocaine and metabolites in tissues were extracted and chromatographed as described in previously published procedures [5,16].

For inhibition studies, a group of 12 rats received cocaine·HCl (40 mg/kg, IP) in saline daily for 13 days. On the 14th day all animals received ^{3}H-cocaine·HCl. Half the rats were injected with SKF-525A (12.5 mg/kg, IP), 30 min prior to receiving the labeled drug. The other half received saline. Thirty min after receiving the ^{3}H-cocaine, all animals were sacrificed under ether anesthesia. The time for sacrificing was chosen to coincide with the peak of stereotypy observed in the chronically cocaine-treated animals (Fig. 2). Metabolic studies were carried out as described above. All data were analyzed for significance using Student's *t*-test.

RESULTS

For behavioral studies, data were divided into three groups for each set of animals: onset period, peak period and offset period, as fully described by Kilbey and Ellinwood [7]. Daily administration of cocaine for 14 days produced a significant increase in the cocaine-induced stereotypy both at the onset period (3–15 min, $p=0.014$) and at the peak period (20–45 min, $p=0.014$) (Fig. 1). This enhancement of behavior persisted 7 days after the discontinuation of treatment. The onset and offset periods remained unaltered 7 days after termination of the drug (Fig. 2).

The levels of unchanged labeled cocaine, norcocaine and benzoylecgonine in brain and plasma are shown in Tables 1 and 2.

Fourteen days of daily treatment resulted in an increase of 38%, 22% and 64% of labeled cocaine, norcocaine and benzoylecgonine, respectively, in brain over the levels observed after one dose of the drug. This increase was still observed on the 14th day after drug discontinuation, as the levels of the three compounds were significantly higher than those of the first day of treatment.

In plasma, changes in levels of labeled cocaine and norcocaine were similar, with a slight decrease of the two compounds on the 14th day of postdrug treatment with respect to the 14th day. However, benzoylecgonine levels were rather erratic at the two intervals.

The respective roles of cocaine and its metabolites in the enhancement of stereotypy could not be differentiated by brain levels of the compounds. SKF-525A was used to reduce cocaine metabolism in anticipation that any behavioral alteration would coincide with changes in concentration in the brain. Animals that received the inhibitor prior to the drug exhibited a significantly lower stereotypy rating ($p=0.029$) at the peak period (20–45 min) than those without SKF-525A pretreatment (Fig. 3). No statistical difference was found for the onset (3–15 min) or offset period (60–90 min). Metabolic studies with SKF-525A revealed that at 30 min the inhibitor produced a significant 28% increase of cocaine and a 25% and 19% decrease of norcocaine and benzoylecgonine, respectively, in brain (Table 3).

DISCUSSION

Our studies confirm the reports of Kilbey and Ellinwood

TABLE 3

INHIBITION OF ^{3}H-COCAINE METABOLISM BY SKF-525A IN CHRONIC RATS

Plasma	Pretreatment	nmole/g or nmole/ml		
		Cocaine	Norcocaine	Benzoylecgonine
Brain	Saline	27.5 ± 1.39	7.70 ± 0.31	6.25 ± 0.33
	SKF-525A	35.1 ± 1.38*	5.80 ± 0.21*	5.07 ± 0.29†
		(28% ↑)	(25% ↓)	(19% ↓)
Plasma	Saline	5.19 ± 0.45	2.45 ± 0.13	18.8 ± 0.96
	SKF-525A	6.27 ± 0.21	1.96 ± 0.07†	18.3 ± 0.8
		(21% ↑)	(20% ↓)	

Each value represents the mean ± SEM of duplicate samples from 6 rats. Significant levels from the respective controls: *$p<0.001$;†$p<0.02$. Values in parentheses are the percent changes from the control group.

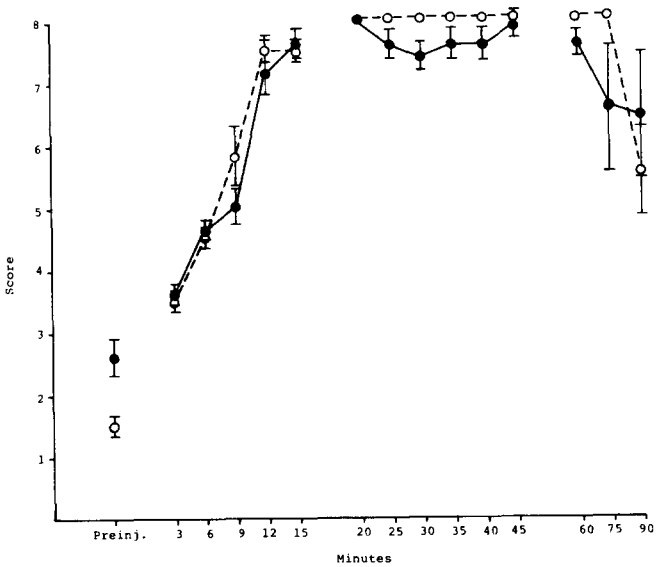

FIG. 3. Effects of SKF-525A on the stereotyped behavior in rats injected daily for 14 days with cocaine HCl (40 mg/kg, IP). Each value represents the mean score ± SEM of 5 animals. ●——●, the SKF-525A-pretreated group; ○——○, the saline group.

[7] and other investigators that repeated administration of high doses of cocaine enhances stereotyped behavior in animals. We have found this potentiation of behavior to coincide with an increase in brain levels of cocaine and its metabolites, norcocaine and benzoylecgonine, at the interval studied. This enhancement of behavior persisted 14 days after discontinuation of drug treatment.

We have previously demonstrated that chronic administration of cocaine causes alteration of the liver enzyme function, with a resultant increase in metabolites of cocaine in brain tissue [4]. In the case of the high convulsant dose (40 mg/kg) used in the present study, the concomitant increase of unchanged cocaine in the brain could be a result of saturation of metabolic enzyme systems beyond their capacity to metabolize the drug.

The effects of ether on cocaine metabolism have not been reported. However, the possibility that it might alter the drug disposition of the drug is considered minimal in this case, since animals were anesthetized for only one minute prior to sacrificing at 45 minutes postinjection. A decrease in the stereotypy rating with the use of the SKF-525A inhibitor, along with a significant decrease of norcocaine in brain, while cocaine levels remained elevated, indicates a major role for this metabolite in the production of stereotyped behavior. Stereotypy ratings for SKF-525A and control animals were not significantly different prior to receiving cocaine. Although repeated cocaine administration, as well as SKF-525A pretreatment, produced changes in benzoylecgonine and norcocaine levels in the brain, benzoylecgonine, in contrast to norcocaine, was behaviorally inactive when given systemically [6,8]. Further investigation is needed for determining the role of benzoylecgonine in the behavioral effects of cocaine.

In this work, the sacrificing time was chosen to coincide with the peak found for the behavioral effect, although this time may not coincide with the peak level of the drug or metabolites on each group under study. We had previously reported the enhancement of locomotor activity in rats chronically treated with cocaine and that the potentiation of this particular behavior corresponded to an increase in norcocaine and a decrease of cocaine in brain [4].

Ortiz [19] reported a difference in the disposition and elimination of ecgonine in male and female rats, whereas the levels of cocaine and benzoylecgonine in brain were not different. Female rats were chosen in the present study to duplicate conditions used in the work of Kilbey and Ellinwood [7], who initially observed the enhancement of stereotyped behavior upon chronic administration of cocaine.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. David Barnes, Mrs. Mary Beth Hansard and Mrs. Mary O'Brien for their technical assistance.

REFERENCES

1. Downs, A. W. and N. B. Eddy. The effects of repeated dose of cocaine in the rat. *J. Pharmac. exp. Ther.* **46:** 199, 1932.
2. Ellinwood, E. H., Jr. and R. L. Balster. Rating the behavioral effects of amphetamine. *Eur. J. Pharmac.* **28:** 35–41, 1974.
3. Estevez, V. S., B. T. Ho and L. F. Englert. Inhibition of the metabolism of cocaine by SKF-525A. *Res. communs. chem. pathol. Pharmac.* **17:** 179–182, 1977.
4. Estevez, V. S., B. T. Ho and L. F. Englert. Enhancement of stimulant activity in rats chronically treated with cocaine. *Res. communs Psychol. Psych. Behav.* **2:** 203–211, 1977.
5. Ho, B. T., D. L. Taylor, V. S. Estevez, L. F. Englert, and M. L. McKenna. *Cocaine and Other Stimulants.* New York: Plenum Press, 1977, p. 229.
6. Just, W. W. and J. Hoyer. The local anesthetic potency of norcocaine, a metabolite of cocaine. *Experientia* **33:** 70–81, 1977.
7. Kilbey, M. M. and E. H. Ellinwood, Jr. The effect of age-related factors on behavior induced by cocaine. *Life Sci.* **20:** 1847–1854, 1977.
8. Misra, A. L., P. K. Nayak, R. Bloch and S. J. Mulé. Estimation and disposition of [^{3}H]-benzoylecgonine and pharmacological activity of some cocaine metabolites. *J. Pharm. Pharmac.* **27:** 784–786, 1975.
9. Post, R. M. and R. T. Kopanda. Cocaine, kindling and reverse tolerance. *The Lancet* **1:** 409–410, 1975.
10. Post, R. M. and R. T. Kopanda. Cocaine, kindling and psychosis. *Am. J. Psychol.* **133:** 627–634, 1976.
11. Post, R. M., R. T. Kopanda and K. E. Black. Progressive effects of cocaine on behavior and central amine metabolism in rhesus monkeys. Relationship to kindling and psychosis. *Biol. Psychiatr.* **11:** 403–419, 1976.
12. Post, R. M. and H. Rose. Increasing effects of repetitive cocaine administration in the rat. *Nature* **260:** 731–732, 1976.
13. Shuster, L., G. Yu and A. Bates. Sensitization to cocaine stimulation in mice. *Psychopharmacology* **52:** 185–190, 1977.
14. Strippling, J. S. and E. H. Ellinwood, Jr. Potentiation of the behavioral and convulsant effects of cocaine by chronic administration in the rat. *Pharmac. Biochem. Behav.* **6:** 571–579, 1977.
15. Tatum, A. L. and M. H. Seevers. Experimental cocaine addiction. *J. Pharmac. exp. Ther.* **36:** 405, 1929.
16. Taylor, D., V. S. Estevez, L. F. Englert and B. T. Ho. Hydrolysis of carbon-labeled cocaine in human serum. *Res. communs. chem. pathol. Pharmac.* **14:** 249–257, 1976.
17. Nayak, P. K., A. L. Misra and J. S. Mulé. Physiological disposition and biotransformation of ^{3}H-cocaine in acutely and chronically treated rats. *J. Pharmac. exp. Ther.* **196:** 556–569, 1976.
18. Misra, A. L., R. B. Pontani and S. J. Mulé. [^{3}H]-Norcocaine and [^{3}H]-pseudococaine: Effect of N-demethylation and C_2-epimerization of cocaine on its pharmacokinetics in the rat. *Experientia* **32:** 895–897, 1976.
19. Ortiz, R. V. Estudio de la distribucion y metabolismo de la cocaine en la rata. *An. Fac. Quim. Farm. Univ. Chile* **18:** 15–19, 1966.