INTRODUCTION

Each country has its own laws regulating recreational drugs. However, sources for novel recreational drugs are increasing, making the application of legislation difficult. Although newly introduced chemicals show similar biological effects to their respective analogs, they are not covered by legislation because of their divergent chemical structures.

Recent years have witnessed a rise in the use of special types of stimulant designer drugs sold to consumers primarily via the Internet or distributed on the street as a “bath salt,” “food plant,” “herb,” or “aroma” to circumvent laws banning their sale as products for human consumption (James et al., 2011; Numazawa, 2013). First-time drug users access to these drugs, one reason why they are called “gateway” drugs. In a previous study, we analyzed these drugs of abuse using GC/MS and found that most contained several compounds determined to be synthetic cannabinoids. Importantly, some “herb” brands contained not only synthetic cannabinoids but also synthetic cathinones, such as are commonly in the stronger drug “bath salts” (Numazawa, 2013).

Cathinone is a monoamine alkaloid found in the shrub Catha edulis (khat). Most of the synthetic cathinone supply originates in China or India and spreads worldwide (Sauer et al., 2009; Marinetti and Antonides, 2013). Cathinone and its derivatives synthetic cathinones are structurally similar to ephedrine, cathine and other phenylethylamines; therefore, their properties closely resemble those of amphetamine (Glennon et al., 1987; Kalix et al., 1992; Widler et al., 1994; Cozzi et al., 1999). Cathinone has served as a structural template for the discovery of compounds with a range of pharmacological activities (Damaj et al., 2004; Meltzer et al., 2006; Carroll et al., 2010), such as antidepressant action and appetite suppression. Recently several synthetic cathinones have become available for purchase through the Internet and in retail shops.
Human use of bath salts in anecdotal and case reports suggests that these substances induce powerful psychological effects including psychotic behavior, paranoia, delusion, and hallucination, possibly self-injury.

1-phenyl-2-(1-pyrrolidinyl)-1-pentanone (α-PVP) is a new designer drug of the cathinone type (Fig. 1). People who have taken drugs containing α-PVP sometimes lose consciousness, develop difficulty breathing, and, in the worst case, die (Marinetti and Antonides, 2013). However, no information is available to date about the effects of α-PVP on the central nervous system (CNS) of experimental animals. The objective of the present study was to investigate the effect of α-PVP on the mouse CNS and compare it with that of methamphetamine (METH, Fig. 1), which is primarily a monoamine transporter inhibitor and a monoamine releaser (Fleckenstein et al., 2000; Rothman et al., 2001).

MATERIALS AND METHODS

Animals and drugs
Balb/c male mice (8 weeks old) were purchased from Sankyo Lab Service Corporation (Tokyo, Japan). Mice were housed in plastic cages in a temperature controlled room (22 ± 1°C) and maintained on a 12-hr light-dark cycle with free access to food and water.

All procedures for animal care were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Showa University. Every effort was made to minimize the number of animals used and their suffering.

(+)-SCH23390 was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sulpiride was purchased from Astellas Pharma Inc. (Tokyo, Japan). Methamphetamine was purchased from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). α-PVP was obtained from a recreational drug vendor (April, 2012) before being controlled by Japanese Government (March, 2013). After 2013, α-PVP was professionally supervised under the control of a narcotic researcher (S. Numazawa) licensed under the Narcotics and Psychotropic Control Act. Its signal and composition as determined by GC-MS, element analysis, and NMR coincided well with those of α-PVP and the purity was greater than 98%.

In this study, we employed a dose of α-PVP as 25 mg/kg, because, other study regarding synthetic cathinones used doses ranging from 20 to 40 mg/kg (Marusich et al., 2012; Angoa-Pérez et al., 2012, den Hollander et al., 2013).

Locomotor activity
All animals were placed in activity chambers (W153 mm x D278 mm x H165 mm) for 40 min prior to drug administration.

For acute α-PVP experiments, mice were orally administered α-PVP (25 mg/kg), METH (5 mg/kg) as a positive control or water as a negative control. Locomotor activity was measured for 120 min after administration. For combination experiments, mice were pretreated with SCH23390 [50 μg/kg, intraperineal, (i.p.)], sulpiride [50 mg/kg, intramuscular, (i.m.)], or saline. Thirty minutes later, the mice were orally administered α-PVP (25 mg/kg) or water and locomotor activity was measured for 60 min. Locomotor activity was measured using the ANY-maze Video Tracking System (Stoelting Co., Wood Dale, IL, USA).

Surgery
Mice were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame. A microdialysis probe (D-I-6-02; 0.22 mm outer diameter, 2 mm membrane length; Eicom Co., Ltd., Kyoto, Japan) was implanted into the striatum at the following coordinates: AP: +0.5 mm, ML: +1.7 mm relative to bregma and DV: -4.4mm from the skull (Zhang et al., 2006; Callahan

![Image]

Fig. 1. Structures of 1-phenyl-2-(1-pyrrolidinyl)-1-pentanone (α-PVP), methamphetamine (METH) and cathinone.
et al., 2011). The probes were secured onto the skull using dental acrylic. The mice were allowed to recover for at least 20 hr before the experiment was begun (Zhang et al., 2006; Callahan et al., 2011).

The probe location was confirmed by microscopic examination after injection of dye solution.

**In vivo microdialysis**

The probes were perfused continuously with artificial cerebrospinal fluid (CSF) at a rate of 2 μl/min (Zhang et al., 2006; Vázquez et al., 2008). Two hours after reflux, the dialyzate was collected in 10-min fractions. Three samples were obtained to establish the baseline levels of extracellular dopamine (DA) before drug administration and 12 samples were obtained thereafter. DA levels were measured using an HTEC-500 HPLC-electron chemical detector system (Eicom Co., Ltd., Kyoto, Japan) with a C18 reversed phase column (4.6 mmφ x 30 mm, Eicompak PP-ODS; Eicom Co., Ltd.).

**Statistical analysis**

Data were analyzed by Kruskal-Wallis followed by Steel test.

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**RESULTS AND DISCUSSION**

The aim of the current study was to investigate the effect of α-PVP on the mouse CNS.

In this study, we employed an α-PVP dose of 25 mg/kg, because other studies regarding synthetic cathinones used doses ranging from 20 to 40 mg/kg (Marusich et al., 2012; Angoa-Pérez et al., 2012; den Hollander et al., 2013). Therefore, results from the present study could be comparable to those of other cathinones. On the other hand, a dose of METH employed herein (5 mg/kg) often used as positive control for psychostimulant activity (Marusich et al., 2012; Angoa-Pérez et al., 2012). Thus, we compared effects of α-PVP and METH with these doses to demonstrate the psychostimulant activity of the former.

**Effect of α-PVP on locomotor activity**

When the mice were placed in the activity chambers, they explored a new environment. It took approximately 10 min for to keep calm (data not shown). They were then administered α-PVP or METH. α-PVP induced significant locomotor activity immediately after the treatment, and the effect continued for the observation period (Fig. 2). The time of onset of action was within 10 min after α-PVP treatment, which was earlier and strong-
er than that after METH treatment. The effect of α-PVP appeared to be more profound than that of METH under these conditions. These results indicate that α-PVP induces a stimulatory effect on the mouse CNS, which comparable to that of METH. It has been suggested that neural dependency and CNS stimulation induced by addictive agents are associated largely with dopaminergic neurons (Pierce et al., 1997; White and Kalivas, 1998).

**Effect of α-PVP on DA level in the striatum**

We accordingly measured the DA level in the striatum by in vivo microdialysis. α-PVP immediately increased extracellular DA levels in the mouse striatum (Fig. 3). A significant increase in the DA level was observed during 120 min after treatment. Although the time of onset of this event appeared to be shorter than that after METH treatment, the effect of α-PVP on extracellular DA level was less profound compared with that of METH. The rapid increase in the DA level induced by α-PVP suggests that the synthetic cathinone increases DA release rather than inhibiting DA transporter.

**Fig. 3.** Extracellular dopamine level in mice striatum of mice. A mouse was placed into an active chamber and the probe was perfused continuously with artificial CSF. The dialysate was collected as the baseline level of extracellular dopamine for 30 min. Mice were orally administered α-PVP (25 mg/kg), METH (5 mg/kg) or water (10 ml/kg), and the dialysate was collected for 120 min. Dopamine level of the dialysate was analyzed by HPLC-ECD system. Values are represent as the percentage changes (the mean ± S.E.M.) from basal levels (control; n = 7, α-PVP and METH; n = 10). Statistical analysis was performed with Kruskal-Wallis followed by Steel test. *p < 0.05, **P < 0.01

**Effect of D<sub>1</sub> and a D<sub>2</sub> receptor antagonist on α-PVP-mediated hyperactivity**

To determine whether an increase in extracellular DA was involved in α-PVP-mediated locomotor activity, the mice were treated with SCH23390 (50 μg/kg, i.p.) or sulpiride (50 mg/kg, i.m.), a D<sub>1</sub> and a D<sub>2</sub> receptor antagonist, respectively, 30 min before α-PVP administration. Both antagonists significantly attenuated the increase in α-PVP-mediated locomotor activity (Fig. 4). Pretreatment with either antagonist decreased locomotor activity to 43% or 54% of the induced level, respectively. These results indicate that the stimulatory effect of α-PVP on CNS mediated, at least in part, by the dopaminergic system via the D<sub>1</sub> and D<sub>2</sub> receptors.

The effect of combined treatment with D<sub>1</sub> and D<sub>2</sub> antagonists on α-PVP-mediated hyperactivity remains to be determined. Such an experiment may clarify the role of the dopaminergic system in α-PVP-mediated hyperactivity. However, a significant level of α-PVP-mediated locomotor activity remained despite the administration of antagonists, suggesting a mechanism other than dopaminergic neurotransmission. The speculation may resolve the question of why α-PVP, a lesser stimulant of the extra-
cellular DA level than METH, induced more locomotor activity than METH.

In conclusion, our results indicate that α-PVP causes CNS stimulation comparable to that of METH. The present study suggests that α-PVP stimulates DA release, causing an increase in locomotor activity, which is mediated, at least in part, by stimulation of D1 and D2 receptors.

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REFERENCE


