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Research report

Brain site- and transmitter-dependent actions of methamphetamine, morphine and antipsychotics

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HIGHLIGHTS

- Morphine induces hyperlocomotion through activation of dopaminergic system.
- Haloperidol potently suppress morphine-induced hyperlocomotion.
- Clozapine more potently suppress hyperactivity induced by methamphetamine.
- Morphine and methamphetamine differentially regulate monoaminergic systems.

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ABSTRACT

While several methamphetamine- and morphine-induced psychotic states are ordinarily treated by antipsychotics, the therapeutic mechanisms of antipsychotic drugs have yet been elucidated. The present study was designed to investigate the mechanisms how antipsychotic drugs suppress the behavioral changes induced by psychoactive drugs in mice. Low to medium doses of methamphetamine produced hyperlocomotion, whereas high dose of methamphetamine induced hypolocomotion. Hyperlocomotion induced by methamphetamine was potently suppressed by clozapine and 5-HT_2 receptor antagonists, but not by the intra-accumbens injection of haloperidol. On the other hand, microinjection of haloperidol into the ventrolateral striatum increased locomotor activity with high dose of methamphetamine. In contrast, morphine-induced hyperlocomotion was suppressed by systemic as well as intra-accumbens injection of haloperidol, whereas relatively resistant to clozapine, compared to its effects in the case of methamphetamine. It has been widely believed that methamphetamine-induced psychosis is an animal model of schizophrenia, which is mediated by activation of accumbal dopamine receptors. Our findings suggest that methamphetamine differentially regulate monoaminergic systems (e.g., dopaminergic vs. 5-HTnergic), and accumbal dopamine receptors are not involved in methamphetamine-induced hyperlocomotion in mice. Thus, our findings may lead to a better understanding of the therapeutic mechanisms that underlie the effects of antipsychotic drugs and behavioral effects of methamphetamine and morphine.

1. Introduction

In humans, methamphetamine induces psychosis, including prominent hallucinations and delusions [1], which are indistinguishable from those schizophrenia, pleasurable sensation ("high") and stereotyped behavior [2]. While morphine can produce a similar psychosis, such as hallucination and delusion, and an intense pleasure "rush" [3], it does not induce stereotyped behavior. It is widely believed that the mesolimbic dopaminergic system, which projects to the prefrontal cortex and nucleus accumbens, is crucially linked to the psychotic state and reward-based learning and motivation [4–6], whereas the nigrostriatal dopaminergic system, which projects to the striatum, is believed to be involved in the volitional control of movement [7]. Furthermore, it is believed that the

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striatum and prefrontal cortex contribute to positive and negative symptoms, respectively.

However, there is little evidence to explain how morphine and methamphetamine can exert totally different pathophysiological changes in humans and behavioral changes in rodents, even though both drugs induces psychosis and reinforcing effects. One possible explanation is that methamphetamine and morphine differentially regulate the activation of dopaminergic systems (e.g., mesolimbic vs. nigrostriatal dopaminergic systems).

Psychotic episodes induced by high doses of methamphetamine or morphine have been traditionally treated by antipsychotics. It is known that dopamine D_2 receptor blockade, particularly in the nucleus accumbens, is critically linked to antipsychotic activity. On the other hand, dysfunction of the nigrostriatal dopaminergic system is related to extrapyramidal symptoms. Typical antipsychotic drugs such as haloperidol and chlorpromazine are effective for the treatment of schizophrenia, however such compounds carry a high risk of extrapyramidal side effects (EPS)[8], whereas atypical antipsychotic drugs, like clozapine and olanzapine, have been used for the treatment of schizophrenic symptoms with relatively few EPS [9]. Previous studies have shown that haloperidol produces a potent increase in c-Fos-positive neurons in the striatum and nucleus accumbens, whereas clozapine produces the induction of c-Fos-positive neurons only in the nucleus accumbens and prefrontal cortex, compared with the striatum [10–12], indicating that these antipsychotics have differential brain region-specific actions.

The analysis of drug effects on locomotor activity in rodents is an important tool in behavioral pharmacology, since changes in this parameter have important consequences for more specific processes, such as the psychotic state, and predict antipsychotic potential [11]. Particularly, high doses of morphine produce compulsive horizontal locomotor activity in mice, whereas high doses of methamphetamine induce phasic hyperlocomotion followed by stereotyped behaviors like licking and self-injurious behavior accompanied by hypolocomotion in mice through the differential action for the dopaminergic systems (mesolimbic vs. nigrostriatal dopaminergic systems) [11]. It is well known that the behavioral changes induced by methamphetamine or morphine, such as an increase in locomotor activity and stereotyped behaviors, are antagonized by dopamine receptor antagonist, and especially by typical antipsychotics. However, little information is available regarding whether or not typical and atypical antipsychotics have different effects on the behavioral changes induced by methamphetamine and morphine. Thus, the mechanisms by which morphine and methamphetamine produce totally different behavioral phenotypes in mice are not yet clear. Thus, the mechanisms by which morphine and methamphetamine produce totally different behavioral phenotypes in mice are not yet clear. In this study we hypothesized here that methamphetamine and morphine differentially regulate the mesolimbic and nigrostriatal dopaminergic systems, therefore typical and atypical antipsychotics distinctly may affect methamphetamine- and morphine-induced behavioral changes.

2. Material and methods

2.1. Animals

Male ddY mice (20 to 25 g) (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) were used. Food and water were available ad libitum for mice in their home cages. Animals were housed in a room maintained at $23 \pm 1\,^{\circ}C$ with a 12 h light-dark cycle (light on 8:00 a.m. to 8:00 p.m.).

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University, as adopted by the Committee on Animal Research of Hoshi University. Every effort was made to minimize the numbers and any suffering of animals used in the following experiments.

2.2. Locomotor assay

The locomotor activity of mice was measured by an ambulometer (ANB-M20, O'Hara Co., Ltd., Tokyo, Japan). A ddY mouse was placed in a tilting-type round activity cage (20 cm in diameter and 19 cm in height). Any slight tilt of the activity cage caused by horizontal movement of the mouse was detected by microswitches. Total activity counts in each 10-min segment were automatically recorded for 30 min prior to the injections and for 180 min following the administration of methamphetamine or morphine. Mice were pretreated with saline, haloperidol (0.03, 0.1 and 0.3 mg/kg i.p.) or clozapine (1, 3 and 10 mg/kg i.p.) 15 min prior to treatment with methamphetamine (1, 2 and 20 mg/kg s.c.) or morphine (10, 20 and 200 mg/kg s.c.). The doses of methamphetamine and morphine were selected based on our previous paper [13].

On the other hand, the locomotor activity of mice at 200 mg/kg of morphine was measured using a circular cage with a 38 cm-diameter and a 15 cm-high cylindrical wall. A circle of 13 cm in diameter was drawn at the center of the cage, and other lines were drawn so as to divide the cage into 4 parts outside the circle. Horizontal movement of the mouse was measured as the total number of times that the right hind paw crossed a line. The total activity counts in each 10-min segment were counted for 90 min after the administration of methamphetamine and morphine.

2.3. Surgery and microinjection

The mice were anesthetized with isoflurane (3%). The anesthetized animals were placed in a stereotaxic apparatus (Kopf Instruments, CA, USA). The skull was exposed and a small hole was made using a dental drill. An infusion cannula (D-1-6-02, Eicom Co., Kyoto, Japan) was bilaterally inserted into the ventrolateral striatum (from bregma: anterior/posterior [A/P] +0.3 mm, lateral [L] ±2.7 mm, ventral [V] −4.6 mm) or into the nucleus accumbens (from bregma: A/P +1.5 mm, L ± 0.9 mm, V −4.9 mm) according to the atlas of Franklin and Paxinos (1997). The animals were injected with vehicle (5%- glucose) or haloperidol (0.27 nmol) in a volume of 60 nl/mouse into the ventrolateral striatum or nucleus accumbens via the infusion cannula using a Hamilton syringe at an infusion rate of 20 nl/min. The mice were then returned to the ambulometer and subjected to the locomotor assay at 15 min after microinjection. After the experiments, brains were removed and fixed in 4% paraformaldehyde in 1 × PBS for 24 h. Brains were cryoprotected by incubation in 30% sucrose, and then sliced using a cryostat. Tissue was subsequently stained with hematoxylin and eosin. Mice with injections in the wrong location were excluded from the study.

2.4. Statistical data analysis

Data are expressed as the mean ± S.E.M. The statistical significance of differences between groups was assessed by one-way or two-way ANOVA followed by the Bonferroni multiple comparisons test, Dunnett's test or Student's *t*-test (unpaired, two-tailed). All statistical analyses were performed using Prism software (version 5.0a, GraphPad Software). A P value of <0.05 was considered to reflect significance.

2.5. Drugs

Methamphetamine hydrochloride (Dainippon-Sumitomo Pharmaceutical Co, Osaka, Japan), morphine hydrochloride (Daiichi-Sankyo, Co., Tokyo, Japan), haloperidol (HAL; Sigma-Aldrich Co., St.