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Drug interaction between methamphetamine and antihistamines: behavioral changes and tissue concentrations of methamphetamine in rats

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Abstract

Methamphetamine is a psychomotor stimulant, whereas first generation antihistamines cause sedation. Several studies have demonstrated that first generation antihistamines potentiate methamphetamine-induced psychomotor activation and two possible mechanisms have been postulated. One is blockage of the central histaminergic neuron system and the other is inhibition of dopamine reuptake. However, the exact mechanism is still controversial. In this study, we examined in behavioral tests the effects of selected antihistamines on methamphetamine-induced psychomotor activation in rats, and measured plasma and brain tissue concentrations of methamphetamine. We found that some antihistamines significantly potentiate methamphetamine-induced psychomotor activation in rats and that plasma and brain tissue concentrations of methamphetamine in rats treated with methamphetamine in combination with D-chlorpheniramine were markedly higher than those in rats treated with methamphetamine alone. These results suggest that the potentiating effects of antihistamines are due to not only central effects but also the alteration of the pharmacokinetics of methamphetamine.

Keywords: Methamphetamine; Antihistamine; Prepulse inhibition; Locomotor activity; Methamphetamine concentration

1. Introduction

Methamphetamine is a potent psychostimulant with abuse potential, while first generation antihistamines are known to cause sedation because they penetrate blood–brain barrier and, to a large extent, block central histamine H1 receptors (Yanai et al., 1995). First generation antihistamines are well known to cause sedation because the histamine H1 receptors can mediate excitatory actions on the whole brain activity (for review, see Haas and Panula, 2003). The initial report that first generation antihistamines potentiate the amphetamine-induced psychomotor activation occurred in the 1970s (Naylor and Costall, 1971), despite their sedative effect. In 1984, the central histaminergic neuron system was identified in the rat brain (Panula et al., 1984; Watanabe et al., 1984), though the physiological functions of histamine neurons were not fully understood in those days. Since first generation antihistamines potentiate methamphetamine-induced psychomotor activation, several studies speculated that histamine neurons might inhibit the functions of dopamine neurons (Itoh et al., 1984; Ito et al., 1997; Watanabe and Yanai, 2001). Other studies reported that antihistamines could inhibit the reuptake of dopamine at the dopaminergic nerve terminals (Shishido et al., 1991; Oishi et al., 1994; Matsunaga et al., 1998). From these studies, two possible mechanisms have been postulated. One is blockage of the central histaminergic neuron system, the other is inhibition of dopamine reuptake at dopaminergic

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nerve terminals. However, the exact mechanism is still controversial.

Recent studies have demonstrated that first generation antihistamines inhibit cytochrome P450 (CYP) 2D6 (Hamelin et al., 1998; He et al., 2002; for review, see Sharma and Hamelin, 2003). This inhibition can interfere with the metabolism of methamphetamine and may explain the potentiating effect of antihistamines on methamphetamine-induced psychomotor activation. However, to date, plasma and tissue concentrations of methamphetamine have not been measured in rats treated with a combination of methamphetamine and a first generation antihistamine.

In most previous studies, behavioral examinations were performed by direct observation and scoring. Prepulse inhibition of acoustic startle is the reduction in startle amplitude when startle stimuli is preceded by a weak non-startle stimulus (Hoffman and Ison, 1980). Disruption in prepulse inhibition is pharmacologically produced by dopamine D2 receptor agonists, serotonin receptor agonists and *N*-methyl-D-asparate (NMDA) antagonists (for review, see Geyer et al., 2001). Prepulse inhibition test is thought to be a more objective method (rather than direct observation) in order to assess methamphetamine-induced psychomotor activation. In this study, to determine whether antihistamines affect the pharmacokinetics of methamphetamine in rats, we examined the effects of selected antihistamines on methamphetamine-induced psychomotor activation in behavioral tests and measured plasma and brain tissue concentrations of methamphetamine in both acute and chronic experiments.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (SLC, Japan) were used in this study. The rats (7 weeks old) arrived at the animal facility at least 7 days prior to the start of the experiments and were housed in groups of four to five in plastic cages under controlled temperature (24±1 °C) and humidity (50±10%) with a 12 h light/dark cycle (light on 0700–1900). Food and tap water were available at all times except during the experiments. Behavioral experiments were performed between 0900 and 1800. All experimental procedures were approved by the Animal Care Committee of Tohoku University School of Medicine. This study was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

2.2.1. Locomotor activity measurement

Locomotor activity was measured by an infrared ray sensor system (SUPER-MEX®, Muromachi-Kikai, Tokyo, Japan) that consists of 8 small compartments divided with walls on a large shelf. The size of each compartment is 40 cm wide×50 cm long×35 cm high, and each compartment is equipped with a ceiling sensor that can detect heat energy radiated from the rats. The system monitors rat movement by measuring changes in heat energy in the covered field. Rats were individually placed in the plastic cages (19 cm wide×27.5 cm long×17 cm high) and put on the shelf.

2.2.2. Prepulse inhibition test

The prepulse inhibition test was conducted in a startle response system (Responder-X®, Columbus Instruments, Ohio, USA). A startle chamber is put in a sound attenuating cabinet (64 cm wide×40 cm long×60 cm high), and illuminated by a 15-W light bulb on the ceiling. The startle chamber consists of a stainless steel platform surrounded by plastic walls on all sides. Rats can move, but only slightly, while on the platform. A piezoelectric accelerometer unit attached to the bottom of the platform detects and transduces rat movement. The signals obtained from the unit are digitized and recorded to a computer. Acoustic stimuli are delivered through two loud speakers placed on the side of the platform. The sound level can be calibrated before the start of every experiment. In this study, a continuous background noise (65 dB) was provided throughout the experiments, and the startle pulse intensity was set to 120 dB with a duration of 40 ms. Prepulse intensity was set to 68, 71, and 77 dB with a duration of 20 ms, and the interval between the prepulse and startle pulse was 100 ms. Settings for the prepulse inhibition test were as follows: After an acclimation period of 5 min, five trials with 120 dB were presented (data discarded). Following these 5 trials, 7 iterations of 8 different types of trials were presented in a random manner; no pulse (0 dB), startle pulse alone (120 dB, 40 ms duration), prepulse alone (68, 71, 77 dB, 20 ms duration), prepulse+startle pulses. The percentage of prepulse inhibition (% prepulse inhibition) was calculated by the following equation: [1−(mean startle amplitude on prepulse+startle pulse trials)/(mean startle amplitude on startle pulse alone trials)]×100.

2.3. Experimental procedures in behavioral tests

In order to keep the number of rats to a minimum in the acute experiment, each rat was given one of the assigned treatments (methamphetamine alone, or methamphetamine and antihistamines) and examined in both prepulse inhibition and locomotor activity tests. The interval between the two tests was at least 1 week, and the testing order was randomized. Locomotor activity was measured every 10 min for 6 h after drug administration. Prepulse inhibition was measured at 30 and 180 min after drug administration.

In the chronic experiment, each rat received one of the assigned treatments for 8 consecutive days. After the treatment, drug administration was discontinued for 6 days. After the withdrawal, rats were treated again with the same

drugs on day 15. Locomotor activity was measured as described in the acute experiment on days 1, 4, 8, and 15.

2.4. Measurement of methamphetamine concentration in plasma and brain tissue

2.4.1. Time course of methamphetamine concentration in plasma in the acute experiment

Another 19 drug-naïve rats were use in this experiment, i.e. nine rats for 3 mg/kg methamphetamine alone, five rats for 3 mg/kg methamphetamine co-administered with 10 mg/kg pyrilamine, and five rats for 3 mg/kg methamphetamine co-administered with 7.5 mg/kg D-chlorpheniramine. Experimental procedures were carried out as described previously (Kitaichi et al., 2003). In brief, 1 day before the start of the experiment, rats were anesthetized with sodium pentobarbital (25 mg/kg body weight, i.p.), and the right juglar vein was cannulated with sterilized polyethylene tubes for drug administration and/or blood sampling. On the day of the experiment, the rats were allowed to move freely, and received each drug as described above. Plasma samples were then collected at 20 min intervals for 180 min, immediately centrifuged at 6000×*g* for 10 min, and stored at −30 °C until further analysis.

Concentrations of methamphetamine in plasma were determined by high-performance liquid chromatography (HPLC) as described elsewhere (Kitaichi et al., 2003). The HPLC apparatus (LC-6A, Shimadzu, Kyoto, Japan) consists of an LC-6A liquid pump, an RF-535 fluorescence detector, an SIL-6A autoinjector, a Cosmosil 5C18 column (4.6×150 mm; Nacalai Tesque, Kyoto, Japan), and a column oven (OTC-6A, Shimadzu) heated to 40 °C. The detection limit of methamphetamine was 5 ng/ml.

2.4.2. Pharmacokinetics data analysis

Time course of methamphetamine concentration in plasma of each rat was analyzed using the non-compartmental method. The area under plasma concentration against time curve (AUC) was calculated by the trapezoidal rule method up to the last measured plasma concentration. Half-life ($T_{1/2}$) of methamphetamine in plasma was calculated as $0.693/k$, where k represents the elimination constant. All computer analyses were performed using WinNonlin (version 2.1, Pharsight Mountain View, CA).

2.4.3. Methamphetamine concentration in brain tissue in both acute and chronic experiments

In the acute experiment, another 15 drug-naïve rats were used for the measurement of methamphetamine concentration in brain tissues. Each rat was decapitated 180 min after drug administration. In the chronic experiment, 7 days after the challenge test, rats were treated with the same drugs as the challenge test, and then decapitated 120 min after drug administration.

Brain dissection was performed as follows: after removing the cerebellum, medulla oblongata and pons, brain samples were divided into two parts at the sagittal cerebral fissure, and the striatum was dissected from the left brain according to the method of Glowinski and Iversen (1966). Blood was collected from the body after decapitation and centrifuged at 3000×*g* for 5 min to obtain plasma samples. All samples were immediately stored at −80 °C until further analysis.

Methamphetamine concentration in brain tissues was measured by gas chromatography/mass spectrometry (GC/MS) after extraction of methamphetamine from brain tissues as described previously (Nakagawa et al., 2003). Briefly, 100 mg of methamphetamine-*d3* was added to tissue samples as an internal standard. Brain tissue samples were then homogenized with 1 ml of methanol and centrifuged at 12,000×*g* for 5 min. Methamphetamine in the tissue samples was extracted using a solid extraction method (Bond Elut Certify, Varian, CA, USA). Finally, methamphetamine concentration in each sample was measured by GC/MS (AutoSpec-Ultima®, Micromass. USA) with a selected ion monitoring method. Ions at *m*/*z* 254 for methamphetamine and at *m*/*z* 257 for methamphetamine-*d3* were monitored with a dynamic resolution of 4000.

2.5. Drugs

Methamphetamine (Dainippon Pharmaceutical, Japan), D- and L-chlorpheniramine maleate (Wako Chemical Japan and Essex Japan Pharmaceuticals, respectively), pyrilamine maleate (ICN Biomedicals USA) and ebastine (a generous gift from Dainippon Pharmaceutical, Japan) were used. All drugs except ebastine were dissolved in physiological saline. Ebastine was suspended in 0.5% CMC. Treatment groups and their abbreviations were as follows.

In the acute experiment, saline (S group, n=10), 7.5 mg/kg D-chlorpheniramine alone (dC7.5 group, n=10), 3 mg/kg methamphetamine alone (M3 group, n=11), 3 mg/kg methamphetamine+1.0 mg/kg D-chlorpheniramine (M3+dC1.0 group, n=11), 3 mg/kg methamphetamine+2.5 mg/kg D-chlorpheniramine (M3+dC2.5 group, n=10), 3 mg/kg methamphetamine+7.5 mg/kg D-chlorpheniramine (M3+dC7.5 group, n=11), 3 mg/kg methamphetamine+7.5 mg/kg L-chlorpheniramine (M3+lC7.5 group, n=10), 3 mg/kg methamphetamine+10 mg/kg pyrilamine (M3+P10 group, n=12), 3 mg/kg methamphetamine+10 mg/kg ebastine (M3+E10 group, n=10).

In the chronic experiment, 1 mg/kg methamphetamine alone (M1 group, n=12), 1 mg/kg methamphetamine+2.5 mg/kg D-chlorpheniramine (M1+dC2.5 group, n=12), 1 mg/kg methamphetamine+5 mg/kg pyrilamine (M1+P5 group, n=12). All drugs were intraperitoneally administered in a volume of 2 ml/kg.

2.6. Statistical analyses

All statistical analyses were performed with SPSS statistical package (Ver. 11.0 for Windows, SPSS, Japan).

The percentage of prepulse inhibition, the cumulative counts, AUC, $T_{1/2}$ and brain tissue concentrations of methamphetamine were examined using one-way analysis of variance (ANOVA). Post hoc analysis was performed with Dunnett's comparison test against saline or methamphetamine alone. Paired-sample test was used for comparison between 30 and 180 min in the prepulse inhibition test of the acute experiment, and between day 1 and the other days in the cumulative counts of the chronic experiment. $P<0.05$ was considered statistically significant. Data are presented as means±S.E.M.

3. Results

3.1. Acute experiment

3.1.1. Methamphetamine-induced hyperactivity and disruption in prepulse inhibition

Methamphetamine significantly increased locomotor activity ($P<0.001$, Dunnett's test, Fig. 1A) and decreased % prepulse inhibition at 71 and 77 dB (68 dB, $P=0.765$; 71 dB, $P=0.035$; 77 dB, $P=0.006$, Dunnett's test, Fig. 1B) when compared with saline. There was no significant difference between D-chlorpheniramine and saline in both locomotor activity ($P=0.354$, Dunnett's test) and prepulse inhibition tests (68 dB, $P=0.550$; 71 dB, $P=0.985$; 77 dB, $P=0.888$, Dunnett's test). Since no significant difference was recorded at 68 dB in % prepulse inhibition between methamphetamine and saline, we excluded the data obtained at 68 dB in % prepulse inhibition from further analysis.

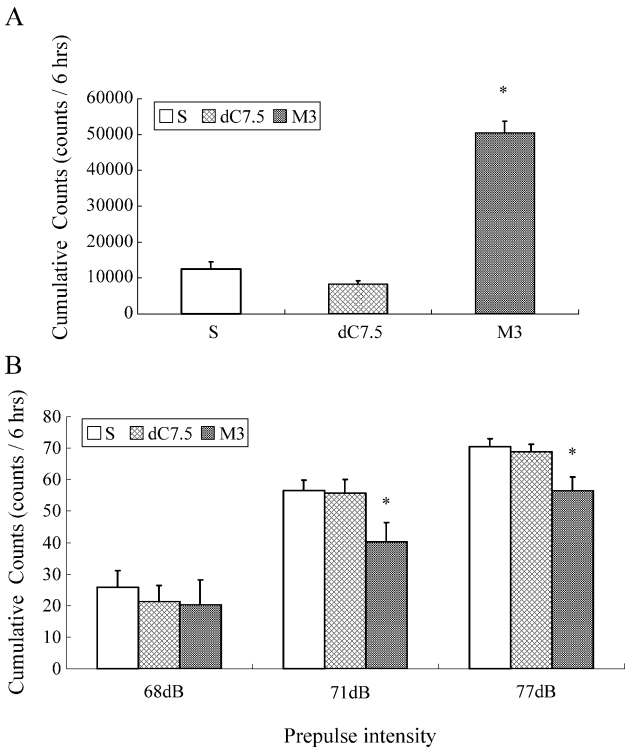

Fig. 1. Effects of D-chlorpheniramine (7.5 mg/kg) and methamphetamine (3 mg/kg) on locomotor activity and prepulse inhibition in rats. (A) Accumulated counts of locomotor activity for 6 h after drug administration. Data represent the mean of accumulated counts (±S.E.M.) of 10–11 rats. (B) % prepulse inhibition. Each bar represents the mean (±S.E.M.) of % prepulse inhibition of 10–11 rats. *Significantly higher (in the locomotor activity test) or lower (in the prepulse inhibition test) than saline as indicated by Dunnett's test.

3.1.2. Effects of various antihistamines on methamphetamine-induced hyperactivity

The cumulative counts for 6 h were significantly different among the treatment groups [F (4,50)=95.358, $P<0.001$, Fig. 2A,B]. The cumulative counts in rats that received 3 mg/kg methamphetamine in combination with pyrilamine (10 mg/kg), D-chlorpheniramine (7.5 mg/kg) or L-chlorpheniramine (7.5 mg/kg) were significantly higher than those in rats that were treated methamphetamine alone ($P<0.001$, $P<0.001$, $P<0.001$, respectively, Dunnett's test). Treatment with ebastine, a second generation antihistamine, did not have any significant effect on the cumulative counts ($P=0.832$, Dunnett's test). In a separate experiment, the effects of D-chlorpheniramine, given at doses of 1 and 2.5 mg/kg, on methamphetamine-induced hyperactivity were also examined in rats. Treatment with methamphetamine (3 mg/kg) in combination with D-chlorpheniramine (1 or 2.5 mg/kg) dose-dependently increased the cumulative counts as compared to treatment with methamphetamine alone (data not shown). The increase in locomotion induced by co-treatment with methamphetamine (3 mg/kg) and D-chlorpheniramine (1, 2.5, or 7.5 mg/kg) was approximately 1.4-, 1.9-, or 2.3-fold that induced by methamphetamine alone, respectively.

3.1.3. Effects of antihistamines on methamphetamine-induced disruption in prepulse inhibition

There were significant differences in the % prepulse inhibition among the treatment groups at 180 min after drug administration, although no significant difference was observed 30 min after drug administration [30 min: 71 dB, F (4, 47)=0.293, $P=0.881$; 77 dB, F (4, 47)=0.729, $P=0.577$, 180 min: 71 dB, F (4, 47)=5.354, $P=0.001$; 77 dB, F (4, 47)=3.313, $P=0.018$, Fig. 3]. Methamphetamine (3 mg/kg) in combination with D-chlorpheniramine (7.5 mg/kg) produced significantly lower % prepulse inhibition values at both prepulse intensities than methamphetamine alone at 180 min after drug administration (71 dB; $P=0.001$, 77 dB; $P=0.021$, Dunnett's test). L-Chlorpheniramine (7.5 mg/kg) produced a similar potentiating effect on methamphetamine-induced disruption in prepulse inhibition at 180 min after drug administration (77 dB; $P=0.035$, Dunnett's test). Neither pyrilamine nor ebastine had any significant effect on methamphetamine-induced disruption in prepulse inhibition at 180 min after drug administration (71 dB, $P=0.995$, 0.715; 77 dB, $P=0.965$, 0.579, respectively; Dunnett's test). The % prepulse inhibition at 180 min after

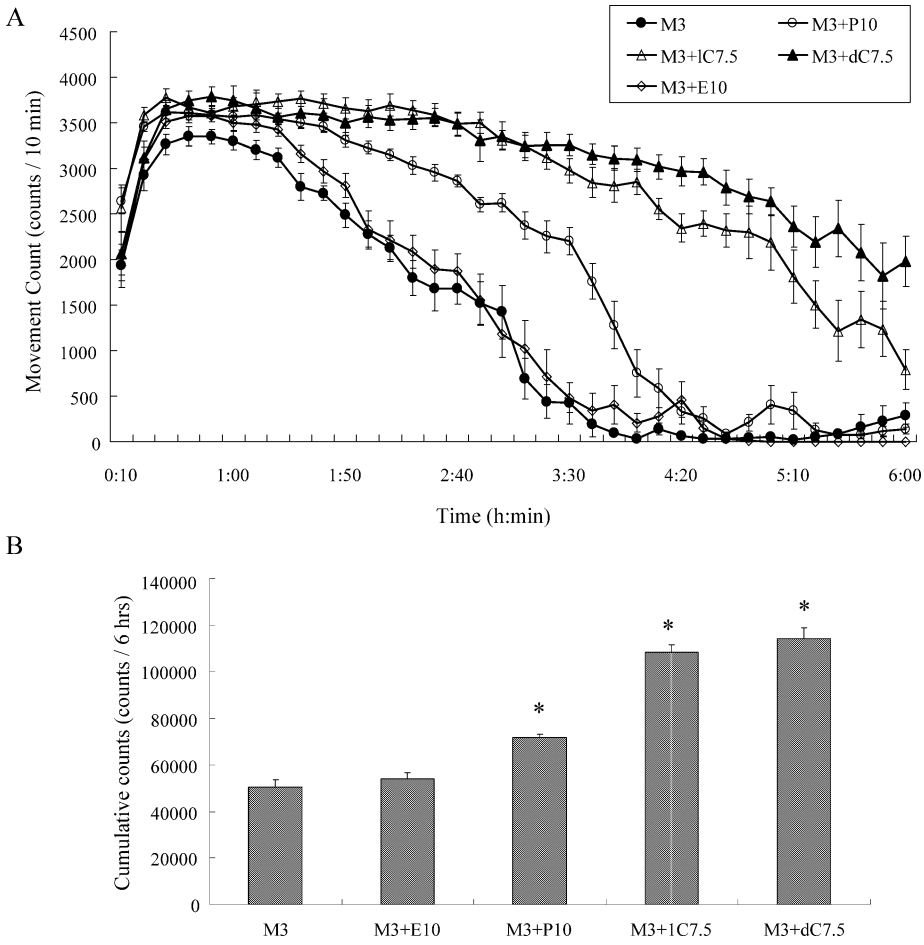

Fig. 2. Effects of antihistamines [D- and L-chlorpheniramine (7.5 mg/kg), pyrilamine (10 mg/kg) and ebastine (10 mg/kg)] on methamphetamine (3 mg/kg)-induced hyperactivity in rats. (A) Time course of locomotion. Data represent the mean of accumulated counts for 10 min and are expressed as means±S.E.M. of 10–12 rats. (B) Accumulated counts for 6 h after drug administration. Data represent the mean of accumulated counts (±S.E.M.) of 10–12 rats. *Significantly higher than methamphetamine alone as indicated by one-way ANOVA followed by Dunnett's test.

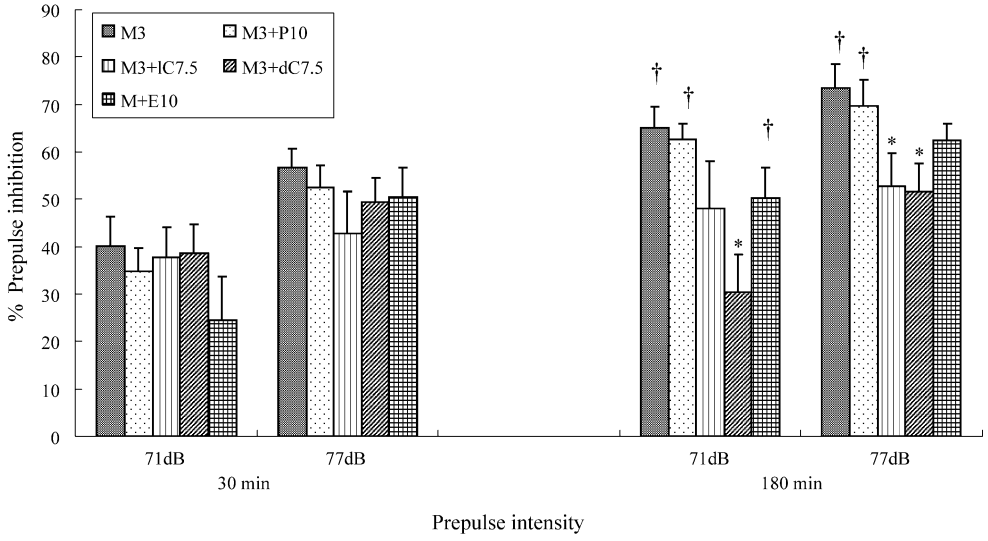

Fig. 3. Effects of antihistamines [D- and L-chlorpheniramine (7.5 mg/kg), pyrilamine (10 mg/kg) and ebastine (10 mg/kg)] on methamphetamine (3 mg/kg)-induced disruption in prepulse inhibition in acoustic startle at 30 and 180 min after drug administration. Each bar represents the mean (±S.E.M.) of % prepulse inhibition of 10–12 rats. *Significantly lower than methamphetamine alone as indicated by one-way ANOVA followed by Dunnett's test. †Significantly higher than 30 min as indicated by paired-sample *t*-test.

drug administration in both methamphetamine alone and methamphetamine in combination with pyrilamine groups were significantly higher than those at 30 min after drug administration at both prepulse intensities (71 dB; P=0.005, <0.001. 77 dB; P=0.035, 0.006, respectively).

3.1.4. Dose-dependent effects of D-chlorpheniramine on methamphetamine-induced disruption in prepulse inhibition

There were significant differences in the % prepulse inhibition at 180 min after drug administration, while no significant changes were observed at 30 min after drug administration [30 min: 71 dB, F (3, 37)=2.480, P=0.076; 77 dB, F (3, 37)=1.657, P=0.193, 180 min: 71 dB, F (3, 37)=5.881, P=0.002; 77 dB, F (3, 37)=3.525, P=0.024, Fig. 4]. Treatment with methamphetamine in combination with D-chlorpheniramine dose-dependently produced lower % prepulse inhibition at 180 min after drug administration than treatment with methamphetamine alone. The % prepulse inhibition in rats treated with 3 mg/kg methamphetamine in combination with 1 mg/kg D-chlorpheniramine at 180 min after drug administration were significantly higher than those 30 min after drug administration at both prepulse intensities (71 dB, P<0.001; 77 dB, P=0.006).

3.2. Chronic effects of antihistamines on methamphetamine-induced hyperactivity

The cumulative counts on day 15 were significantly higher than those on day 1 in all treatment groups (M1 group, P<0.001; M1+P5 group, P<0.001; M1+dC2.5 group, P<0.001), indicating sensitization. As in the acute experiment, there were significant differences in the cumulative counts between groups on all testing days [day 1, F (2, 33)=45.680, P<0.001; day 4, F (2, 33)=64.574, P<0.001; day 8, F (2, 33)=49.662, P<0.001; day 15, F (2, 33)=96.887, P<0.001, Fig. 5]. The cumulative counts in rats that received 1 mg/kg methamphetamine in combination with 2.5 mg/kg D-chlorpheniramine were significantly higher than those in rats that were treated with methamphetamine alone on all testing days (day 1, 4, 8, and 15, P<0.001, Dunnett's test). However, there was no significant difference in the % prepulse inhibition in the chronic experiment (data not shown). This is probably due to the use of lower doses of methamphetamine in the chronic experiment.

3.3. Methamphetamine concentration in plasma and brain tissue

3.3.1. Methamphetamine concentration in plasma in the acute experiment

Fig. 6A shows the time course of methamphetamine concentration in plasma. AUC in rats that received methamphetamine in combination with pyrilamine or D-chlorpheniramine was significantly higher than that in rats treated with methamphetamine alone (M3+P10, P=0.018; M3+dC7.5, P<0.001, Dunnett's test, Fig. 6B). Half-life of methamphetamine in plasma of rats treated with methamphetamine in combination with D-chlorpheniramine was significantly longer than that of methamphetamine alone (P<0.001, Dunnett's test, Fig. 6C). Pyrilamine had no significant effect on half-life of methamphetamine in plasma (P=0.505, Dunnett's test, Fig. 6C).

3.3.2. Concentrations of methamphetamine in brain tissue in the acute and chronic experiments

In the acute experiment, concentration of methamphetamine in both brain tissues and plasma was significantly higher in rats treated with methamphetamine in combination with D-chlorpheniramine than in rats that received methamphetamine alone (whole brain, striatum, the rest, plasma,

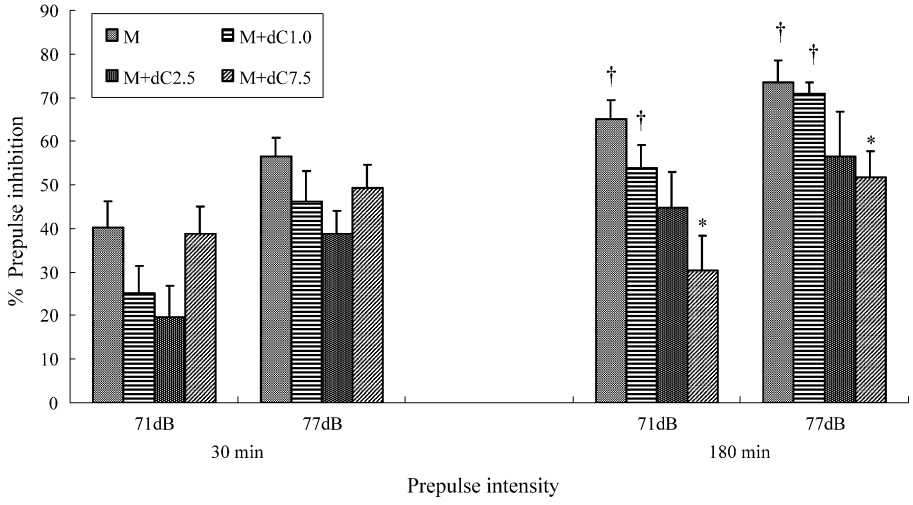

Fig. 4. Effects of D-chlorpheniramine given at different doses (1, 2.5, 7.5 mg/kg) on methamphetamine (3 mg/kg)-induced disruption in prepulse inhibition in acoustic startle at 30 and 180 min after drug administration. Each bar represents the mean (±S.E.M.) of % prepulse inhibition of 10–12 rats. *Significantly lower than methamphetamine alone as indicated by one-way ANOVA followed by Dunnett's test. †Significantly higher than 30 min as indicated by paired-sample t-test.

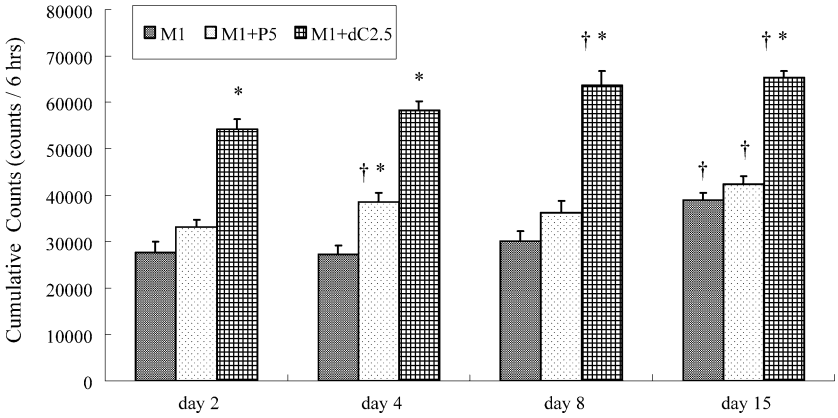

Fig. 5. Chronic effects of antihistamines on methamphetamine-induced hyperactivity. Data represent the average of accumulated counts (±S.E.M.) of 12 rats on days 1, 4, 8 and 15. *Significantly higher than methamphetamine alone as indicated by one-way ANOVA followed by Dunnett's test. †Significantly higher than on day 1 as indicated by paired-sample *t*-test.

$P<0.001$, Dunnett's test, Table 1). However, no significant difference was observed in rats treated with methamphetamine in combination with pyrilamine (whole brain, $P=0.741$; striatum, $P=0.438$; the rest, $P=0.847$; plasma, $P=0.377$, Dunnett's test, Table 1).

In the chronic experiment, concentration of methamphetamine in brain tissue, but not in plasma, was significantly higher in rats treated with methamphetamine in combination with D-chlorpheniramine than in rats treated with methamphetamine alone (whole brain, striatum, the rest, $P<0.001$; plasma, $P=0.090$, Dunnett's test, Table 1). In rats treated with methamphetamine in combination with pyrilamine, significant difference was observed only in striatum (striatum, $P=0.002$; whole brain, $P=0.417$; the rest, $P=0.930$; plasma, $P=0.733$, Dunnett's test, Table 1).

4. Discussion

In this study we found that, in both acute and chronic experiments, the cumulative counts of locomotor activity were significantly higher in rats treated with methamphetamine in combination with first generation antihistamines than in rats treated with methamphetamine alone (Figs. 2 and 5). We also found that disruption in prepulse inhibition continued for up to 180 min after drug administration in rats treated with 3 mg/kg methamphetamine in combination with 7.5 mg/kg L- or D-chlorpheniramine (Fig. 3). These results indicate that the effects of methamphetamine, given in combination with antihistamines, last longer than those of methamphetamine given alone.

The results of our behavioral experiments show that 7.5 mg/kg D-chlorpheniramine not only increased the cumulative counts of methamphetamine-induced hyperactivity but also decreased % prepulse inhibition at 180 min after drug administration. On the other hand, pyrilamine increased the cumulative counts of methamphetamine-induced hyperactivity, but did not decrease % prepulse inhibition at 180 min after drug administration. These behavioral data are in agreement with our pharmacokinetic data showing that D-chlorpheniramine increased both AUC and $T_{1/2}$, whereas pyrilamine increased only AUC (Fig. 6B,C). Like in the plasma of rats that received combination therapy, concentrations of methamphetamine in brain tissues were higher in rats treated with methamphetamine in combination with D-chlorpheniramine than in those treated with methamphetamine alone in both acute and chronic experiments (Table 1). These findings suggest that antihistamines alter the pharmacokinetics of methamphetamine and that this alteration may play an important role in their potentiating effect on methamphetamine-induced psychomotor activation in both acute and chronic experiments.

It is not clear why plasma and brain tissue concentrations of methamphetamine increased when this drug was co-administered with D-chlorpheniramine or pyrilamine. Theoretically, an increase in methamphetamine $T_{1/2}$ by D-chlorpheniramine suggests a decrease in its and/or renal excretion. Indeed, several studies reported that antihistamines including D-chlorpheniramine inhibit the activity of CYP2D6, which are responsible for catalyzing methamphetamine (Lin et al., 1997; Hamelin et al., 1998; He et al., 2002). Taken together, these results suggest that inhibition of CYP2D6 by D-chlorpheniramine might contribute to the prolongation of methamphetamine $T_{1/2}$. Methamphetamine has also been reported to be largely excreted into urine via the organic cation transport system (Riviere et al., 1999; Kitaichi et al., 2003). As chlorpheniramine has been shown to inhibit the function of organic cation transporters (Urakami et al., 2001), it is possible that D-chlorpheniramine inhibits methamphetamine renal excretion. Contrary to D-chlorpheniramine, pyrilamine increased AUC, but not $T_{1/2}$ of methamphetamine, suggesting that pyrilamine might affect the distribution of methamphetamine from the peritoneal cavity to blood circulation. Taking together these findings suggest that antihistamines differentially affect the disposition of methamphetamine.

Two other possible mechanisms for the potentiating effects of antihistamines on methamphetamine-induced

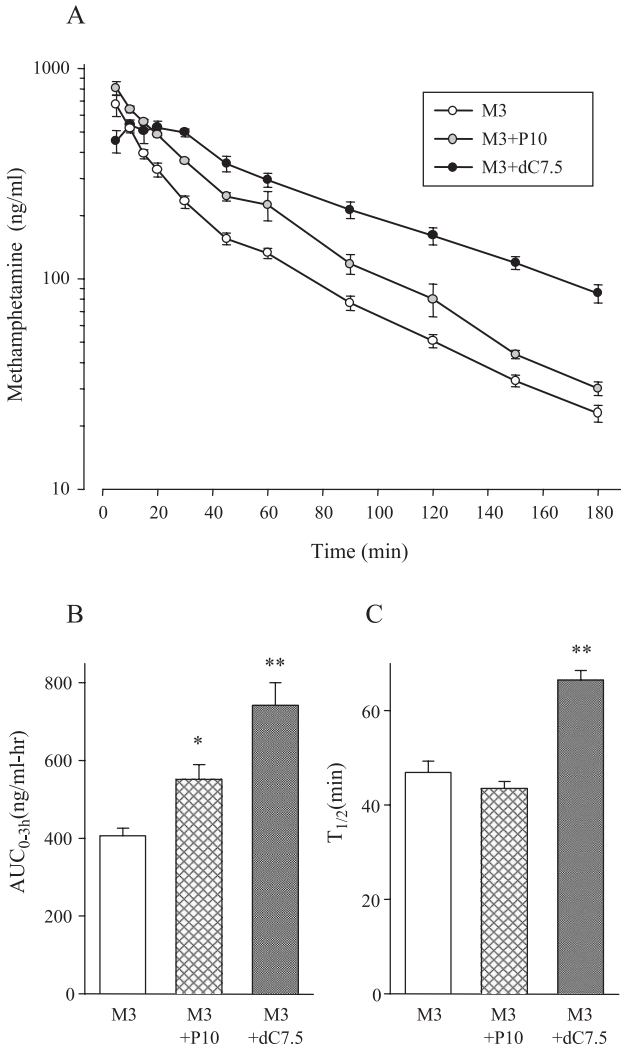

Fig. 6. (A) Time course of methamphetamine concentration in plasma. (B) Area under the curve (AUC) of methamphetamine concentration in plasma. (C) Half-life of methamphetamine concentration ($T_{1/2}$) in plasma. Data represent means±S.E.M. of 5–9 rats. *, **Significantly higher than methamphetamine alone as indicated by one-way ANOVA followed by Dunnett's test (*P<0.05, **P<0.01).

psychomotor activation have been postulated. The first is blockage of the central histaminergic neuron system (Itoh et al., 1984; Ito et al., 1997). The central histaminergic neuron system originates in the tuberomamillary nucleus of the posterior hypothalamus and its efferent fibers are widely distributed in the brain (Panula et al., 1984; Watanabe et al., 1984). The central histaminergic system modulates many biological functions including arousal, anxiety, antinociception, and appetite control (for review, see Brown et al., 2001), and might inhibit reinforcement and mnemonic process (Huston et al., 1997). We have previously reported that methamphetamine-induced hyperactivity and the development of behavioral sensitization were facilitated in histidine decarboxylase (HDC) gene-knockout mice and histamine H1/H2 receptor gene-double knockout mice than in their wild-type mice (Kubota et al., 2002; Iwabuchi et al., 2004). These findings suggest that brain histamine inhibits methamphetamine action through both histamine H1 and H2 receptors. As there was a slight difference in the potentiating effects of D- and L-chlorpheniramine on methamphetamine-induced hyperactivity, blockage of the central histaminergic neuron system as a possible mechanism for the potentiating effects of antihistamines on methamphetamine-induced psychomotor activation cannot be excluded.

The second mechanism is inhibition of dopamine reuptake by antihistamines (Shishido et al., 1991; Oishi et al., 1994; Matsunaga et al., 1998). Previous studies have shown that D-chlorpheniramine and pyrilamine at doses higher than 2 mg/kg inhibit dopamine reuptake in vivo, although D-chlorpheniramine at a dose of 1 mg/kg does not (Oishi et al., 1994). However, in this study, we found that D-chlorpheniramine (1 mg/kg), given in combination with methamphetamine, significantly increased (1.4-fold) the cumulative counts of locomotor activity as compared to methamphetamine alone. Since K_D (equilibrium dissociation constant) values for chlorpheniramine and cocaine at the dopamine transporter system are about 1060 and 220 nM, respectively (Tatsumi et al., 1997, 1999), it is suggested that chlorpheniramine needs a relatively large dose to act as a dopamine transporter blocker. Therefore, the results of our behavioral experiments cannot be explained by inhibition of dopamine reuptake alone.

Other studies have reported that scopolamine (Yui and Miura, 1996), methysergide (Balsara et al., 1979) and

Table 1
Concentration of methamphetamine in plasma and brain tissues in the acute and chronic experiments

	Plasma	Whole brain	Striatum	The rest
Acute experiment				
M3	11.1±2.7	135.6±47.5	95.1±11.9	80.9±13.1
M3+dC7.5	88.5±6.5[a]	649.6±69.3[a]	808.2±92.2[a]	664.4±62.3[a]
M3+P10	18.7±2.4	182.0±20.7	34.8±8.6	106.1±5.8
Chronic experiment				
M1	16.1±2.5	149.4±16.0	87.8±12.0	193.8±43.8
M1+dC2.5	38.1±12.1	591.6±37.3[a]	355.9±23.5[a]	480.1±28.0[a]
M1+P5	22.9±1.3	191.0±15.0	200.1±18.7[a]	179.3±21.2

Blood and brain tissue samples in the acute and chronic experiments were collected from non-sensitized rats 180 min after drug administration and from sensitized rats 120 min after drug administration, respectively. In the acute experiment, M3 indicates rats treated with methamphetamine (3 mg/kg) alone, M3+dC7.5 indicates rats treated with a combination of methamphetamine (3 mg/kg) and D-chlorpheniramine (7.5 mg/kg), and M3+P10 indicates rats treated with a combination of methamphetamine (3 mg/kg) and pyrilamine (10 mg/kg). In the chronic experiment, M1 indicates rats treated with methamphetamine (1 mg/kg) alone, M1+dC2.5 indicates rats treated with a combination of methamphetamine (1 mg/kg) and D-chlorpheniramine (2.5 mg/kg), and M1+P5 indicates rats treated with a combination of methamphetamine (1 mg/kg) and pyrilamine (5 mg/kg). Concentrations are expressed in ng/g.

[a] Significantly higher than methamphetamine alone as indicated by one-way ANOVA followed by Dunnett's test (p<0.01). Data are expressed as the mean±S.E.M. of 3–5 rats.

phenytoin (Izumi et al., 1984) also potentiate methamphetamine-induced psychomotor activation. Since antihistamines have various functions including anticholinergic, antiserotonergic and local anesthetic function besides their histamine H1 receptor antagonism (Katzung and Julius, 2001), it is not clear whether these functions contribute to the potentiating effects of antihistamines on methamphetamine-induced psychomotor activation.

In this study, we found that the potentiating effects of various antihistamines on methamphetamine-induced hyperactivity were not equal. For example, D- and L-chlorpheniramine had the strongest effect, whereas the effect of pyrilamine was weaker than that of D- or L-chlorpheniramine. Ebastine, a second generation antihistamine, had almost no effect on methamphetamine-induced psychomotor activation. The difference in the effect of antihistamines may be attributed to difference in the following factors: 1—inhibition of CYP 2D6 and/or OCT, 2—affinity for the histamine H_1 receptor, 3—inhibition of dopamine reuptake, 4—ability to penetrate blood–brain barrier, and 5—other functions of antihistamine besides histamine H1 receptor antagonism. It is also believed that these factors have a complex interaction with one another and that further studies are needed to clarify which mechanism is the most important.

In conclusion, our findings indicate that first generation antihistamines significantly potentiate methamphetamine-induced psychomotor activation not only by their central effects but also by their peripheral effects in both acute and chronic experiments. We propose a third mechanism, namely alteration of methamphetamine pharmacokinetics by antihistamines, in addition to the two central mechanisms postulated in previous studies.

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