Complicated Interaction Between Psychostimulants and Morphine in Expression of Phenotype of Behavior in the Dopaminergic System of BALB/c Mice

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Abstract. It is believed that BALB/c mice appear to be less sensitive to the locomotor effects of abused drugs compared to other strains, and several behaviors induced by abused drugs depend on genetic factors. The present study was designed to investigate the effects of the interaction between psychostimulants and morphine on behavior in BALB/c mice. Morphine and cocaine induced hyperlocomotion and hypolocomotion, respectively, while methamphetamine did not affect locomotor activity and high doses of methamphetamine significantly increased self-injurious behavior. Cocaine or methamphetamine increased the effects of morphine on locomotor behavior. Haloperidol (a dopamine-receptor antagonist) attenuated the hyperlocomotion induced by the combination of cocaine or methamphetamine plus morphine. These results indicate that the synergistic effects of methamphetamine or cocaine and morphine on locomotor activity are mediated through enhancement of the dopaminergic system and that combinations of psychostimulants and morphine enhance the locomotor activity in BALB/c mice. On the other hand, morphine completely attenuated methamphetamine-induced self-injurious behavior. Furthermore, a low dose (0.01 mg/kg) of haloperidol significantly increased the effects of methamphetamine and morphine on the locomotor activity. Hyperlocomotion induced by psychostimulants is mediated by the mesolimbic dopaminergic system, whereas stereotyped behaviors is mediated by the nigrostriatal dopaminergic system. Our findings suggest that balances of the activation of dopaminergic neurons (between mesolimbic and nigrostriatal systems) may play an important role to engender corresponding behavioral outcomes in BALB/c mice.

Keywords: methamphetamine, cocaine, morphine, BALB/c, self-injurious behavior

Introduction

Polydrug abuse has become a major drug-abuse problem worldwide. The combination of cocaine and opioids (heroin) (“speedball”) is quite common and is reportedly used to produce a more intensely pleasurable “rush” (1 – 3). In addition, the simultaneous administration of amphetamine and heroin, a “bombitas”, has been noted (4, 5). Therefore, some investigators have sought to characterize the interactions of these psychostimulants (cocaine and methamphetamine) and opioids (morphine and heroin) (6 – 13).

It is well known that methamphetamine, cocaine, and morphine induce hyperlocomotion and stereotyped behavior in most strains of mice, which might be mediated by the activation of the dopaminergic system. Combining psychostimulants and morphine or heroin could enhance their locomotor-activating and rewarding effects in rodents (6, 9, 12, 14, 15). Furthermore, Suzuki et al. (10, 11) previously demonstrated that morphine could enhance the discriminative stimulus effects of cocaine. On the other hand, previous studies demonstrated that some opioid receptor agonists have an antagonistic effect on the dopamine-related behavior,
especially stereotyped behaviors, in mice (15 – 17). We interestingly demonstrated that haloperidol (a dopamine-receptor antagonist) increased the locomotor activity on combined effects of psychostimulants (methamphetamine and cocaine) and reduced methamphetamine-induced self-injurious behavior accompanied by an induction in increase of locomotor activity (12, 13). Thus, the mechanism(s) for expression of the phenotype of dopamine-related behavior induced by combination of psychostimulants and opioids in mice is not clear.

The analysis of drug effects on locomotor activity and exploratory behavior is an important tool in behavioral pharmacology. Alternations in these parameters have important consequences for more specific process, such as memory reinforcing effects, and provide a better understanding of drug interactions (18). A large growing body of evidence suggests that there are clear strain differences in some behavioral effects. Furthermore, it has been noted that several behaviors induced by abused drugs depend on genetic factors. Previous studies showed that hyperlocomotion was observed following the administration of morphine, but not with methamphetamine and cocaine in BALB/c mice, unlike in other strains (6, 19 – 21). Thus, BALB/c mice appear to be less sensitive to the locomotor activating effects of abused drugs compared to other strains.

Kita et al. (22) noticed that the order of intensity in stereotypy induced by methamphetamine was BALB/c>DBA>C57BL. Relatively high dose of methamphetamine engendered self-injurious behavior in BALB/c mice as compared with other strains without affecting the locomotor activity (23) (personal communication). Thus, phenotypes of the behaviors induced by several abused drugs in BALB/c mice are different from other strains of mice. It is known that the hyperlocomotion induced by psychostimulants is mediated by the mesolimbic dopaminergic system, whereas stereotyped behavior including self-injurious behavior is mediated by the nigrostriatal dopaminergic system. We believed that investigations on the effects of the interaction between psychostimulants and morphine on locomotor activity and/or self-injurious behavior in BALB/c mice would lead to better understanding of the mechanism(s) for expression of the phenotype of behavior induced by cocaine/methamphetamine and morphine and relationship of genetic factor on behaviors induced by abused drugs. Therefore, the present study was designed to investigate the effects of the interaction between psychostimulants and morphine on locomotor activity and self-injurious behavior in BALB/c mice.

Materials and Methods

Animals

Male BALB/c mice (Charles River Japan Inc., Atsugi) weighing 18 – 23 g were used for the following experiments. The animals were housed at a room temperature of 20°C – 25°C and under a 12-h light-dark cycle (lights on at 7:00 AM). Food and water were available ad libitum. All of the following procedures were conducted in accordance with the guiding principles for the care and use of laboratory animals by the Japanese Pharmacological Society and with the guidelines for animal care in our laboratories, as approved by the Tokyo Women’s Medical University Committee on animal care and use.

Locomotor activity and self-injurious behavior score

Locomotor activity was measured while each mouse was in a transparent acrylic cage (270 x 440 x 187 mm, w x l x h) on 0.5-cm-deep sawdust using an MK-ANIMEX activity meter (Muromachi Kikai Co., Tokyo). After an exploratory period of 1 h, the mice were taken out of the cage, injected s.c. or i.p. with various doses of drugs, and place back in the cage. Locomotor activities were monitored immediately in 15-min intervals for a period of 120 min.

After the administration of methamphetamine, self-injurious behavior, especially skin-picking or self-biting around the chest, was measured over 3 min at 15-min intervals. A score of 0 was given for no self-injurious behavior; 1, for very mild self-injurious behavior (less than 1 min); 2, for at least 1 min of self-injurious behavior; and 3, for continuous self-injurious behavior almost throughout the 3-min observation period. A saline control was also tested as a placebo. The doses used were 1.0 – 20 mg/kg of methamphetamine (s.c.), 5.0 – 20 mg/kg of morphine (s.c.), and 5.0 – 20 mg/kg of cocaine (i.p.). In combination tests, animals were co-administered methamphetamine (2.0 and 20 mg/kg) or cocaine (20 mg/kg) and morphine (20 mg/kg). These drugs were dissolved in saline in a volume of 10 ml/kg. In antagonism tests, animals were pretreated with vehicle or haloperidol (0.01 and 0.03 mg/kg, i.p.) 15 min prior to the administration of methamphetamine or cocaine plus morphine. The doses and the pretreatment time were based on our previous papers (12, 21). The doses of methamphetamine were selected based on our previous papers (13) indicating that methamphetamine induced hyperlocomotion and self-injurious behaviors in ddY mice.

Drugs

The drugs used in the present study were metham-
amphetamine hydrochloride (Dainippon Pharmaceutical Co., Osaka), morphine hydrochloride (Sankyo Co., Tokyo), cocaine hydrochloride (Takeda Pharmaceutical Industries, Inc., Osaka), and haloperidol (Serenace Injection®; Dainippon Pharmaceutical Co., Osaka).

**Statistical analysis**

Data are expressed as the mean with S.E.M. The Kruskall Wallis test was used to evaluate the significance of differences. A \( P \) value of <0.05 was considered to reflect significance.

**Results**

**Effects of morphine and cocaine on locomotor activity**

Morphine (5.0 – 20 mg/kg) initially decreased locomotor activity, while the highest dose of morphine (20 mg/kg) significantly increased locomotor activity from 60 to 120 min (Fig. 1A). Cocaine (5.0 – 20 mg/kg) significantly attenuated locomotor activity (Fig. 1B).

**Effects of methamphetamine on locomotor and self-injurious behaviors**

The doses of methamphetamine used in the present study did not induce hyperlocomotion (Fig. 2A), and high doses of methamphetamine induced self-injurious behavior such as skin-picking and biting (Fig. 2B). Continuous self-injurious behavior was observed in 4 of 7 mice and 6 of 7 mice with the administration of 8.0 and 20 mg/kg of methamphetamine, respectively.

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Fig. 1. Effects of morphine (5 – 20 mg/kg: A) and cocaine (5 – 20 mg/kg: B) on spontaneous locomotor activity in BALB/c mice. Each point represents the mean counts with S.E.M. of 7 animals. \(*P<0.05, **P<0.01, vs\) saline control.

Fig. 2. Effects of methamphetamine (2 – 20 mg/kg) on spontaneous locomotor activity (A) and self-injurious behavior (SIB) (B) in BALB/c mice. Each point represents the mean counts with S.E.M. of 7 animals. \(*P<0.05, **P<0.01, vs\) saline control.
Effects of the cocaine, methamphetamine, and morphine on locomotor behaviors

In combination tests, although morphine (20 mg/kg) initially and cocaine (20 mg/kg) induced hyperactivity, the combination of these drugs significantly increased locomotor activity (Fig. 3A). The co-administration of methamphetamine (2.0 mg/kg) and morphine (20 mg/kg) significantly enhanced the hyperlocomotion induced by morphine (Fig. 3B).

Effects of morphine on methamphetamine: self-injurious and locomotor behaviors

Furthermore, morphine (20 mg/kg) completely and significantly attenuated methamphetamine (20 mg/kg)-induced self-injurious behavior (Fig. 4A), and this was accompanied by hyperlocomotion (Fig. 4B). In addition, 5 of 7 mice died within 24 h with the combination of methamphetamine (20 mg/kg) and morphine.

Fig. 3. Effects of the combination of cocaine (20 mg/kg: A) or methamphetamine (2.0 mg/kg: B) and morphine (20 mg/kg) on spontaneous locomotor activity in BALB/c mice. Each point represents the mean counts with S.E.M. of 7 animals. *P<0.05, **P<0.01, vs saline control; *P<0.05, **P<0.01, vs morphine control; $P<0.01$, vs cocaine or methamphetamine control.

Fig. 4. Effects of morphine (20 mg/kg) on methamphetamine (20 mg/kg)-induced self-injurious behavior (SIB) (A) and locomotor activity induced by methamphetamine (20 mg/kg) (B) in BALB/c mice. Each point represents the mean counts with S.E.M. of 7 animals. **P<0.01, vs saline control; **P<0.01, vs methamphetamine control.
**Effects of haloperidol on locomotor activity**

In antagonism tests, haloperidol (0.01 and 0.03 mg/kg) significantly reduced the locomotor-activating effects of the combination of cocaine (20 mg/kg) and morphine (20 mg/kg) (Fig. 5A). Similar to the results with the combination of cocaine and morphine, 0.3 mg/kg of haloperidol significantly attenuated the locomotor-activating effects of the combination of methamphetamine (2.0 mg/kg) and morphine (20 mg/kg), whereas 0.1 mg/kg of haloperidol significantly increased such effects induced by methamphetamine and morphine (Fig. 5B) and attenuated the imbalanced motion at the hind paw.

**Discussion**

Different inbred strains of mice, each with their own homogenous genetic material, display different behavioral sensitivities to amphetamine (24), methamphetamine, and cocaine (25, 26). There was a significant correlation between locomotor stimulation and the brain concentration of cocaine, suggesting that the differences between strains with regard to their locomotor responses to cocaine are related to the disposition of cocaine in the brain (27). On the other hand, it has been demonstrated that some specific strains increased the methamphetamine-induced stereotypy and self-injurious behavior compared to other strains (22) (T. Kita, personal communication). Thus, previous studies indicate that genetic factors play crucial roles in the behavioral effects induced by abused drugs.

With genetically controlled inbred strains of mice, it is generally recognized that there are clear strain differences in some behavioral effects induced by abused drugs (19, 20, 22). Consistent with previous results, morphine, but not methamphetamine or amphetamine, induced hyperlocomotion in BALB/c mice (24–26), whereas cocaine significantly attenuated locomotor activity. However, we found that the combination of cocaine or methamphetamine and morphine could enhance synergistically locomotor activity in BALB/c mice that are less sensitive to psychostimulants. Furthermore, haloperidol attenuated the hyperlocomotion induced by the combination of cocaine or methamphetamine plus morphine. On the other hand, low dose (0.01 mg/kg) of haloperidol significantly increased the effects of methamphetamine and morphine on the locomotor activity. Our findings suggest that locomotor activity induced by abused drugs might be varied due to their differential modulation of dopaminergic neuronal systems in BALB/c mice.

Although methamphetamine or cocaine itself did not increase the locomotor activity, the combination of cocaine or methamphetamine and morphine significantly increased locomotor activity in BALB/c mice. In fact, hyperlocomotion-induced by methamphetamine (2.0 mg/kg) was attenuated by its combination with morphine (10 or 20 mg/kg) in ddY mice (12), unlike in the case of BALB/c mice. In another recent study, it was that the co-administration of a µ-opioid receptor agonist and cocaine increased extracellular dopamine levels in the nucleus accumbens as measured by microdialysis.

![Fig. 5. Effects of haloperidol (HAL) (0.01 and 0.03 mg/kg) on the effects of the combination of cocaine (A) or methamphetamine (B) and morphine (20 mg/kg) on locomotor activity in BALB/c mice. Each point represents the mean counts with S.E.M. of 7 animals. *P<0.05, **P<0.01, vs methamphetamine or cocaine plus morphine control.](image-url)
techniques (28). Morphine potentiated the amphetamine-induced increase in the extracellular dopamine level (29). In the present study, high dose of haloperidol (a dopamine-receptor antagonist) attenuated the hyperlocomotion induced by the combination of cocaine or methamphetamine plus morphine. Therefore, the synergistic effects of methamphetamine or cocaine and morphine on locomotor activity in BALB/c mice may be mediated through enhancement of the dopaminergic system. These results may be informative for understanding the mechanism(s) of polydrug abuse, especially the abuse of “speedball” and “bombitas”.

It has been noted that the hyperlocomotion induced by psychostimulants is mediated by the mesolimbic dopaminergic system, whereas stereotyped behavior is mediated by the nigrostriatal dopaminergic system (30 – 32). It has been reported that haloperidol preferentially affects the nigrostriatum dopaminergic system rather than mesolimbic dopaminergic system (33, 34). Consistent with our previous results in ddY mice (12), the combination of morphine and cocaine potently increased locomotor activity in a dopamine-related manner, and the effects of the combination of morphine and methamphetamine were significantly increased by a low dose (0.01 mg/kg) of haloperidol. Therefore, the later results suggest that excess dopaminergic (especially nigrostriatum) activation may mask the effect of the interaction of methamphetamine and morphine on locomotor activity, which might be related to the nucleus accumbens. In fact, a strain difference exists in the pattern of dopamine release from the nucleus accumbens and striatum induced by cocaine, and the magnitude of the cocaine-induced increase in the extracellular level of dopamine in the nucleus accumbens is not always related to the behavioral outcome (32). Therefore, we propose that appropriate dopaminergic activation (between the nucleus accumbens and striatum) induced by abused drugs might engender corresponding behavioral outcomes.

Self-injurious behavior in humans has been observed in several neuropsychiatric disorders, including schizophrenia, Lesch-Nyhan syndrome (35 – 37), Tourette’s syndrome (38, 39), and Cornelia de Lange syndrome (40). In addition, such behavior is also found after the administration of psychostimulants. Kita et al. previously showed that multiple administration of methamphetamine (8 mg/kg) induced self-injurious behavior in BALB/c mice (23). We showed that single administration of methamphetamine (20 mg/kg) induced self-injurious behavior in BALB/c mice, indicating that blood levels are important in the expression of methamphetamine-induced self-injurious behavior. On the other hand, morphine completely attenuated methamphetamine-induced self-injurious behavior in BALB/c mice, accompanied by hyperlocomotion. With regard to this result, continuous self-injurious behavior induced by high methamphetamine doses might be related to activation of the nigrostriatum dopaminergic system (13). On the other hand, morphine produced an increase in spontaneous locomotor activity without an increase in spontaneous rearing, and a high dose of morphine produced a “compulsive” or “robotic” appearance in locomotion, which might be mediated by the mesolimbic dopaminergic system (12). Thus, morphine-induced activation of the mesolimbic dopaminergic system may mask methamphetamine-induced self-injurious behavior and enhance locomotor activity in combination of methamphetamine and morphine.

We recently showed that methamphetamine-induced self-injurious behavior was accompanied by neurotoxicity, which is mediated by dopaminergic and NMDAergic systems (41). Furthermore, the incidence of methamphetamine-induced self-injurious behavior might be a valid marker of long-term toxicity (23). A recent study showed that coadministration of 20 mg/kg of methamphetamine and 20 mg/kg of morphine induced lethality in BALB/c mice, which may be mediated by activation NMDA receptors (42). Methamphetamine-induced hyperthermia is one of the markers of methamphetamine-induced neurotoxicity; interestingly, morphine (which itself induced hyperthermia) enhanced hyperthermia induced by 20 mg/kg of methamphetamine (42). However, it is not yet clear whether morphine can affect methamphetamine-induced neurotoxicity or not. Therefore, further examination is needed to determine if morphine can affect the methamphetamine-induced neurotoxicity and whether the hyperthermia induced by morphine is involved in the attenuation of methamphetamine-induced self-injurious behavior.

Similar to the present results using BALB/c mice, the combination of psychostimulants and morphine enhanced locomotor activity, and methamphetamine induced self-injurious behavior in outbred (ddY) mice (12, 13). Therefore, this suggests that abused drug-induced behaviors might not be specific for each strain. Thus, it would be expected that the nigrostriatal dopaminergic system in BALB/c mice might be hyperactive, whereas the mesolimbic dopaminergic system might be hypoactive when compared to outbred mice.

References
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