Pharmacology Biochemistry & Behavior, Vol. 41, pp. 227–230. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Cocaine Binding Sites in Mouse Striatum, Dopamine Autoreceptors, and Cocaine-Induced Locomotion

MAARTEN E. A. REITH[1] AND GABOR SELMECI

Division for Neurochemistry, N. S. Kline Institute for Psychiatric Research
Building 39, Oak and Plaza Streets, Orangeburg, NY 10962

Received 28 May 1991

REITH, M. E. A. AND G. SELMECI. *Cocaine binding sites in mouse striatum, dopamine autoreceptors, and cocaine-induced locomotion.* PHARMACOL BIOCHEM BEHAV **41**(1) 227–230, 1992.—BALB/cByJ mice received cocaine (25 mg/kg IP) once a day for 3 days, resulting in a greater locomotor response to cocaine on day 3 than on day 1. On day 4, a dose (0.03 mg/kg SC) of apomorphine, targeted at dopamine autoreceptors, caused the same degree of locomotor depression in cocaine- as in saline-pretreated mice. In addition, no change was found in either the affinity or density of cocaine binding sites in their striatum as measured by the binding of [^{3}H]CFT. C57BL/6ByJ mice displayed a greater locomotor response to cocaine than BALB/cByJ mice, but had the same number of striatal [^{3}H]CFT binding sites with the same affinity. Factors other than striatal cocaine binding sites, or dopamine autoreceptors as measured by apomorphine-induced depression of locomotion, should be considered for the explanation of the enhancement of the locomotor response upon daily cocaine administration in BALB/cByJ mice, or for the different locomotor response to cocaine of this strain compared with the C57BL/6ByJ strain of mice.

Cocaine Locomotion Cocaine binding Dopamine autoreceptors Mouse striatum

COCAINE administration to rodents stimulates locomotor behavior, and this effect increases over time with repeated intermittent doses of moderate size (11, 14, 15). The locomotor response to acute administration of cocaine varies in different strains of rats (5) and mice (6, 21, 22, 24). In addition, different mouse strains respond differently to subchronic treatment with cocaine (19,22). Current theories implicate mesencephalic dopaminergic systems in cocaine's stimulatory activity (4,25); this effect is associated with blockade of the neuronal uptake of dopamine by binding of cocaine to the dopamine transporter (12, 16, 20). Therefore, cocaine binding sites are potential candidates for mediators of the differences in the responsiveness to cocaine between strains, or the sensitization following repeated cocaine administration. Additional candidates are dopamine autoreceptors, known to be subject to regulation by cocaine administration depending on the dosage schedule and functional assay employed (1, 3, 9, 26).

The present study focusses on cocaine binding sites and dopamine autoreceptors in two mouse strains, the BALB/cByJ and C57BL/6ByJ. Enhancement of the locomotor effect of cocaine is produced by daily injection of cocaine for three days according to our previously described model (15); on day 4 the animals are examined for either the binding of [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)-tropane (also known as WIN 35,428 or CFT), a cocaine analog that binds with high affinity to dopamine uptake sites (12), or the locomotor depressing effect of a low dose of apomorphine, selectively targeted at dopamine autoreceptors (2,17).

METHOD

Animals

The experiments were conducted in the previous location of our Institute on Ward's Island, NY 10035. Male BALB/cByJ and C57BL/6ByJ mice were obtained from the laboratory's own breeding colonies derived from the stocks of Jackson Laboratories (Bar Harbor, ME). "Adult" BALB/cBy mice (weight 21–28 g) and "adult" C57BL/6ByJ mice (weight 23–28 g) were 3 months old. "Aged" C57BL/6ByJ mice (weight 28–35 g) were 16 months old. The animals were held on a 12-h light/dark cycle (7 a.m./7 p.m.), with food and water available ad lib.

Plan of Experiments

In the experiments with repeated administration, BALB/cByJ mice, housed individually, received 25 mg/kg IP of cocaine hy-

[1]Requests for reprints should be addressed to M. E. A. Reith at his present address: University of Illinois Medical College at Peoria, Department of Basic Sciences, Box 1649, Peoria, IL 61656.

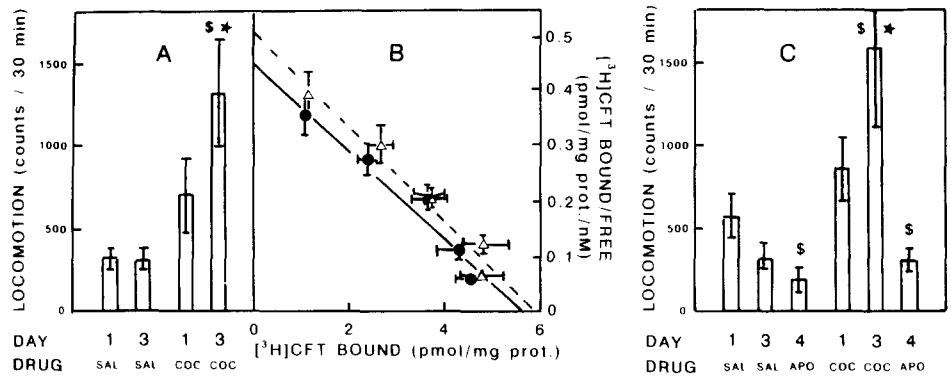

FIG. 1. Binding of [^{3}H]CFT to striatal membranes and locomotor response to apomorphine following three days treatment with cocaine or saline. Mice received once a day cocaine (25 mg/kg IP) or saline for 3 days and locomotion was measured on day 1 and 3 (panel A and C). On day 4, [^{3}H]CFT binding was measured (panel B) or the locomotor depression following apomorphine (0.03 mg/kg SC) (panel C). Locomotor data reflect postinjection activity measured for 30 min. The data shown in panels (A) and (B) are from one set of animals, the data in panel (C) from another set. Values are mean ± S.E.M. (vertical or horizontal bar, not shown when smaller than the data point itself) for 8–11 animals. Abbreviations used are: SAL, saline, COC, cocaine, and APO, apomorphine. *$p<0.02$ compared with COC on day 1 (2-tailed Wilcoxon signed-ranks test), †$p<0.02$ compared with preinjection locomotor counts on same day (2-tailed Wilcoxon signed-ranks test).

drochloride (Sigma, St. Louis, MO) (dissolved in saline, 0.15 ml per 20 g body weight) once a day for three days. Control animals received saline. Locomotor activity was monitored for 30 min before and 30 min after injection. On day 4, twenty-four h after the last injection, animals were either sacrificed for the determination of [^{3}H]CFT binding to striatal membranes, or received a challenge injection of 0.03 mg/kg SC of apomorphine (Sigma Chemical Co., St. Louis, MO) (dissolved in warm H_2O and diluted with saline) (0.15 ml per 20 g bodyweight) for the measurement of locomotor depression.

In the experiments with acute administration, adult or aged C57BL/6ByJ or BALB/cByJ mice were injected with either cocaine (25 mg/kg IP) or saline and monitored for locomotor activity for a period of 50 min.

Spontaneous Locomotor Activity and [^{3}H]CFT Binding

The detailed procedures for measuring locomotion of individually housed mice have been described elsewhere (17–19). Only consecutive interruptions of the infrared beams of the Opto-Varimex-Minor activity monitors (Columbus Instruments, Columbus, OH) were counted and totaled for 10-min periods. As reported previously (17–19) no development of stereotyped behavior was observed in the mice under the conditions used.

In the experiment involving repeated cocaine and saline pretreatment, striatal tissue from each animal was dissected and placed in 0.32 M sucrose. The following steps were as described by Hardy et al. (8) for optimal preservation of monoamine uptake sites. Briefly, the samples were frozen slowly at −20°C, and then transferred to a −80°C freezer. After 6–10 days, samples were thawed rapidly. Four Scatchard analyses were run each day on two samples from cocaine-pretreated mice and two samples from saline-pretreated mice. In the experiment comparing different strains and ages, fresh striatal tissue was used. Downscaled binding assay conditions permitted a Scatchard analysis on striatal tissue from each mouse separately, except for the analysis of the 7- and 18-day-old mice that required pooling of striatal tissue from two animals. The tissue was homogenized in 15 volumes of ice cold 0.32 M sucrose in a glass homogenizer with a motor driven Teflon pestle. The homogenizer and pestle were rinsed with 30 volumes 0.32 M sucrose. This fluid and the homogenate were combined and centrifuged at 1,000 × g for 10 min at 0–4°C. The supernatant fraction was subsequently centrifuged at 17,000 × g for 20 min. The resulting pellet (P_2) was homogenized in ice cold 25 mM sodium phosphate (48 mM Na^+, pH 7.7) (approximately 0.08 ml per mg initial tissue weight), with a Brinkmann Polytron (setting 6, 15 s). Portions of the P_2 preparation (50 μl, approximately 40 μg of protein) were incubated in triplicate at 0–4°C for 2 h with 3 nM [^{3}H]CFT (79.4 Ci/mmol, Dupont-New England Nuclear) and varying concentrations (0, 1.2, 3, 7.5, 15 nM) of unlabeled CFT naphthalene sulfonate (Research Biochemicals Inc., Natick, MA) in a total volume of 200 μl in 1-ml ministrip tubes of Skatron Inc. (Sterling, VA). Binding assays were terminated by the addition of 1 ml of ice cold buffer (same as used in assay) and rapid filtration over Skatron receptor binding filtermats (pretreated with 0.05% w/v poly-L-lysine) with a miniharvesting apparatus (Type 11021, Skatron Inc.). The filters were washed with three 1-ml portions of ice cold buffer and assayed for radioactivity by liquid scintillation counting (18). Protein was estimated by the method of Lowry et al. as described previously (16). Nonspecific binding was defined with 30 μM cocaine, and was approximately 1.5% of total binding. Binding to filters was negligible.

Data Analysis and Statistics

Equilibrium binding data were analyzed with the nonlinear computer fitting program LIGAND of Munson and Rodbard [see ref. in Reith et al. (16)] (Elsevier-Biosoft, Cambridge, UK). For parametric tests, locomotor data were subjected to logarithmic transformation for homogeneity of variance. To compare the shape of the time course of cocaine-induced activity of two groups measured for 50 min, two-way ANOVA was performed with the pretreatment and activity in each 10-min segment (% of total activity) as the two factors. As described previously (18), the interaction term was used as a statistical indicator for a dif-

TABLE 1

COCAINE-INDUCED LOCOMOTION AND BINDING OF [^{3}H]CFT TO STRIATAL MEMBRANES IN BALB/CBYJ AND C57BL/6BYJ MICE

	Locomotion*		[^{3}H]CFT Binding	
	Saline	Cocaine	K_d (nM)	B_{max} (pmol/mg prot.)
Group	(counts per 50 min)			
7-day C57			10 ± 0.5	0.58 ± 0.14‡(2)
18-day C57			14 ± 2.2	1.79 ± 0.18‡(2)
Adult C57	780 ± 185 (16)	2863 ± 316 (19)	13 ± 1.2	3.76 ± 0.36 (4)
Aged C57	544 ± 124 (17)	2654 ± 408 (19)	10 ± 0.7	3.66 ± 0.13 (6)
Adult BALB	850 ± 155 (16)	1609 ± 362†(19)	13 ± 0.3	3.89 ± 0.08 (4)

C57BL/6ByJ (C57) and BALB/cByJ (BALB) mice received cocaine (25 mg/kg IP) or saline and were monitored for locomotor activity for 50 min. Separate animals were used for the determination of [^{3}H]CFT binding to their striatal membranes. Values are mean ± S.E.M. for the number of animals given between parentheses. Only in the case of 7- and 18-day-old mice, striatal tissue of 2 animals was pooled and the average reflects the data for 2 of such independent preparations.

*$F(1,100) = 40.52$, $p<0.0001$ (drug factor in two-way ANOVA); $F(2,100) = 1.33$, $p=0.27$ (animal group factor); $F(2,100) = 5.312$, $p<0.01$ (interaction).

†$p<0.05$ compared with adult or aged C57 (Newman-Keul's test following one-way ANOVA).

‡$p<0.01$ compared with all other groups (Newman-Keul's test following one-way ANOVA).

ference in the shape of the curves; for multiple comparisons between three groups the p value was multiplied by 3 according to the Bonferroni method.

RESULTS AND DISCUSSION

Repeated Cocaine Administration, Locomotor Activation, and [^{3}H]CFT Binding

Animals that received repeated cocaine injections produced more locomotor counts following the injection on day 3 than on day 1 ($p<0.02$), in contrast to animals treated with saline (Fig. 1A). There were no statistically significant differences in the preinjection locomotor counts between days or treatment groups (data not shown). An increase in the locomotor stimulatory effect of cocaine upon its repeated administration has been observed previously (11, 14, 15, 19, 22); in the BALB/cBy mice under the conditions used in this study, the locomotor activity is as high on day 4 as on day 3, or even higher (15). With the use of one challenged dose of cocaine, as in the present report, no distinction can be made between horizontal and vertical shifts in the dose-response curve.

Striatal tissues dissected one day after the last injection bound [^{3}H]CFT with a K_d of 12.4±0.7 nM (mean±S.E.M. for 8 saline-pretreated animals) and 11.5±0.6 nM (for 9 cocaine-pretreated animals), and a B_{max} of 5.6±0.5 pmol/mg of protein (saline) and 5.9±0.6 pmol/mg of protein (cocaine) (Fig. 1B). Under the present conditions, [^{3}H]CFT binding was homogeneous, reflecting a single site; analysis with the LIGAND program could not detect multiple components in experiments with [^{3}H]CFT ranging from 0.1 to 80 nM, or 3 nM [^{3}H]CFT with cold CFT varying from 0 to 1.5 μM, (manuscript submitted) in contrast to the experiments of Madras et al. (12) on monkey striatal membranes. Therefore, saturation curves with only 5 points were considered appropriate in the present study for the estimation of the single site parameters. There was no effect of repeated daily cocaine injections on [^{3}H]CFT binding to striatal membranes of mice that did show an enhancement of cocaine's locomotor stimulatory effect. This is reminiscent of the lack of effect of cocaine administration (once a day for 8 days) on striatal binding of [^{3}H]nomifensin, another ligand for dopamine uptake sites, measured 24 h after the last injection in rats that showed sensitization to the effect of cocaine on locomotor and stereotyped behavior (13). It appears that enhancement of locomotor activation can occur without a concomitant change in binding sites for dopamine uptake blockers including the cocaine analog CFT in the striatum. The present experiments do not address the possibility that dopaminergic systems in nigrostriatal and mesolimbic pathways are regulated in a different manner. Some evidence in support of this has been reported for dopamine uptake sites, but accompanying behavioral data are lacking (10).

Repeated Cocaine Administration, Locomotor Activation, and Apomorphine-Induced Locomotor Depression

As in the above experiment, repeated treatment with cocaine resulted in an increase in its locomotor stimulatory effect ($p<0.02$) as opposed to treatment with saline (Fig. 1C). When all animals were challenged with apomorphine (0.03 mg/kg) 24 h after the last cocaine or saline injection, a significant depression of locomotion occurred ($p<0.02$), but there was no statistically significant difference between cocaine- and saline-pretreated mice (Fig. 1C). The same conclusion was drawn from the apomorphine data expressed as a ratio of postinjection over preinjection locomotor counts: the cocaine group had a ratio (mean±S.E.M.) of 0.67±0.14 (n=11), and the saline group 0.48±0.16 (n=9) ($p=0.21$, Mann-Whitney U-test). There were no differences in the preinjection locomotor counts between day 1, 3, and 4, or between treatment groups (data not shown).

The dose of apomorphine, 0.03 mg/kg, was chosen based on a dose-response curve determined in separate animals: at 0.01 mg/kg the postinjection over preinjection ratio of locomotor counts (mean±S.E.M. for 8 mice) was 0.90±0.25, at 0.03 mg/kg 0.33±0.08, and at 0.1 mg/kg 0.28±0.15. Thus it was reasoned that subsensitivity of dopamine autoreceptors as measured with 0.03 mg/kg of apomorphine, if it occurred, would be detectable. The present results did not give strong support for

such a phenomenon, although full dose-response curves for apomorphine would be necessary to firmly rule out a subsensitivity to the compound. There was no indication for supersensitivity in the present data in contrast to the findings reported in a preliminary presentation of similar experiments with apomorphine-challenged rats (23); differences in time schedules, environmental context, and species may all be important. In addition, the possibility should be considered that the locomotor depression following a low dose of apomorphine does not reflect its exclusive action at dopamine autoreceptors.

Different Strains and Ages, Cocaine-Induced Locomotion, and [^{3}H]CFT Binding

Adult C57BL/6ByJ, aged C57BL/6ByJ, and adult BALB/cByJ mice did not differ in their locomotor response to saline, but showed a different sensitivity to cocaine; BALB/cBy mice were less responsive to cocaine than C57BL/6ByJ mice (Table 1). Aging in the C57 animals did not change the responsiveness as measured by total locomotor counts 50 min following cocaine. However, analysis of time courses revealed significant differences in their shapes between those for aged C57 mice on the one hand and adult C57 [interaction term pretreatment × time in two-way ANOVA, $F(4,180)=3.80$, $p<0.02$] or adult BALB/c, $F(4,180)=4.19$, $p<0.01$, mice on the other hand, with the peak of activity occurring in the 20- to 30-min segment for aged animals and in the 0- to 10-min segment for adult mice.

The relatively lower responsiveness in BALB/cByJ mice was not correlated with [^{3}H]CFT binding to their striatal membranes (Table 1). In fact, the K_d and B_{max} of this binding was the same in adult BALB/cByJ and adult C57B/6ByJ mice, and did not change upon aging. The density of binding sites was dependent upon the stage of development with the lowest value measured in 7-day-old mice and intermediate values in 18-day olds (Table 1).

The difference between BALB/cByJ and C57BL/6ByJ mice in their locomotor response to cocaine appears to be accounted for by a factor other than the number of cocaine binding sites or their affinity in the striatum. A variable to be considered is the pharmacokinetics of cocaine resulting in different brain levels of cocaine in the two strains (24). Similar to the present results, the different locomotor response of the SS and LS mouse strains to cocaine was not paralleled by a difference in the binding of [^{3}H]mazindol to dopamine uptake sites in their striatum (6). Of course, the possibility exists that changes during or after treatment with cocaine occur in systems other than dopaminergic circuitries, such as GABAergic (7) or serotonergic (5, 18, 19) pathways.

ACKNOWLEDGEMENT

Supported by USPHS grant DA 03025.

REFERENCES

1. Ackerman, J. M.; White, F. J. A10 somatodendritric dopamine autoreceptor sensitivity following withdrawal from repeated cocaine treatment. Neurosci. Lett. 117:181–187; 1990.
2. Dichiara, G.; Porceddu, M. L.; Vargiu, L.; Argiolas, A.; Gessa, G. L. Evidence for dopamine receptors mediating sedation in the mouse brain. Nature 264:564–567; 1976.
3. Dwoskin, L. P.; Peris, J.; Yasuda, R. P.; Philpott, K.; Zahniser, N. R. Repeated cocaine administration results in supersensitivity of striatal D-2 dopamine autoreceptors to pergolide. Life Sci. 42:255–262; 1988.
4. Gawin, F. H. Cocaine addiction: Psychology and neurophysiology. Science 251:1580–1586; 1991.
5. George, F. R. Cocaine produces low dose locomotor depressant effects in NBR and F344 rats. Pharmacol. Biochem. Behav. 37:795–798; 1990.
6. George, F. R.; Ritz, M. C. Cocaine produces locomotor stimulation in SS but not LS mice: Relationship to dopaminergic function. Psychopharmacology (Berlin) 101:18–22; 1990.
7. Goeders, N.; Bell, V.; Guidroz, A.; McNulty, M. Dopaminergic involvement in the cocaine-induced upregulation of benzodiazepine receptors in the rat caudate nucleus. Brain Res. 515:1–9; 1990.
8. Hardy, J. A.; Dodd, P. R.; Oakley, A. E.; Perry, R. H.; Edwardson, J. A.; Kidd, A. M. Metabolically active synaptosomes can be prepared from frozen rat and human brain. J. Neurochem. 40:608–614; 1983.
9. Henry, D. J.; Greene, M. A.; White, F. J. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Repeated administration. J. Pharmacol. Exp. Ther. 251:833–839; 1989.
10. Izenwasser, S.; Cox, B. M. Daily cocaine treatment produces a persistent reduction of [^{3}H]dopamine uptake in vitro in rat nucleus accumbens but not in striatum. Brain Res. 531:338–341; 1990.
11. Kalivas, P. W.; Duffy, P.; Dumars, L. A.; Skinner, C. Behavioral and neurochemical effects of acute and daily cocaine administration in rats. J. Pharmacol. Exp. Ther. 245:485–492; 1988.
12. Madras, B. K.; Spealman, R. D.; Fahey, M. A.; Neumeyer, J. L.; Saha, J. K.; Milius, R. A. Cocaine receptors labeled by [^{3}H]2β-carbomethoxy-3β-(4-fluorophenyl)tropane. Mol. Pharmacol. 36:518–524; 1989.
13. Peris, J.; Boyson, S. J.; Casss, W. A.; Curella, P.; Dwoskin, L. P.; Larson, G.; Lin, L. H.; Yasuda, R. P.; Zahniser, N. R. Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J. Pharmacol. Exp. Ther. 253:38–44; 1990.
14. Post, R. M.; Contel, N. R. Human and animal studies of cocaine: Implications for development of behavioral pathology. In: Creese, I., ed. Stimulants: Neurochemical, behavioral, and clinical perspectives. New York: Raven Press; 1983:169–203.
15. Reith, M. E. A. Effect of repeated administration of various doses of cocaine and WIN 35,065-2 on locomotor behavior of mice. Eur. J. Pharmacol. 130:65–72; 1986.
16. Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem. Pharmacol. 35:1123–1129; 1986.
17. Reith, M. E. A.; Lajtha, A. Locomotor depression in mice by norcocaine does not involve central α_2-adrenergic or presynaptic dopamine receptors. Pharmacol. Biochem. Behav. 24:305–307; 1986.
18. Reith, M. E. A.; Wiener, H. L.; Fischette, C. T. Sertraline and cocaine-induced locomotion in mice. I. Acute studies. Psychopharmacology (Berlin) 103:297–305; 1991.
19. Reith, M. E. A.; Fischette, C. T. Sertraline and cocaine-induced locomotion in mice. II. Chronic studies. Psychopharmacology (Berlin) 103:306–313; 1991.
20. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223; 1987.
21. Ruth, J. A.; Ullman, E.; Collins, A. C. An analysis of cocaine effects on locomotor activities and heart rate in four inbred mouse strains. Pharmacol. Biochem. Behav. 29:157–162; 1988.
22. Shuster, L.; Yu, G.; Bates, A. Sensitization to cocaine stimulation in mice. Psychopharmacology (Berlin) 52:185–190; 1977.
23. Weiss, F.; Hurd, Y. L.; Ungerstedt, U.; Koob, G. F. Repeated cocaine administration potentiates the hypomotility effects of apomorphine. Soc. Neurosci. Abstr. 15:250; 1989.
24. Wiener, H. L.; Reith, M. E. A. Correlation between cocaine-induced locomotion and cocaine disposition in the brain among four inbred strains of mice. Pharmacol. Biochem. Behav. 36:699–701; 1990.
25. Wise, R.; Bozarth, M. A. A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469–492; 1987.
26. Yi, S. J.; Johnson, K. M. Chronic cocaine treatment impairs the regulation of synaptosomal ^{3}H-DA release by D_2 autoreceptors. Pharmacol. Biochem. Behav. 36:457–461; 1990.