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BRIEF COMMUNICATION

Enhanced Locomotor Reactivity to Apomorphine Following Repeated Cocaine Treatment

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KIYATKIN, E. A. *Enhanced locomotor reactivity to apomorphine following repeated cocaine treatment.* PHARMACOL BIOCHEM BEHAV **49**(1) 247–251, 1994.—To study the involvement of postsynaptic dopamine (DA) receptors in cocaine-induced behavioral sensitization, locomotor responses to apomorphine (APO, 360 μg/kg, IP), a direct DA agonist, were compared in rats repeatedly treated with cocaine (15 mg/kg, IP × 5 and one challenge injection 4 days later) and saline under different environmental conditions. When cocaine was injected under activated conditions, immediately before animal placement in activity chamber, more powerful locomotor response to the initial drug and a significant effect of repeated treatment (sensitization) were found compared to drug administration under quiet conditions, 2 h after animal placement in activity chamber. In this case, locomotor response to the initial drug was similar to that of saline, and the effect of repeated injections on locomotion was absent (no sensitization). Locomotor stimulation induced by APO was significantly enhanced in both groups of cocaine-treated animals compared to saline controls. This effect, however, was more powerful in animals treated with cocaine under activated conditions. Present data suggest that enhanced responsiveness of postsynaptic DA receptors developed due to chronic cocaine treatment may contribute to stable alteration of DA transmission thought to mediate cocaine sensitization.

Cocaine Behavioral sensitization Dopamine receptors Receptor plasticity Adaptation

THE locomotor stimulation and stereotyped behavior produced by cocaine and other psychostimulants are progressively enhanced or sensitized following repeated drug administration [for review, see (15)]. An increase in mesocorticolimbic dopamine (DA) transmission has been proposed as the main mechanism mediating this phenomenon (15,23,26). Subsensitivity of impulse-regulating somatodendritic (1,9) and supersensitivity of release-modulating axonal terminal DA autoreceptors found in cocaine-treated animals (4,8,21) may be responsible for hyperreactivity of DA neurons and the more intense increases in DA concentration induced by subsequent drug injections (2,14). However, several reports provide evidence for desensitization of the same release-modulating autoreceptors (7,13). In our previous study, significant changes in nucleus accumbens DA-dependent electrochemical signal were found to accompany repeated cocaine injections (17). These data suggest that hyperesponsiveness of mesolimbic DA cells is developed following repeated cocaine treatment. Augmentation of the behavioral effects of cocaine may also be associated also with hyperresponsiveness of postsynaptic DA receptors, which bind released DA. Upregulation of postsynaptic D_1 receptors was shown in an electrophysiological study (9,10), although no change in quantity and affinity of D_1 receptors has been found in radioreceptor studies (18,21).

To assess the involvement of a postynaptic component regulating DA transmission in the mediation of cocaine-induced

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behavioral sensitization, the locomotor stimulatory effects of the direct DA agonist apomorphine (APO), used at a dose sufficient to activate postsynaptic D_1 receptors (3,12), were compared in rats previously repeatedly administered either cocaine or saline. Because the locomotor stimulatory effects of cocaine and intensity of their changes following repeated drug treatment depend significantly upon the environmental conditions of drug administration (11,22), responses to APO were evaluated in rats treated with cocaine under different environmental conditions.

METHOD

Experiments were performed on 24 Long-Evans male rats (Charles River, St.-Constant, Quebec) weighing 270–290 g. Horizontal locomotion was measured in individual activity chambers (42 × 30 × 20 cm; two pairs of photobeams at the level of 3.5 cm from cage floor) under indirect light. Treatment regimen (cocaine 15 mg/kg or saline, IP; five repeated injections once a day for 5 days and the challenge injection 4 days later) was the same in the following two experiments. In Experiment 1, cocaine and saline injections were performed under quiet conditions, 2 h after animal placement in the activity chamber (acclimated group). In experiment 2, rats were injected with cocaine and saline immediately before their placement in the activity chamber (nonacclimated group). Duration of each session was 4 h. The animals of both groups were returned to their home cages between tests. The changes in locomotor stimulatory effects of cocaine and saline (total amount of counts for 60 min after injection) were analyzed using a one-way ANOVA for repeated measures. The between-group differences in locomotion were compared using a Mann–Whitney *U*-test.

Apomorphine HCl (APO, 360 μg/kg, IP dissolved in saline; Sigma, MO) was injected in rats of all four groups on the day 12 (2 days after the last cocaine or saline injection) under quiet conditions, 2 h after their placement in activity chambers. The between-group differences in responses to APO were analyzed using subsequent ten min values of counts and total amount of counts for 10–70 min after drug injection (approximate duration of APO effect).

RESULTS

As can be seen in Fig. 1, repeated injections of cocaine in both experimental conditions resulted in the enhancement of the drug's effectiveness to stimulate locomotion (significant differences against saline). When cocaine was injected under quiet conditions (A), locomotor stimulation to the initial drug was not significantly different vs. the response to saline, and there was no significant effect of repeated treatment on locomotion changes [$F(6, 41) = 1.31$, $p = 0.29$]. However, significant changes in the time course of locomotor activation induced by cocaine, a quick start and early peak, were found in drug-treated rats (Fig. 2A). In contrast, in animals that were repeatedly treated with cocaine under behaviorally activated conditions (B) locomotor response to the initial drug was significantly higher compared to saline control and drug injection under quiet conditions. In addition, an enhancing effect of repeated treatment was significant [$F(5, 35) = 3.47$, $p < 0.001$], with locomotor response to cocaine challenge significantly higher compared to the initial drug effect. As can be seen in Fig. 2B, locomotor enhancing effect of repeated treatment was maximal during the first 20 min after cocaine injection.

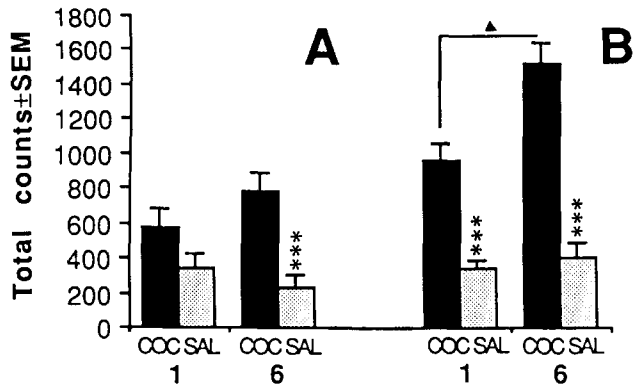

FIG. 1. Magnitude of locomotion responses (total activity counts for 60 min ± SEM) induced by the first [1] and sixth [6] cocaine (C, black columns) or saline (S, hatched columns) injections performed under quiet (A) and activated (B) conditions. ***Significance of differences between cocaine and saline-treated rats ($p < 0.001$; Mann-Whitney *U*-test). $^{+}p < 0.01$; Scheffe *F*-test.

Locomotion response induced by APO was significantly enhanced in rats of both groups previously treated with cocaine compared to saline-treated controls (Fig. 3). In rats injected with cocaine under quiet conditions (A), locomotor enhancement was significant at 50–70 min after APO injection, although there were no significant differences between cocaine- and saline-treated rats in total amount of counts. In animal treated with cocaine under activated conditions (B), locomotor enhancement was significant at 30–40 min as well as a difference between cocaine and saline groups in total amount of counts ($p < 0.01$).

DISCUSSION

The present data indicate that repeated cocaine administration results in an enhancement of the drug's effectiveness to stimulate behavior and a more intense locomotor responsivity to the direct acting DA agonist APO. Taking into account that locomotion induced by APO in a high dose (>100 μg/kg) is mediated by activation of postsynaptic DA receptors (3,12), these data may suggest that the enhanced effectiveness of cocaine to stimulate behavior developed with repeated treatment is associated with increased sensitivity of postsynaptic DA receptors.

Our finding seems contradict to previous two reports (5,6), suggesting the decreased responsiveness to APO after repeated cocaine treatment. Obviously, the differences in both cocaine treatment regimen (20 mg/kg for 9 days and 120 mg/kg for 10 days, respectively), time interval between termination of cocaine treatment and APO test (24 h and 3 days), dose of apomorphine (1.0 and 2.0 mg/kg with no effect at 0.25 and 0.5 mg/kg), and the analyzed parameter (stereotypic licking and gnawing scores) compared to our protocol may account to these differences in results.

It is well known that APO is a mixed D_1 and D_2 agonist. At low doses (<100 μg/kg), APO activates D_2 somatodendritic and terminal autoreceptors, thus inhibiting the DA cells and decreasing DA release (3,27,28). This depressive action on DA transmission is considered the main mechanism for the locomotor inhibition produced by low dose APO. Therefore, decreased responsiveness of somatodendritic, as well as both

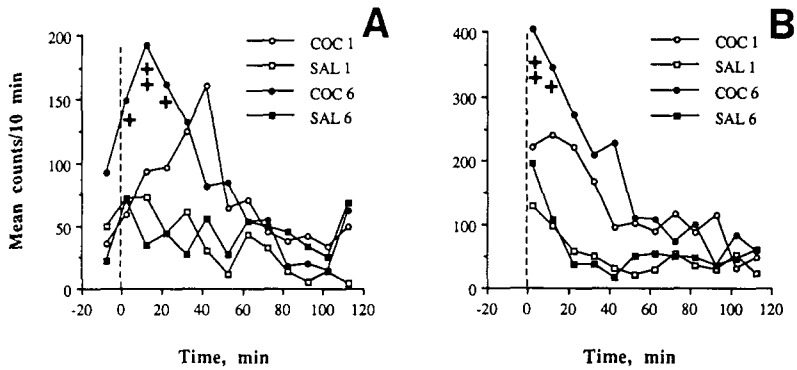

FIG. 2. Time course of locomotor activation (mean counts/10 min) induced by the first and six cocaine or saline injections performed under quiet (A) and activated (B) conditions. White circle = first cocaine, black circle = sixth cocaine, white square = first saline, black square = sixth saline. Hatched line represent the time of injections. $^{+,++}$Significance of differences between the initial and challenge cocaine with $p < 0.05$ and $p < 0.01$, respectively (Mann-Whitney U-test).

increased (1,8,9,21) or decreased (7,13) responsiveness of terminal autoreceptors found in chronic cocaine-treated animals cannot explain the enhanced locomotion seen following high dose APO in the present experiments. Alternatively, the most parsimonious explanation for enhanced responsiveness to APO is upregulation of postsynaptic D_1 receptors. Although it is impossible to exclude the involvement of other postsynaptic DA receptors [D_2 see: (3)], their influence on locomotion may be only indirect through modulating the DA cell firing rate and DA release. Because both the impulse activity and terminal DA release are strongly inhibited by APO even at low doses (27,28), changes in presynaptic DA receptors cannot explain the enhancement in APO-induced locomotion seen in cocaine-treated rats. It is obviously impossible to explain enhanced locomotion as a consequence of enhanced animal reactivity to the procedure of injection (as a conditioned, drug-related stimulus). In animals repeatedly injected with cocaine under quiet conditions, when the injection was the event preceding the drug's effect, an atypical bimodal response to APO was found. An immediate increase in locomotion seen in cocaine-treated rats after APO injection (0–20 min) may be partly or exclusively enhanced response to the procedure of injection as a cocaine-related cue (conditioned locomotion). However, significant locomotor enhancement was evident vs. saline control at 50–70 min after APO injection. In rats treated with cocaine under activated conditions, the influence of the injection as a conditioned stimulus was eliminated, because the drug's effect developed as an association to the orienting locomotor response to novelty. In this case, the curve of APO-induced locomotion had a normal unimodal shape, and the values in cocaine-treated rats were significantly higher (30–40 min) compared to control animals.

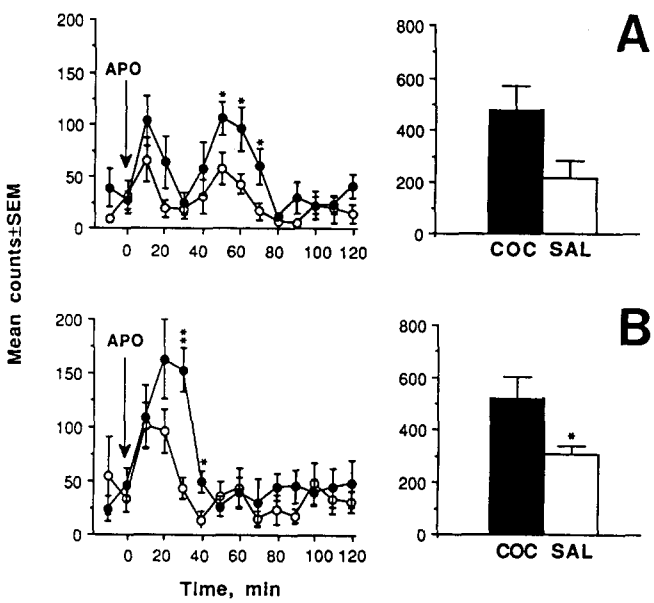

FIG. 3. Time-course (left, counts/10 min ± SEM) and magnitude of locomotor response (right, counts/from 10 to 70 min ± SEM) caused by apomorphine (APO 360 μg/kg) in rats previously treated with cocaine under quiet (A) and activated (B) conditions. Black circles = cocaine-treated rats, white circles = saline-treated rats. *, **Significance of between-group differences with $p < 0.05$ and $p < 0.01$, respectively (Mann-Whitney U-test). Arrow shows the moment of APO injection.

Differences in treatment conditions can best explain differences found in both the initial effect of cocaine as well as in dynamic changes in locomotion seen following repeated administrations. When cocaine was injected in nonacclimated conditions (drug + activated state associated with an animal's placement from home to the test cage), the locomotor response to the initial drug was significantly higher vs. saline and cocaine injected under quiet wakefulness. Apparently, a combination of the drug's effect with orienting response to novelty and stress of handling resulted in the development of enhanced responses to cocaine seen in these conditions (sensitization). A weaker response to the initial cocaine injection and a nonsignificant effect of repeated treatment (no sensitization) were found when cocaine was injected to acclimated animals under quiet conditions. Based on the correlation found between the intensities of locomotion elicited by cocaine and APO, it appears reasonable to suppose an interrela-

tion between these changes. More powerful behavioral activation to cocaine injections (nonacclimated conditions) may be associated with more prominent changes in DA receptors and vise versa (acclimated conditions). Hence, our observations may suggest that not only the drug's dose, duration of treatment, and type of drug administration, but the environmental context and animal's functional state associated with drug's effect, are the reasons for compensatory changes in receptors developed due to repeated cocaine treatment.

The fact that compensatory changes in postsynaptic DA receptors may contribute to enhanced locomotor reactivity to cocaine developed following repeated treatment is in general agreement with the literature. First, high responsiveness of striatal and nucleus accumbens cells to microiontophoretic applications of selective D_1 agonists has been found in cocaine-treated rats (10). This effect was observed even 1 month after termination of cocaine treatment, while the changes in D_2 autoreceptors were found to be short-lasting, disappearing several days after the last drug injection (1,19). These temporal differences are especially important, because numerous observations have shown that behavioral sensitization to cocaine is a long-lasting phenomenon and should be mediated by long-term changes in receptors (23). Second, some microdialysis data does not support the hypothesis on enhanced DA release elicited by cocaine as a main mechanism of behavioral sensitization (25). Third, both animal (7,16,20,24) and human studies (29) suggest that repeated cocaine treatment may result in a decreasing DA concentration in the target fields of mesocorticolimbic DA cells. These findings not only strongly support the hypothesis on an enhanced responsiveness of postsynaptic DA receptors developed following repeated cocaine treatment, but suggest that these receptor changes may be the compensatory mechanism to normalize the decreased DA release.

Apparently, adaptive changes in DA receptors is an essential but not the main mechanism mediating behavioral sensitization phenomenon. Results of our previous electrochemical study (17) suggest that an increases in nucleus accumbens DA levels occur with repeated injections more rapidly, reach peak quicker and become stronger compared to the initial drug administration. Thus, combined changes in both responsiveness of DA cells with more acute and pronounced DA release and DA receptors may be responsible for dramatic alterations in DA neurotransmission thought to mediate cocaine-induced behavioral sensitization.

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REFERENCES

1. Ackerman, J. M.; White, F. J. Continuous and intermittent cocaine treatment decrease sensitivity of the A10 somatodendritic autoreceptors. Soc. Neurosci. Abstr. 16:1050; 1990.
2. Akimoto, K.; Hahamura, T.; Otsuki, S. Subchronic cocaine treatment enhances cocaine-induced dopamine efflux, studied by in vivo intracerebral dialysis. Brain Res. 490:339-344; 1989.
3. Cools, A. R. Basal ganglia and Pakinson's disease: Neurobiological and pharmacological aspects in animals and man. Clin. Neurol. Neurosurg. 86:178-195; 1984.
4. Dwoskin, L. P.; Peris, J.; Yasuda, R. P.; Philpott, K.; Zahnisher, N. R. Repated cocaine administration results in supersensitivity of striatal D_2 dopamine autoreceptors to pergolide. Life Sci. 42:255-262; 1988.
5. Epstein, P. N.; Altshuler H. L. Altered response to apomorphine and haloperidol after nine days of cocaine injections. Pharmacol. Biochem. Behav. 10:189-193; 1979.
6. Gale, K. Catecholamine-independent behavioral and neurochemical effects of cocaine in rats. NIDA Res. Monogr. 54:323-332; 1984.
7. Galloway, M. P. Neurochemical interaction of cocaine with dopaminergic system. Trends Pharmacol. Sci. 9:451-454; 1988.
8. Goeders, N. E.; Kuhar, M. J. Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. Alcohol Drug Res. 7:207-216; 1987.
9. Henry, D. J.; Greene, M. A.; White, F. E. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Repeated administration. J. Pharmacol. Exp. Ther. 251:833-839; 1989.
10. Henry, D. J.; White, F. J. Repeated cocaine administration causes persistent enhancement of D_1 dopamine receptor sensitivity within the rat nucleus accumbens. J. Pharmacol. Exp. Ther. 258:882-890; 1991.
11. Hinson, R. E.; Poulos, C. X. Sensitization to the behavioral effects of cocaine: Modification of Pavlovian conditioning. Pharmacol. Biochem. Behav. 15:559-562; 1981.
12. Iversen, S. D.; Frey, P. J. Brain catecholamines in relation to affect. In: Mac Fadden, D., ed. Neural mechanisms of nehavior. New York: Springer; 1980:229-269.
13. Kalivas, P. W.; Duffy, P.; DuMars, L. A.; Skinner, C. Behavioral and neurochemical effects of acute and daily cocaine administration. J. Pharmacol. Exp. Ther. 245:485-492; 1988.
14. Kalivas, P. W.; Duffy, P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48-58; 1990.
15. Kalivas, P. W.; Stewart, J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16:223-244; 1991.
16. Karoum, F.; Fawcett, R. M.; Wyatt, R. J. Chronic cocaine effects on peripheral biogenic amines: A long-term reduction in peripheral dopamine and phenylethylenamine production. Eur. J. Pharmacol. 148:381-388; 1988.
17. Kiyatkin, E. A. Changes in dopamine-dependent electrochemical signal in the nucleus accumbens associated with repeated cocaine injections in rats. Brain Res. 642:228-236; 1994.
18. Mayfield, R. D.; Larson, G.; Zahniser, R. Cocaine-induced sensitization and D_1 dopamine receptor function in rat nucleus accumbens and striatum. Brain Res. 573:331-335; 1992.
19. Ng, J. B.; Hubert, G. W.; Justice, J. B. Increased stimulated release and uptake in nucleus accumbens after repeated cocaine administration as measured by in vivo voltammetry. J. Neurochem. 56:1485-1492; 1991.
20. Parsons, L. H.; Smith, A. D.; Justice, J. B., Jr. Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine. Synapse 9:60-95; 1991.
21. Peris, J.; Boyson, S. J.; Cass, W. A.; Curella, P.; Dwoskin, L. P.; Larson, G.; Lin, L.-H.; Yasuda, R. P.; Zahnisher, N. R. Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J. Pharmacol. Exp. Ther. 253: 38-44; 1990.
22. Post, R. M.; Lockfeld, A.; Squillace, K. M.; Contel, N. R. Drug environment interaction: Context dependency of cocaine-induced behavioral sensitization. Life Sci. 28:755-760; 1981.
23. Robinson, T. E. Stimulant drugs and stress: Factors influencing individual differences in the susceptibility to sensitization. In: Kalivas, P. W.; Barnes, C. D., eds. Sensitization of the nervous system. Caldwell, NJ: Telford Press; 1988:145-173.
24. Rossetti, Z. L.; Hmaidan, Y.; Gessa, G. L. Marked inhibition of mesolimbic dopamine release: A common feature of ethanol,

morphine, cocaine and amphetamine abstinence in rats. Eur. J. Pharmacol. 221:227–234; 1992.
25. Segal, D. S.; Kuczenski, R. Repeated cocaine administration induces behavioral sensitization and corresponding decreased extracellular dopamine response in caudate and accumbens. Brain Res. 577:351–355; 1992.
26. Stewart, J.; Vezina, P. Conditioning and behavioral sensitization. In: Kalivas, P. W.; Barnes, C. D., eds. Sensitization of the nervous system. Caldwell, NJ: Telford Press; 1988:207–224.
27. Suaud-Chagny, M. F.; Ponec, J.; Gonon, F. Presynaptic autoinhibition of the electrically evoked dopamine release studied in the rat olfactory tubercle by in vivo electrochemistry. Neuroscience 45:641–652; 1991.
28. Trulson, M. E. Activity of dopamine-containing substantia nigra neurons in freely moving cats. Neurosci. Biobehav. Rev. 9:283–297; 1985.
29. Wilson, J. M.; Deck, J.; Shannak, K.; Chang, L. J.; Di Stefano, L. M.; Kish, S. J. Markedly reduced striatal dopamine levels in brain of a chronic cocaine abusers. Soc. Neurosci. Abstr. 16:252; 1990.