Modification by Nicotine of the Sensitization to Methamphetamine-induced Ambulatory Stimulation in Mice: Possibility of Increase in Methamphetamine Dose of Abuse and Enhancement of Its Psychotoxicity

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Abstract: Since methamphetamine (MAP) has been frequently inhaled simultaneously with nicotine, i.e., cigarette smoking mixed with MAP, the modification by nicotine of the behavioral sensitization to MAP was evaluated in terms of ambulatory activity in mice. During the 5 repeated co-administrations of MAP (2 mg/kg s.c.) with nicotine (0.03, 0.1, 0.3 and 1 mg/kg s.c.) at 3 day intervals, nicotine dose-dependently inhibited the progressive enhancement of MAP-induced ambulatory stimulation. However, such pre-treatments did not modify the induction of ambulatory sensitization to MAP (2 mg/kg s.c.). In both the drug-naive and MAP-sensitized mice, nicotine reduced the ambulatory stimulant effect of MAP. These results suggest that, although nicotine reduces the expression of ambulatory stimulant effect of MAP, nicotine does not protect, but rather accelerate, the induction of psychotoxic effect of MAP.
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Key words: Methamphetamine-nicotine interaction, Ambulatory activity, Mice, Repeated co-administration, Behavioral sensitization, Psychotoxicity

Introduction

Methamphetamine (MAP) abuse is the most serious drug abuse problem in Japan. Although MAP has traditionally been administered through intravenous route, an inhalation of MAP vapor, namely ABURI, or smoking MAP mixed with tobacco, namely MOKU, is increasing not only because to avoid infections and the trace of picking needle, but also because of easy way for taking the drug.

The central stimulant effect of amphetamines is caused by an acceleration of dopamine release from the cytoplasmic pool of neurons at nucleus accumbens and striatum (McMillen, 1873). It has been known that the repeated administration of MAP induces the sensitization to its behavioral stimulant effect in animals (Tadokoro and Kuribara, 1986) and causes the increased risk of psychotoxic symptoms such as delusion and hallucination in humans (Wise and Bozarth, 1987).

Nicotine, an agonist of nicotinic acetylcholine receptors (Fuxe et al., 1986; Imperato et al., 1986; Marks et al., 1986), also accelerates dopamine release in the brain (Marks et al., 1986; Sershen et al., 1991; Jutkiewicz et al., 2008), and shows an ambulatory stimulant effect in rodents, particularly in rats (Kita et al., 1992; Ann-Sophie et al., 2006). Such neurochemical and behavioral characteristics of nicotine are partially similar to those of amphetamines. However, there are some reports which suggest an antagonistic effect of nicotine on the amphetamine-induced ambulatory stimulation and stereotyped behaviors (Stevens et al., 1995), and impairment of auditory sensory gating (Stevens et al., 1995).

Since MAP is inhaled simultaneously with nicotine following the smoking MAP mixed with tobacco, it is important to behaviorally assess the combined effect of MAP and nicotine, although the combined effects of nicotine and N-cyanomethylmethamphetamine, a main pyrolysis product of smoking methamphetamine mixed with tobacco, have been reported (Sekine et al., 1997; Kuribara, 2010).

The aim of the present study was to evaluate the modification by nicotine of the induction and expression of behavioral sensitization to MAP in mice. The following two experiments were conducted: 1) Repeated co-administrations of MAP with nicotine, and followed by the challenge administration of MAP alone. 2) The induction of MAP-sensitization and followed by co-administration of MAP with nicotine. 3) Repeated administration of nicotine and was followed by the challenge administration of MAP.

Materials and Methods

Animals

Male mice of ddY strain (Japan Laboratory Animals, Tokyo) were used at the age of 6 weeks (weighing 25-30 g). These mice were housed in groups of 10 in polycarbonate cages (20W X 25L X 10H cm) in a controlled room (temperature; 23 ± 2 ℃ , relative humidity; 55 ± 3 %, and light on between 05:00-19:00 hr). They could eat a solid diet (MF: Oriental Yeast, Tokyo) and drink tap water except during the behavioral tests.

Apparatus

The ambulatory activity of 10 mice was individually and simultaneously measured with a tilting-type "ambulometer" (SMA-10: O'hara & Co., Tokyo). This apparatus has 10 bucket-like activity cages of 20 cm in diameter and 15 cm in height. Each slight tilt of the activity cage generated by a horizontal movement (ambulation), but not by any vertical movements or turning, of the mouse was detected with one of 3 microswitches attached to the activity cage.

Drugs

The drugs used were methamphetamine HCl (MAP: Phylopone; Dainippon Pharm., Osaka) and nicotine free base (Nakarai Chemical., Tokyo). MAP and nicotine were dissolved in physiological saline, and the concentration of each drug solution was adjusted so that the volume injected (s.c.) was always constant at 0.1 ml/10 g body weight of the mouse. The dose of MAP was fixed to 2 mg/kg in the salt form which was optimum for increasing the ambulation without producing any strong stereotyped behaviors throughout the 5 repeated administrations at 3-day intervals in the ddY strain mice (Kuribara and Hirabayashi, 1985; Kuribara et al., 1996a, b).

Experimental schedules

Prior to the drug administration, mice were adapted to the activity cage for 10 min. After the drug administration, the ambulatory activity of each mouse was measured for 3 hr. All the behavioral tests were carried out between 09:00-16:00 hr.

Experiment 1. Repeated co-administrations of MAP with nicotine, and then challenge administration of MAP alone

Five groups of mice (10 each) were first treated with 5 repeated administrations of either MAP alone (nicotine dose=0) or MAP in combination with nicotine (0.03, 0.1, 0.3 or 1 mg/kg) at 3-day intervals. Three days after the 5th treatment, all mice were challenge-administered with MAP alone. In addition, the administration of MAP to the drug-naive mice (n=10) that were age-matched to the mice treated with the co-administrations of MAP with nicotine was also conducted.

Experiment 2. Induction of MAP-sensitization and then challenge administration of MAP in combination with nicotine

To induce the sensitization to MAP, 5 groups of mice (10 each) were first treated with 5 repeated administrations of MAP at 3-day intervals in the same way as in experiment 1. Three days after the 5th treatment, all groups of mice were challenged with either MAP alone (nicotine dose=0), or combination of MAP with nicotine (0.03, 0.1, 0.3 or 1 mg/kg).

Experiment 3. Repeated administrations of nicotine and then challenge administration of MAP alone, or MAP in combination with nicotine

Two set of 2 groups of mice (10 each) were administered saline (nicotine dose=0), or nicotine (1 mg/kg s.c.) once a day for 10 days in their home cages. These drug treatments were not followed by the measurement of ambulatory activities of mice. The days after the 10th treatment, 2 groups of mice of the first and second set were challenge-administered with MAP alone and MAP with nicotine (1 mg/kg).

Ethical consideration for experimental animals

All the experimental treatments of mice mentioned above were carried out according to the "*Guiding Principles for the Care and Use of Laboratory Animals*" of The Japanese Pharmacological Society.

Statistical analysis

Mean 3-hr overall ambulatory activity counts after the drug administrations were first analyzed by one- or two-way analysis of variance. In cases of significant variance, post-hoc analyses were carried out by Bonferoni test. Values of p less than 0.05 were considered significant.

Results

Experiment 1. Repeated co-administrations of MAP with nicotine, and then challenge administration of MAP alone

As shown in Table 1, the repeated co-administrations of MAP with nicotine induced a progressive enhancement of the ambulatory stimulant effect in all groups of mice. Nicotine reduced the stimulant effect of MAP in a dose-dependent manner in the repeated administration phase.

However, following the challenge administration of MAP, there was no significant difference in the activity counts among the groups of mice that had been treated with MAP alone or combination of MAP with nicotine.

Experiment 2. Combined administration of MAP with nicotine to the MAP-sensitized mice

Five repeated administrations of MAP (2mg/kg s.c.) at 3-day intervals resulted in an sensitization to the ambulatory stimulant effect of MAP in all groups of mice; the activity counts at the 1st and 5th administration being 1500-1600 and 5000-5200, respectively (data are not shown).

Table 2 shows mean 3-hr activity counts after the administration of MAP alone (nicotine dose=0) or MAP in combination with nicotine (0.03-1 mg/kg) to the MAP-

Table 1. Mean 3-hr ambulatory activity counts ± SEMs after 5 repeated administrations of methamphetamine (MAP: 2 mg/kg s.c.) alone, and MAP with nicotine (NICO: 0.03, 0.1, 0.3 and 1 mg/kg s.c.) at 3-day intervals, and the challenge administration of MAP.

Doses of drugs	1st	2nd	3rd	4th	5th	MAP-challenge
MAP only	1595 ± 220	2206 ± 311	4189 ± 593*	4630 ± 651*	4772 ± 707*	5001 ± 617#
MAP+NICO (0.03)	1570 ± 238	2122 ± 274	3620 ± 582*	4747 ± 630*	4699 ± 650*	5199 ± 630#
MAP+NICO(0.1)	1278 ± 194	1807 ± 239	2752 ± 374*,$	4139 ± 511*	4254 ± 630*	5166 ± 619#
MAP+NICO(0.3)	1110 ± 121$	1460 ± 190$	2754 ± 410*,$	3680 ± 517*	3535 ± 520*	5234 ± 704#
MAP+NICO(1)	912 ± 130$	1104 ± 148$	1906 ± 293*,$	3005 ± 404*,$	2918 ± 445*,$	4906 ± 539#
MAP						1549 ± 207

*: Significantly different vs. the count at the first administration within each group (p<0.05). $: Significantly different from the count of MAP alone-treated group at the same administration number (p<0.05). #: Significantly different from the count following the administration of MAP to the drug-naive mice (p<0.05). N=10 in each group.

Table 2. Mean 3-hr ambulatory activity counts ± SEMs after the administration of methamphetamine (MAP: 2 mg/kg s.c.) alone, co-administration of MAP with nicotine (NICO: 0.03-1 mg/kg s.c.), or nicotine alone to the MAP-sensitized mice.

Doses of drugs	Drug naive	MAP-sensitized
MAP alone	1595 ± 220	5120 ± 656
MAP + NICO (0.03 mg/kg)	1570 ± 238	4660 ± 591
MAP + NICO (0.1 mg/kg)	1273 ± 194	3570 ± 581*
MAP + NICO (0.3 mg/kg)	1110 ± 121*	3033 ± 453*
MAP + NICO (1 mg/kg)	912 ± 130*	2605 ± 349*

*: Significantly different from the activity count following the challenge administration of MAP (p<0.05). N=10 in each group.

sensitized mice. For comparison, the activity counts in the drug-naive mice, which are shown in Table 1 (the counts at the 1st administration), are also presented.

The ambulatory stimulant effect of MAP was reduced by nicotine in both the drug-naive and MAP-sensitized mice. The activity counts following the co-administration of MAP with 0.3-1 mg/kg nicotine in the drug-naive and MAP-sensitized mice were significantly lower than those following the administration of MAP alone.

Experiment 3. Repeated administrations of nicotine and then challenge administration of MAP alone, or MAP in combination with nicotine

As shown in Table 3, the pretreatment with nicotine (1 mg/kg s.c., 10 times) did not change the sensitivity to MAP or MAP + nicotine.

Discussion

In agreement with our previous reports (Kuribara and Hirabayashi, 1985), the repeated administrations of MAP induced significant sensitization to the ambulatory stimulant effect of MAP. The central stimulant effect of MAP is caused by acceleration of dopamine release from the cytoplasmic pool (McMillen, 1983). It is generally considered that the nicotine-induced behavioral stimulation is caused by an acceleration of dopaminergic neurotransmission through stimulation of acetylcholine release in the brain (Kita et al., 1992; Sershen et al., 1991). Amphetamine and nicotine interact to enhance their behavioral and neurochemical effects in rats (Huston-Lyons et al., 1993; Anne-Sophie et al., 2006).

However, it is notable that the ambulatory stimulant effect of MAP was significantly reduced by nicotine in both the drug-naive and MAP-sensitized mice. These results are in consistent with the inhibitory effects of nicotine on the amphetamine-induced locomotor stimulation (Stolerman et al., 1973), stereotyped behavior (Arnfred and Rundrup, 1968; Klawans et al., 1972), and impairment of auditory gating in rats (Stevens et al., 1995). Some mechanisms can be considered to be involved in the antagonistic effect of nicotine on the behavioral stimulant effect of amphetamines.

The possible mechanism is the nicotine-induced dopamine release through a stimulation of the nicotinic acetylcholine receptors in the brain, which may reduce the amphetamine-induced dopamine release (Sershen et al., 1991). Anne-Sophie et al. (2006) suggested a role of serotonergic mechanism in the nicotine-induced locomotor effect in mice.

Mesolimbic dopaminergic systems (Van der Heuval and Pasterkamp, 2008) play significant roles not only in the reward effect of drugs, i.e., substance abuse liability (Ikemoto, 2007; Piercem and Kumaresan 2006; Berridge, 2007), but also in the behavioral and psychological activities, particularly, motivation (Matsumoto and Hikosaka, 2009). Janhunen and Ahtee (2007) reported differential nicotinic regulation of the nigrostriatal and mesolimbic dopaminergic pathways. It is therefore important to assess the changes in the behavioral effect following the repeated combined administration of central stimulants including MAP and related drugs with nicotine.

The behavioral sensitization to amphetamines has been considered to be related not only to the risk of the abuse liability (Wise and Bozarth, 1987) but also to the risk of the induction of psychotoxic symptoms, namely amphetamine psychosis, following the repeated abuse of amphetamines (Tadokoro and Kuribara, 1986; Robinson and Becker,

Table 3. The activity counts following the challenge administration of methamphetamine (MAP: 2 mg/kg s.c.) to the mice pretreated with saline or nicotine (NICO: 1 mg/kg s.c.) daily for 10 days.

Pretreatment (10 times)	Challenge administration	
	MAP	MAP + NICO
Saline	1552 ± 271	891 ± 93*
NICO (1 mg/kg)	1598 ± 259	922 ± 129*

*: Significantly different from the activity count following the challenge administration of MAP.

1986). The present study showed that, although nicotine inhibited the acute ambulatory stimulant effect of MAP, the sensitization to MAP was not modified by nicotine. N-cyanomethylmethamphetamine, a main pyrolysis product of smoking methamphetamine mixed with tobacco, inhibited the behavioral stimulant effect of MAP (Sekine et al., 1997), but enhanced the induction of behavioral sensitization to MAP (Kuribara, 2010).

These results suggest that nicotine does not change the process of the behavioral sensitization to MAP. It is therefore highly probable that the repeated smoking MAP mixed with tobacco may not protect the liability of MAP abuse. This result also indicates another problem of MAP abuse mixed with tobacco. Thus, to recover the reward effect of these drugs, the combined MAP abuse mixed with tobacco may increase the MAP dose, and may accelerate the risk of the MAP-induced psychotoxic symptoms.

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NicotineによるMethamphetamineのマウス移所運動促進効果に対する増感現象の修飾
－Methamphetamine乱用量の増加と精神毒性増強の可能性－

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抄録： タバコに覚せい剤（methamphetamine: MAP）を混入して喫煙する乱用例がしばしばみられることから、MAPとnicotineの相互作用の検討が必要である。すでに著者らは、MAPを反復投与すると中枢刺激作用に対する増加現象が引き起こされ、この現象は乱用後の精神毒性の発現と共通することを報告した。本研究では、マウスの移所運動を指標に、nicotineの併用によるMAPに対する増感現象の修飾を検討した。MAP（2 mg/kg s.c.）とnicotine（0.03, 0.1, 0.3および1 mg/kg s.c.）の併用を3日間隔で5回反復投与すると、移所運動促進効果はnicotineの用量に依存して軽減された。しかし、6回目にMAP（2 mg/kg s.c.）を単独投与してみると、nicotineの用量に関係なく、MAPに対する増感現象が誘発された。MAPに対する増感を形成したマウスに対してMAP＋nicotineを併用投与すると、nicotineの用量に依存して、MAPの運動促進作用が軽減された。一方、nicotine（1mg/kg s.c.）の反復投与は、MAPに対する感受性の変化を引き起こさなかった。本実験結果は、nicotineはMAPの精神運動効果に対して抑制的に働くが、覚せい剤乱用による精神毒性の誘発を防止することはなく、むしろ増強する可能性を示唆している。
（別刷請求先：栗原　久）

キーワード： Methamphetamine/Nicotine 相互作用、反復投与、マウスの移所運動、行動的増感現象、精神毒性