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Effect of Repeated Methamphetamine Pretreatment on Freezing Behavior Induced by Conditioned Fear Stress

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TSUCHIYA, K., T. INOUE AND T. KOYAMA. *Effect of repeated methamphetamine pretreatment on freezing behavior induced by conditioned fear stress.* PHARMACOL BIOCHEM BEHAV **54**(4) 687–691, 1996.—The present study examined the effect of methamphetamine (MA) pretreatment on conditioned fear stress in male Wistar–King rats. Rats received MA or the vehicle according to the repeated escalating dose schedule (1.25, 2.5, 3.75, and 5 mg/kg SC × 2/every other day for a week). After a 5-day drug abstinent period, the rats were exposed to conditioned fear stress (exposure to an environment paired previously with foot shock). Repeated but not single MA pretreatment significantly increased conditioned freezing behavior, suggesting that rats previously exposed to chronic MA are hypersensitive to subsequent stress than control rats. Repeated MA treatment did not decrease basal dopamine or serotonin concentrations in the brain. Furthermore, coadministration of MK-801 (noncompetitive NMDA antagonist), amfonelic acid (dopamine reuptake inhibitor), or fluoxetine (serotonin reuptake inhibitor) with MA did not alter the enhanced freezing behavior. Taken together, it seems that MA-induced hypersensitivity to stress is not due to the neurotoxic effect of MA. Coadministration of nemonapride ($D_{2/3/4}$ antagonist) with MA prevented the MA-induced increase in freezing. These results suggest that $D_{2/3/4}$ receptors play an important role for MA-induced enhancement of fear or anxiety.

Methamphetamine Anxiety Conditioned fear stress Dopamine$_2$ receptors Schizophrenia

IT has been generally recognized that schizophrenia-like psychosis occurs following long-term use of methamphetamine (MA) or amphetamine (2,4,19,25). In humans with a history of MA psychosis, a paranoid psychotic state can readily be reinduced not only by low doses of MA but also by psychological stressors after a long remission period (19,25). Psychological stress also plays an important role in relapse in schizophrenic patients (3). Consistent with these clinical observations, previous animal studies have reported that rats previously exposed to amphetamine are more sensitive to subsequent stress: behavioral and neurochemical responses to stress are augmented in amphetamine-sensitized rats (1,8,18).

This study was proposed to confirm the idea that animals previously exposed to chronic MA are hypersensitive to subsequent stress. We used conditioned fear stress (CFS; exposure to an environment paired previously with foot shock) as a stressor. CFS is regarded as psychological stress and a simple animal model of anxiety or fear (5,6,10). Furthermore, to explore the mechanism of development of MA-induced hypersensitivity to stress, we administered MK-801 [noncompetitive NMDA antagonist; (13)], amfonelic acid [dopamine reuptake inhibitor; (7)], fluoxetine [serotonin reuptake inhibitor; (23)], or nemonapride [$D_{2/3/4}$ antagonist; (24)] to rats prior to MA treatment and observed the freezing behavior induced by CFS.

METHOD

Animals

Male Wistar–King rats, weighing 200–250 g at the start of the experiment, were housed four per cage and maintained in a 12 L:12 D, temperature-controlled environment, with free access to food and water. Experiments began after a 7-day period of acclimatization.

Drugs

The following drugs were used: methamphetamine hydrochloride (Dainippon Pharmaceutical Co., Osaka, Japan); MK-

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TABLE 1
METHAMPHETAMINE TREATMENT REGIMEN

	Day	1	3	5	7
Methamphetamine	Low dose	1.25	2.5	3.75	5
mg/kg,SC×2/day	Middle dose	2.5	5	7.5	10
	High dose	5	10	15	20

Rats received methamphetamine twice a day on alternate days according to the three different escalating dose schedules; low dose group, middle dose group, high dose group.

801 (RBI, Natick); amfonelic acid (Sigma Chemical Co., St. Louis, MO); fluoxetine hydrochloride (Eli Lilly and Company, Indianapolis, IN); nemonapride (formerly YM-09151-2, Yamanouchi Pharmaceutical Co., Tokyo, Japan). MA and MK-801 were dissolved in 0.9% NaCl solution. Fluoxetine was dissolved in distilled water. Amfonelic acid was dissolved in propyleneglycol:2N K_2CO_3 (9:1 v/v). Nemonapride was dissolved in 0.15% tartaric acid.

Conditioned Fear Stress

Rats were individually exposed to inescapable electric foot shock [2.5 mA of scrambled shock (10 ms shock every 100 ms), on a variable interval schedule with a mean intershock interval of 60 s (35–85 s) and shock duration of 30 s, for 30 min] in a shock chamber with a grid floor (19 × 22 × 20 cm, Medical Agent Co., Kyoto, Japan) for 2 days. Electric shock was provided by a Model SGS-02D Shock Generator (Medical Agent Co., Kyoto, Japan). This provides a high-voltage, high-resistance circuit with resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5 mA, this generator actually gives the shock level equivalency of 0.2 mA for scrambled constant current to rats. Twenty-four hours after the last foot shock session, the rats were again placed in the shock chamber without shocks and observed for 5 min. Behavior was recorded using a time-sampling procedure (5). Every 10 s, the behavior in which the animal was currently engaged was classified as either freezing or activity. Freezing was defined as the lack of all observable movement of the body and vibrissae except those related to respiration. All other behavior was scored as activity. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee and in compliance with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

Experiment 1

Rats received MA twice a day on alternate days according to the three different escalating dose schedules; low dose group, middle dose group, and high dose group (shown in Table 1). Following the same schedule, controls were injected subcutaneously with 1 ml/kg saline. After a 5-day drug abstinent period, the rats were subjected to electric foot shock for 2 days. Twenty-four hours after the last foot shock session, the rats were again placed in the shock chamber without shocks and observed for 5 min.

Experiment 2

To determine whether a single administration of MA is sufficient to induce enhanced freezing behavior or not, rats were given a single injection of MA (5 mg/kg or 10 mg/kg SC) or saline (1 ml/kg SC). After a 5-day drug abstinent period, the rats were subjected to electric foot shock for 2 days. Twenty-four hours after the last foot shock session, the rats were exposed to CFS and observed for 5 min.

Experiment 3

MA treatment was performed according to the low dose group schedule shown in Table 1. The rats received nemonapride 1 mg/kg SC, amfonelic acid 0.5 mg/kg IP, fluoxetine 10 mg/kg IP, or MK-801 0.5 mg/kg IP 30 min prior to the injection of MA. After a 5-day drug abstinent period, the rats were subjected to electric foot shock for 2 days. Twenty-four hours after the last foot shock session the rats were exposed to CFS and observed for 5 min. To determine whether the vehicles affect freezing behavior, the rats received saline, distilled water, propyleneglycol:2N K_2CO_3 (9:1 v/v) or 0.15% tartaric acid twice a day on alternate 4 days, then exposed to CFS on the same schedule and observed for 5 min.

Experiment 4

Rats received nemonapride 1 mg/kg SC, amfonelic acid 0.5 mg/kg IP, fluoxetine 10 mg/kg IP, MK-801 0.5 mg/kg IP or saline 1 ml/kg SC twice a day on alternate 4 days. After a 5-day drug abstinent period, the rats were subjected to electric foot shock for 2 days. Twenty-four hours after the last foot shock session, the rats were exposed to CFS and observed for 5 min.

Experiment 5

Rats received MA or the vehicle according to the low dose group schedule shown in Table 1. The animals were killed by decapitation 7 days after the last treatment, and the brains were immediately removed, frozen and stored at −70°C. The following brain regions were punched out with small stainless steel needles according to the method of Palkovits et al. (16): the medial prefrontal cortex, nucleus accumbens, and striatum. Serotonin, dopamine, and noradrenaline were determined by the high-pressure liquid chromatography with electrochemical detection (HPLC-ECD) method, as described previously (10,11).

Data Analysis

Results are expressed as means ± SEM of the individual values of rats from each group. Multiple group comparisons were made using the one-way analysis of variance followed by Dunnett's two-tailed multiple comparison test. Comparison between two groups was performed with the nonpaired two-tailed Student's *t*-test.

RESULTS

Experiment 1

In the low dose group, repeated administration of MA significantly increased freezing behavior induced by CFS ($p < 0.01$). However, in the middle dose group or high dose group, there were no significant changes relative to the control group (Fig. 1).

Experiment 2

A single injection of MA did not change freezing behavior at the doses of 5 or 10 mg/kg (Fig. 2), $F(2, 21) = 0.028, p < 0.98$.

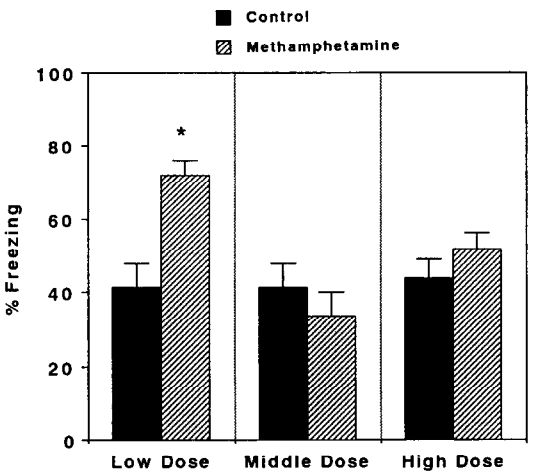

FIG. 1. Effect of repeated administration of methamphetamine according to three different dose schedules on CFS-induced freezing. Rats received methamphetamine or the vehicle according to the dose schedules shown in Table 1. After a 5-day drug abstinent period, the rats were subjected to electric foot shock for 2 days. Twenty-four hours after the last foot shock session, they were again placed in the shock chamber without shocks and observed for 5 min. Values are mean percent ± SEM of freezing obtained on eight rats. Behavior was sampled at 10-s intervals. $*p < 0.01$, significantly different from control group.

Experiment 3

Coadministration of nemonapride (1 mg/kg) with MA significantly prevented the MA-induced increase in freezing behavior ($p < 0.01$), $F(5, 90) = 6.382$, $p < 0.0002$, but amfonelic acid (0.5 mg/kg), fluoxetine (10 mg/kg), or MK-801 (0.5 mg/kg) had no effect on this increase (Fig. 3). There were no differences in conditioned freezing among four vehicle-treated groups [saline group, 47.9% ± 9.5; distilled water group, 47.5% ± 8.3; tartaric acid group, 41.7% ± 6.9; propyleneglycol:2N K_2CO_3 (9:1 v/v) group, 46.3% ± 5.7; $F(3, 28) = 0.137$, $p < 0.94$].

Experiment 4

Repeated injection of nemonapride (1 mg/kg), amfonelic acid (0.5 mg/kg), fluoxetine (10 mg/kg), or MK-801 (0.5 mg/kg) produced no significant change on freezing behavior (Fig. 4), $F(4, 35) = 0.083$, $p < 0.99$.

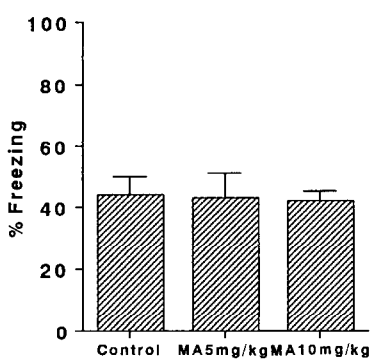

FIG. 2. Effect of single administration of methamphetamine (5 mg/kg or 10 mg/kg SC) on CFS-induced freezing. Values are mean percent + SEM of freezing obtained on eight rats. Behavior was sampled at 10-s intervals. MA: methamphetamine.

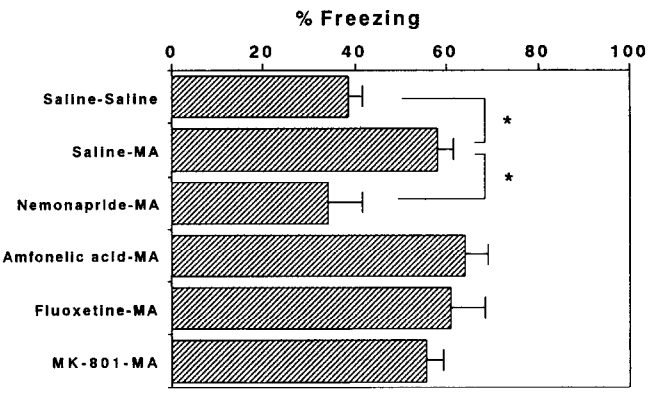

FIG. 3. Effect of coadministration of nemonapride (1 mg/kg), amfonelic acid (0.5 mg/kg), fluoxetine (10 mg/kg), and MK-801 (0.5 mg/kg) with methamphetamine (MA) on enhanced freezing induced by repeated MA treatment. Values are mean percent ± SEM of freezing obtained on 32 rats (saline-saline group and saline-MA group) or eight rats (other groups). Behavior was sampled at 10-s intervals. $*p < 0.01$, significantly different from the saline-MA group.

Experiment 5

Repeated MA treatment did not change dopamine, noradrenaline, or serotonin concentrations in any of the brain regions examined (data not shown).

DISCUSSION

In the present study, we examined the effect of repeated MA treatment on CFS. Experiment 1 demonstrated that repeated MA treatment enhanced freezing behavior induced by CFS after a 5-day drug abstinent period. This MA-induced hypersensitivity to stress was observed in the low dose group, but not in the higher dose groups. The reason for differences between the low and higher dose groups in enhanced conditioned freezing is unclear. However, because the previous study reported that animals given toxic doses of amphetamine

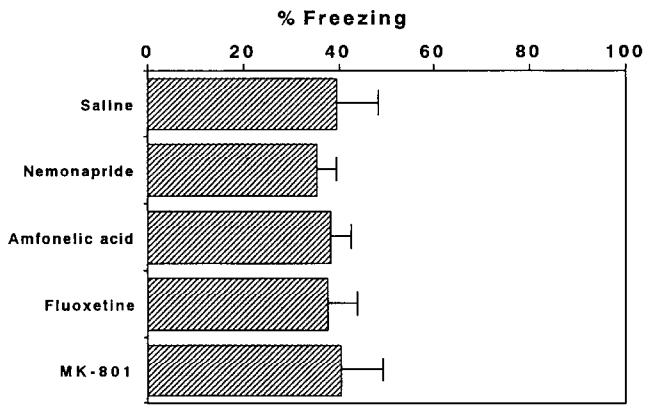

FIG. 4. Effect of repeated administration of nemonapride (1 mg/kg), amfonelic acid (0.5 mg/kg), fluoxetine (10 mg/kg), and MK-801 (0.5 mg/kg) on CFS-induced freezing. Values are mean percent ± SEM of freezing obtained on eight rats. Behavior was sampled at 10-s intervals.

are not hypersensitive to later amphetamine challenge (14), toxic effects of higher doses of MA might influence MA-induced behavioral hypersensitivity to CFS. Furthermore, Experiment 2 demonstrated that this behavioral enhancement did not occur after a single MA injection, suggesting that repeated treatment with MA are necessary for the development of hypersensitivity to stress.

There have been some previous reports indicating that animals previously exposed to amphetamine or MA show enhanced responses to subsequent stress. Antelman et al. (1) reported that amphetamine pretreatment reduced the ability of haloperidol to suppress tail-pinch induced behavior 3–30 days later in rats. Robinson et al. (18) reported that repeated amphetamine pretreatment produced a more rapid onset in foot shock-induced increases in 3,4-dihydroxyphenylacetic acid/dopamine ratios in the medial prefrontal cortex. Hamamura et al. (8) reported that amphetamine-pretreated rats showed greater foot shock-induced increases in extracellular dopamine in the medial prefrontal cortex than controls. These reports are consistent with our results.

Fanselow (5) reported that rats exhibited freezing behavior when tested in the same location where they had been shocked following a 24-h interval after delivery of electric foot shock. This finding suggested that postshock freezing was produced by conditioned fear elicited by cues associated with shock. Recently, two classes of anxiolytics, diazepam and ipsapirone, have been reported to reduce conditioned-freezing response (6,12,17). Thus, CFS is regarded as an animal model of anxiety or fear. Therefore, enhanced freezing behavior in MA-pretreated rats reflects the augmentation of fear or anxiety.

Repeated administration of high doses of MA decreases neuronal concentrations of dopamine and serotonin and their metabolites as well as the activity of their biosynthetic enzymes, tyrosine hydroxylase, and tryptophan hydroxylase, respectively (21). MK-801, fluoxetine, and amfonelic acid have been known to have neuroprotective effects against neurotoxicity of MA (9,15,20,22). However, in the present study, coadministration of MK-801, fluoxetine, or amfonelic acid with MA did not prevent the enhanced freezing behavior induced by MA. Furthermore, enhanced behavioral response to CFS was shown in the low dose group, but not in the higher dose groups. This MA treatment according to the low dose schedule did not decrease basal dopamine and serotonin concentrations in the brain. Taken together, it seems that MA-induced hypersensitivity to anxiety is not due to the toxic effect of MA.

Coadministration of nemonapride ($D_{2/3/4}$ antagonist) with MA prevented the MA-induced increase in freezing behavior. Because repeated injection of nemonapride alone had no effect on freezing behavior, the protective effect of nemonapride can be ascribed to blocking the pharmacological effect of MA. Therefore, these results suggest that $D_{2/3/4}$ receptors plays an important role for MA-induced enhancement of fear or anxiety.

In summary, the present study has shown that repeated administration of MA induces behavioral hypersensitivity to stress. This behavioral hypersensitivity indicates that animals previously exposed to repeated MA tend to be anxious or fearful. Moreover, the result that $D_{2/3/4}$ antagonist prevented this tendency toward anxiety suggests that the development of the hypersensitivity to stress might be mediated by $D_{2/3/4}$ receptors.

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REFERENCES

1. Antelman, S. M.; Eichler, A. J.; Black, C. A.; Kocan, D. Interchangeability of stress and amphetamine in sensitization. Science 207:329–331; 1980.
2. Bell, D. S. Comparison of amphetamine psychosis and schizophrenia. Br. J. Psychiatry 111:701–707; 1965.
3. Dohrenwend, B. P.; Egri, G. Recent stressful life events and episodes of schizophrenia. Schizophr. Bull. 7:12–23; 1981.
4. Ellinwood, E. H. Amphetamine psychosis: Description of the individuals and processes. J. Nerv. Ment. Dis. 144:273–283; 1969.
5. Fanselow, M. S. Conditional and unconditional components of postshock freezing. Pavlov. J. Biol. Sci. 15:177–182; 1980.
6. Fanselow, M. S.; Helmstetter, F. J. Conditioned analgesia, defensive freezing, and benzodiazepines. Behav. Neurosci. 102:233–243; 1988.
7. Fuller, R. W.; Perry, K. W.; Bymaster, F. P.; Wong, D. T. Comparative effects of pemoline, amfonelic acid and amphetamine on dopamine uptake and release in vitro and brain 3, 4-dihydroxyphenylacetic acid concentration in spiperone-treated rats. J. Pharm. Pharmacol. 30:197–198; 1978.
8. Hamamura, T.; Fibiger, H. C. Enhanced stress-induced dopamine release in the prefrontal cortex of amphetamine-sensitized rats. Eur. J. Pharmacol. 237:65–71; 1993.
9. Hotchkiss, A. J.; Gibb, J. W. Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. J. Pharmacol. Exp. Ther. 214:257–262; 1980.
10. Inoue, T.; Koyama, T.; Yamashita, I. Effect of conditioned fear stress on serotonin metabolism in the rat brain. Pharmacol. Biochem. Behav. 44:371–374; 1993.
11. Inoue, T.; Tsuchiya, K.; Koyama, T. Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. Pharmacol. Biochem. Behav. 49:911–920; 1994.
12. Inoue, T.; Tsuchiya, K.; Koyama, T. Serotonergic activation reduces defensive freezing in the conditioned fear paradigm. Pharmacol. Biochem. Behav. 53:825–831; 1996.
13. Kemp, J. A.; Foster, A. C.; Wong, E. Noncompetitive antagonists of excitatory amino acid receptors. Trends. Neurosci. 10:294–298; 1987.
14. Nelson, L. R.; Ellison, G. Enhanced stereotypies after repeated injections but not continuous amphetamines. Neuropharmacology 17:1081–1084; 1978.
15. Ohmori, T.; Koyama, T.; Muraki, A.; Yamashita, I. Competitive and noncompetitive *N*-methyl-D-apartate antagonists protect dopaminergic and serotonergic neurotoxicity produced by methamphetamine in various brain regions. J. Neural Transm. 92:97–106; 1993.
16. Palkovits, M.; Brownstein, M. J. Maps and guide to microdissection of the rat brain. New York: Elsevier; 1988.
17. Rittenhouse, P. A.; Bakkum, E. A.; O'Connor, P. A.; Carnes, M.; Bethea, C. L.; van de Kar, L. D. Comparison of neuroendocrine and behavioral effects of ipsapirone, a 5-HT_{1A} agonist, in three stress paradigms: Immobilization, forced swim and conditioned fear. Brain Res. 508:205–214; 1992.
18. Robinson, T. E.; Becker, J. B.; Young, E. A.; Akil, H.; Castaneda, E. The effects of foot shock stress on regional brain dopamine metabolism and pituitary β-endorphin release in rats previously sensitized to amphetamine. Neuropharmacology 26:679–691; 1987.
19. Sato, M.; Numach, Y.; Hamamura, T. Relapse of paranoid psychotic state in methamphetamine model of schizophrenia. Schizophr. Bull. 18:115–122; 1992.

20. Schmidt, C. J.; Gibb, J. W. Role of the dopamine uptake carrier in the neurochemical response to methamphetamine: Effects of amfonelic acid. Eur. J. Pharmacol. 109:73–80; 1985.
21. Seiden, L. S.; Ricaurte, G. A. Neurotoxicity of methamphetamine and related drugs. In: Meltzer, H. Y., ed. Psychopharmacology, the third generation of progress. New York: Raven Press; 1987: 359–366.
22. Sonsalla, P. K.; Nicklas, W. J.; Heikkila, R. E. Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. Science 243:398–400; 1989.
23. Stark, P.; Fuller, R. W.; Wong, D. T. The pharmacological profile of fluoxetine. J. Clin. Psychiatry 46:7–13; 1985.
24. Tang, L.; Todd, R. D.; Heller, A.; O'Mally, K. L. Pharmacological and functional characterization of D_2, D_3 and D_4 dopamine receptors in fibroblast and dopaminergic cell lines. J. Pharmacol. Exp. Ther. 268:495–502; 1994.
25. Utena, H. Behavioral aberration in methamphetamine-intoxicated animals and chemical correlates in the brain. In: Tokizane, T.; Schade, J. P., eds. Progress in brain research, vol. 21B. Correlative neuroscience-clinical studies. Amsterdam: Elsevier; 1966: 192–207.