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The Abuse Potential of α-Piperidinopropiophenone (PIPP) and α-Piperidinopentiothiophenone (PIVT), Two New Synthetic Cathinones with Piperidine Ring Substituent

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Abstract

A diversity of synthetic cathinones has flooded the recreational drug marketplace worldwide. This variety is often a response to legal control actions for one specific compound (e.g. methcathinone) which has resulted in the emergence of closely related replacement. Based on recent trends, the nitrogen atom is one of the sites in the cathinone molecule being explored by designer type modifications. In this study, we designed and synthesized two new synthetic cathinones, (1) α-piperidinopropiophenone (PIPP) and (2) α-piperidinopentiothiophenone (PIVT), which have piperidine ring substituent on their nitrogen atom. Thereafter, we evaluated whether these two compounds have an abuse potential through the conditioned place preference (CPP) in mice and self-administration (SA) in rats. We also investigated whether the substances can induce locomotor sensitization in mice following 7 days daily injection and challenge. qRT-PCR analyses were conducted to determine their effects on dopamine-related genes in the striatum. PIPP (10 and 30 mg/kg) induced CPP in mice, but not PIVT. However, both synthetic cathinones were not self-administered by the rats and did not induce locomotor sensitization in mice. qRT-PCR analyses showed that PIPP, but not PIVT, reduced dopamine transporter gene expression in the striatum. These data indicate that PIPP, but not PIVT, has rewarding effects, which may be attributed to its ability to affect dopamine transporter gene expression. Altogether, this study suggests that PIPP may have abuse potential. Careful monitoring of this type of cathinone and related drugs are advocated.

Key Words: Synthetic cathinones, Conditioned place preference, Self-administration, Locomotor sensitization, Abuse potential

INTRODUCTION

In the past few years, products that contain synthetic stimulants have appeared in the recreational drug markets worldwide(Brandt *et al*., 2011; German *et al*., 2014). These products, often sold as "plant foods", "researched chemicals" or "bath salts", have psychoactive synthetic cathinones that can be purchased on the Internet or retail shops as legal alternative to psychoactive drugs (Coppola and Mondola, 2012). Synthetic cathinones are beta-ketone compounds derived from cathinone, the active stimulant in the khat plant (Catha edulis)(Carroll *et al*., 2012). Recently, there has been increasing reports regarding the abuse of these psychoactive drugs, which have resulted into serious or even fatal outcomes (European Monitoring Centre for Drugs and Drug Addiction, 2015). In response to this public health threat, legal authorities enacted drug laws that prohibit the use of synthetic cathinones. However, sources for novel synthetic cathinones are still increasing, making the application of drug laws difficult. Although the newly introduced compounds have similar behavioral or neurological effects to their respective analogs, they are still not covered by drug laws because of their diver-

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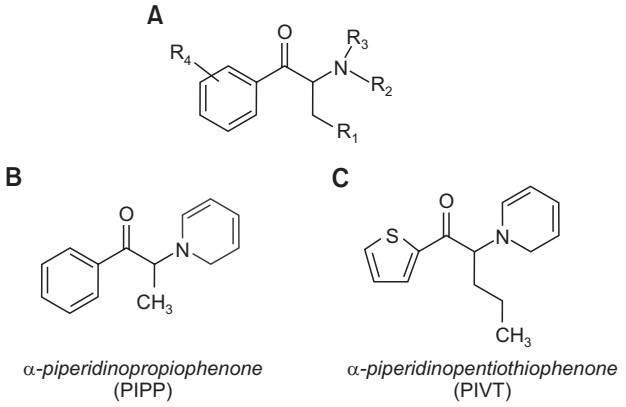

Fig. 1. The general chemical structure of synthetic cathinones (A). Chemical structures of α-piperidinopropiophenone (PIPP) (B) and α-piperidinopentiothiophenone (PIVT)(C).

gent chemical structures (Prosser and Nelson, 2012; Karila *et al*., 2015).

Chemical alterations to the basic structure of cathinone or its analogs (e.g. methcathinone) can create a broad array of designer drugs. In Fig. 1A, the general structure of a cathinone derivative shows the substitution patterns at four locations of the cathinone molecule; for example,on the alkyl side chain (R1), on the nitrogen atom (R2 and R3), and on the aromatic ring (R4). Based on current trends, all these sites are being explored by designer-type modifications (Paillet-Loilier *et al*., 2014). Furthermore, some of these substituted cathinones have been reported to be recreationally used by humans and produce rewarding effects in preclinical studies similar to psychostimulants like amphetamine, cocaine, and methamphetamine (German *et al*., 2014; Paillet-Loilier *et al*., 2014). In addition, these synthetic cathinones have the ability to influence dopaminergic neurotransmission in the brain reward circuit (Watterson and Olive, 2014). Given these observations, it appears that these novel synthetic cathinones have a high potential for abuse.

Here, as part of the continuing effort of the Drug Abuse Research Institute of Korea (DARC) to hasten the regulation of new synthetic cathinones and to predict abuse potential of future synthetic cathinones entities with similar modifications, we designed and synthesized two novel synthetic cathinones with piperidine ring substituent on the nitrogen atom (1) α-piperidinopropiophenone (PIPP) and (2) α-piperidinopentiothiophenone (PIVT) (Fig. 1B, 1C). PIPP and PIVT are analogs of methcathinone and alpha-PVT, respectively. Then, we evaluated their rewarding and reinforcing effects through the conditioned place preference (CPP) and self-administration (SA) tests. We also examined whether these new synthetic cathinones can induce locomotor sensitization following 7 days of drug treatment and challenge. Furthermore, we evaluated the effects of PIPP and PIVT on dopaminergic activity by analyzing the expression of dopamine-related genes, such as dopamine transporter, dopamine receptor D1, and dopamine receptor D2, in the striatum of the mice through quantitative real-time polymerase chain reaction (qRT-PCR). These dopamine-related genes were measured because they are the most common genes that are usually altered by drugs of abuse (Goldstein and Volkow, 2002; Koob and Le Moal, 2002). The striatum was chosen because dopaminergic neurons in this region are closely related to drug reward and reinforcement (Everitt and Robbins, 2013).

MATERIALS AND METHODS

Animals

Male ICR (22 to 27 g) mice were used in the CPP and locomotor sensitization test and were housed 8 per cage. Male Sprague-Dawley rats (200-300 g) were utilized for the SA test and housed individually. All animals were purchased from Hanlim Animal Laboratory Co (Hwasung, Korea). They were kept in a temperature- (22 ± 2°C) and humidity-controlled (55 ± 5%) animal room on a 12/12h light/dark (07:00-19:00h light) schedule. They were habituated to the laboratory setting for five days before any experiments. They had ad libitum access to food and water during acclimatization and experiments, except for the rats during lever training and SA sessions. All tests were performed in adherence with the Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985) and the Animal Care and Use of Guidelines of Sahmyook University, Seoul, Korea.

Drugs

α-piperidinopropiophenone (PIPP): PIPP was synthesized in two steps from propiophenone as described previously (Lagoja *et al*., 2003; Meltzer *et al*., 2006). Briefly, propiophenone was brominated with bromine. The resulting compound was reacted with piperidine to afford PIPP•HCl. Its structure was confirmed by the following spectroscopic analyses. 1H NMR (400 MHz, CD3OD) δ 8.07 (d, J=7.8 Hz, 2H), 7.75 (t, J=7.4 Hz, 1H), 7.60 (t, J=7.8 Hz, 2H), 5.21 (q, J=6.7 Hz, 1H), 3.83-3.35 (m, 2H), 3.12 (s, 2H), 2.19-1.68 (m, 6H), 1.59 (d, J=7.2 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 197.6, 135.7, 133.0, 129.5 (2C), 129.1 (2C), 65.4, 53.9, 50.4, 23.1, 22.7, 21.4, 14.0; HR-Mass calced for (C14H20NO+) 218.1539, found 218.1512.

α-piperidinopentiothiophenone (PIVT): PIVT was synthesized in three steps from thiophene as described previously (Meltzer *et al*., 2006; Gao *et al*., 2008). Briefly, thiophene was treated valeryl chloride and then brominated with bromine to give 2-bromo-1-(thiophen-2-yl)pentan-1-one. The resulting compound was reacted with piperidine to afford PIVT Its structure was confirmed by the following spectroscopic analyses. 1H NMR (400 MHz, CD3OD) δ 8.20 (dd, J=4.0, 0.9 Hz, 1H), 8.14 (dd, J=4.9, 0.9 Hz, 1H), 7.35 (dd, J=4.9, 4.0 Hz, 1H), 5.08 (dd, J=7.7, 4.0 Hz, 1H), 3.74 (brs, 1H), 3.42 (brs, 1H), 3.15 (brs, 2H), 2.03 (m, 3H), 1.90 (m, 4H), 1.60 (brs, 1H), 1.30 (m, 2H), 0.96 (t, J=7.3 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 188.5, 142.6, 138.4, 135.9, 129.3, 68.7, 53.2, 51.1, 30.8, 23.0, 22.7, 21.6, 18.3, 13.1; HR-Mass calced for (C14H22NOS+) 252.1417, found 252.1325.

Methamphetamine (METH) was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). All drugs were diluted in 0.9% sterile saline solution and administered intraperitoneally (i.p.) (CPP and locomotor sensitization) or intravenously (SA). The dosages used in the experiment were based on previous studies that examined the rewarding and reinforcing effects of synthetic cathinones (Gatch *et al*., 2013; Hwang *et al*., 2015; Xu *et al*., 2016; Botanas *et al*., 2017).

Conditioned place preference test

The CPP apparatus was composed of two large compartments (17.4×12.7×12.7 cm). Each compartment had distinct visual and tactile cues: one compartment was white with 6.352 mm stainless steel mesh floor while the other was black with a stainless steel grid floor (3.2 mm diameter rods placed 7.9 mm apart). Each compartment has a Plexiglas cover and illuminating light. A guillotine door provided access to both compartments. Movement and position of animals in the apparatus were detected by infrared beams and were analyzed, quantified, and recorded by a computer program.

This procedure was patterned after our previous study (Tampus *et al*., 2015). The test was composed of three phases: (1) habituation and pre-conditioning, (2) conditioning, and (3) post-conditioning. During the habituation, mice were allowed free access to both compartments for 20 minutes once a day for two days. On the following day, the time spent on each side was recorded (pre-conditioning). The data from the pre-conditioning phase was used to separate animals into groups with approximately equal time spent in each compartment. Mice that spent over 840 sec in one compartment were excluded from the test. The conditioning phase followed wherein subjects received i.p. PIPP or PIVT (3, 10, 30 mg/kg), METH (1 mg/kg) or saline and confined to a randomly designated compartment for 45 min. On alternate days, the mice received saline and were confined to the opposite compartment of the drug-paired compartment. Immediately after the last conditioning day, the post-conditioning phase followed in which mice were drug-free and would be allowed to access both compartments for 20 minutes, similar to the pre-conditioning phase.

Self-administration test

The standard operant chambers (Coulbourn Instruments, Allentown, PA, USA) were placed inside sound-attenuating boxes with ventilation fans. Each operant chamber has a food pellet dispenser, two 4.5 cm wide response levers (left and right), a stimulus light located 6 cm above the left lever, and a centrally positioned house light (2.5 W, 24 V) at the top of the chamber. Downward pressure (approximately 25 g) on a lever would result in a programmed consequence. Located beside the operant chamber was a motor-driven syringe pump that delivered solutions at a rate of 0.01 ml/s. Solutions flowed through a Teflon tubing connected to a swivel, which was mounted on a counterbalanced arm at the top of the chamber. This swivel system allowed animals to move freely around the chamber. The tubing was connected to the animal's intravenous catheter system. The Graphic State Notation software (Coulbourn Instruments) controlled the experimental parameters and collected the data.

Rats were trained to press the active (drug-paired) lever (30 min/day for three days) for a contingent food pellet reward on a continuous schedule of reinforcement. Only rats that earned at least 80 pellets in the last session of training were selected and prepared for surgery. Surgical and post-surgical techniques were described in detail in our previous studies (de la Peña *et al*., 2012). After the recovery period, rats were maintained on a 20 g diet per day and subjected to the 2-hour daily SA session under a fixed-ratio (FR) 1 schedule for seven days. During the SA sessions, both levers were present, and press on the left lever (active lever) would result in an infusion of 0.1 ml of PIPP or PIVT (0.1, 0.3, 1, or 3 mg/kg/infusion) or saline. At the same time, the house light was switched off, and the stimulus light was illuminated,which remained lit for another 20 s after the end of the infusion (time-out period). Lever presses during the time-out periods were recorded but did not have any corresponding effects. As a control for general activity, presses on the right lever (inactive lever) were recorded but not reinforced. Rats were allowed a maximum of 30 drug infusions per session, although lever presses were still recorded until the end of the 2 h session. Catheter patency was assessed by injecting 0.1 ml of thiopental sodium (10 mg/kg), a day before and on the last day of the SA test. Rats that did not lose muscle tone within 3-5 sec were excluded from the experiment.

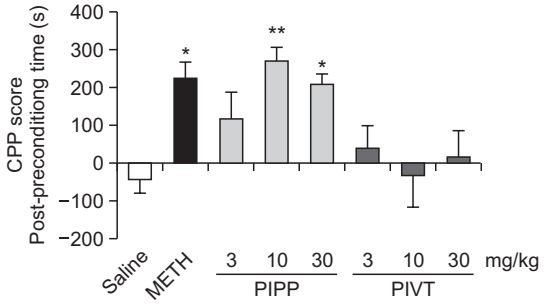

Fig. 2. The effects of PIPP and PIVT on the CPP test in mice. Each bar represents the mean ± SEM of the CPP score(s) calculated as the difference in the time spent in the drug-paired during the post- minus pre-conditioning phases.Values are mean ± SEM. n=6-8 animals per group. *p<0.05, **p<0.01 significantly different from the saline group (Dunnett's posttest).

Locomotor sensitization

Mouse locomotor activity was assessed in a square black Plexiglass container with an open field measuring 42×42×42 cm. A computer system (Ethovision, Noldus, Netherlands) was used to record the total distance moved (cm) and movement duration (sec) of each mouse.

This procedure was patterned after our previous study (Botanas *et al*., 2017). For the first two days, mice were habituated to the apparatus for 30 minutes. On the third day, the locomotor activity [distance moved (cm) and movement duration (sec)] was recorded as a baseline. Thereafter, the mice were treated with i.p. PIPP (1, 2, 4, 30 mg/kg) or PIVT (1, 2, 4 mg/kg), METH (1 mg/kg) or saline for seven days, and then challenged with the same drug and dose after a 7-day withdrawal. Locomotor activity was assessed for 30 minutes immediately after the first, third, and seventh day of drug treatment and on the challenge day.

Tissue collection, RNA preparation, and quantitative RT-PCR (qRT-PCR)

Mice treated PIPP or PIVT (30 mg/kg), METH (1 mg/kg), saline (n=6 animals per group) for 7 days were sacrificed and brains were removed for qRT-PCR analyses. After decapitation, brains were rapidly harvested and placed in ice-cold saline.The striatum was dissected and immediately frozen at −70°C until further use. Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA was further purified using the Hybrid-R™ kit (Geneall Biotechnology, Seoul, Ko-

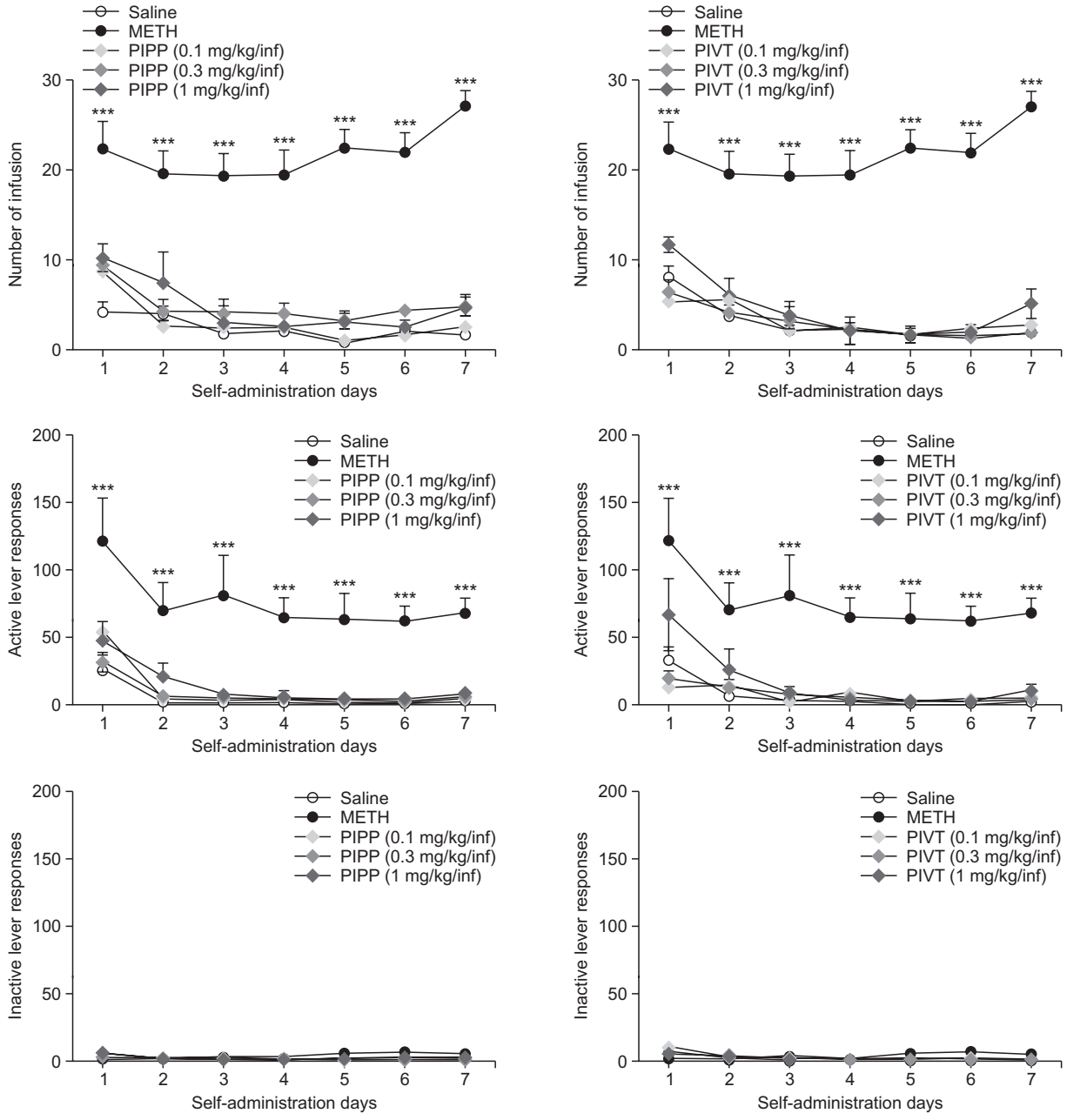

Fig. 3. The effects of PIPP and PIVT on the SA test in rats. Active and inactive lever responses made and number of infusions obtained during the 2-h, 7 days SA sessions under the FR1 schedule. Values are mean ± SEM. *n*=6-8 animals per group. ***p<0.001 relative to the saline group (Bonferroni's post hoc test).

rea). The total RNA concentration was determined using Colibri Microvolume Spectrometer (Titertek-Berthold, Pforzheim, Germany).

Quantitative real-time PCR (qRT-PCR) was conducted to determine the expression of dopamine-related genes−dopamine transporter (DAT), dopamine receptor 1 (DRD1), and dopamine receptor 2 (DRD2) - in the striatum of the mice. Briefly, 1 μg of total RNA was reverse transcribed into cDNA using AccuPower® CycleScript RT PreMix (Bioneer, Seoul, Korea), according to the manufacturer's instructions. The cDNA was amplified using a set of custom sequence-specific primers (Cosmogenetech, Seoul, Korea) (see Supplementary Table 1) and detected with SYBR Green (Solgent, Daejeon, Korea). The qRT-PCR reactions were performed in triplicates. Values were normalized to the relative amounts of GAPDH mRNA. Results are shown as a relative expression level calculated using the $2^{-\Delta\Delta CT}$ method (VanGuilder *et al*., 2008).

Data analysis

All data were presented as means and standard error of the mean (SEM). The CPP results were expressed as the difference in time spent in the drug-paired compartment during the

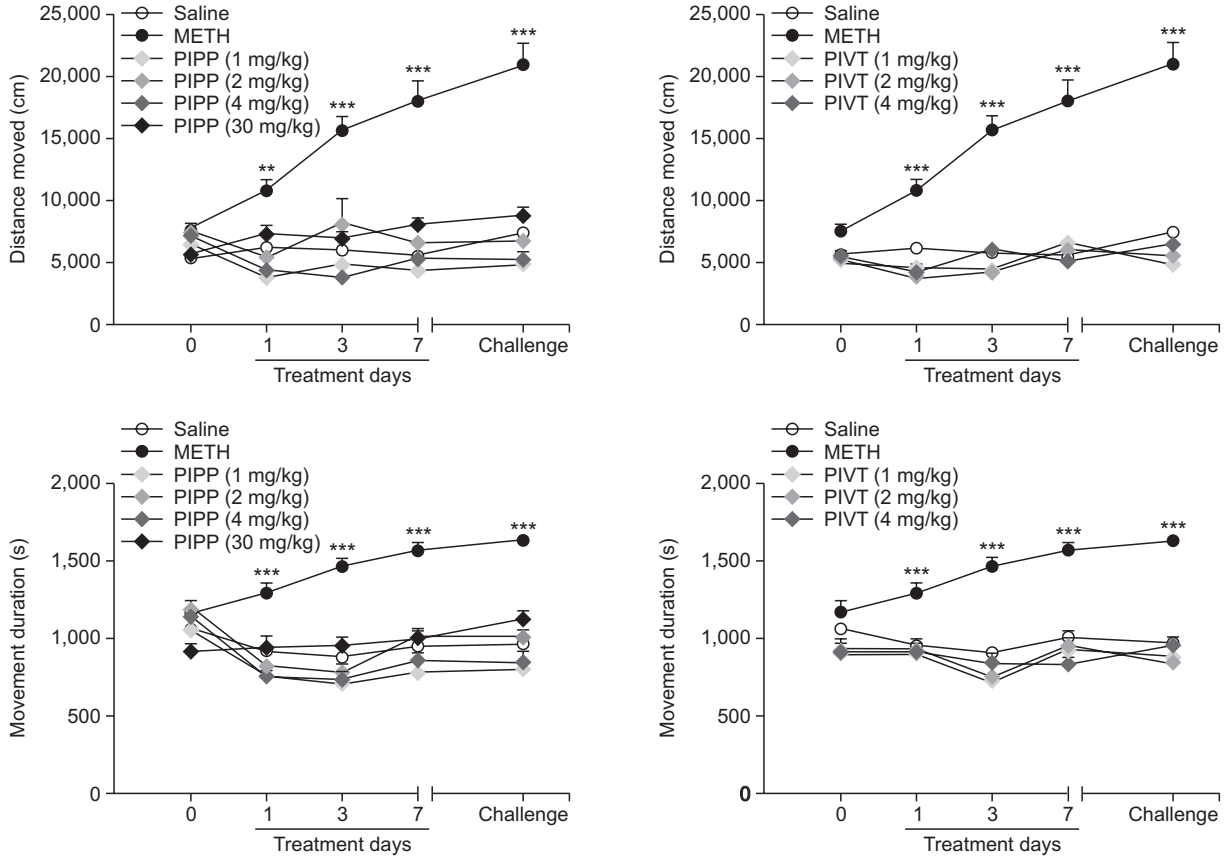

Fig. 4. The effects PIPP and PIVT on the locomotor activity (distance moved and movement duration) of the mice on the first, third, and seventh day of treatment and drug challenge following 7 days of abstinence. Values are mean ± SEM. *n*=10 animals per group. ***p*<0.01,****p*<0.001 relative to the saline group (Bonferroni's post hoc test).

pre- and post-conditioning phases. One-way analysis of variance (ANOVA) was used to determine the variation between treatment groups, followed by Dunnett's test to compare each group to the control group. The SA results were expressed as the number of lever responses (active and inactive lever) made and infusions obtained during the 2-hour daily SA sessions. A two-way repeated measures ANOVA with dosages as between-subject factor and SA days as a within-subject factor were employed. When significant results were obtained, posthoc comparisons were performed using Bonferroni's test. In locomotor sensitization, a two-way ANOVA with repeated measures was used with treatments as between-subject factor and test days as a within-subject factor during the periods of drug treatment and drug challenge. Bonferroni's test was used for further analyses. The results in qRT-PCR were analyzed using one-way ANOVA to determine the effects of treatments on the expression of dopamine-related genes. The accepted level of significance was setat $p<0.05$. GraphPad Prism software v. 4.01 (San Diego, CA, USA) was used for all statistical analyses.

RESULTS

Conditioned place preference

To evaluate the rewarding effects of PIPP and PIVT, CPP test was performed (Fig. 2). One-way ANOVA showed a significant variation between treatment groups [F (7, 62)=4.608, $p<0.001$]. Post-hoc testing revealed that mice treated with and PIPP at 10 ($p<0.01$) and 30 mg/kg ($p<0.01$) and METH ($p<0.05$) showed place preference.

Self-administration

To determine the reinforcing effects of PIPP and PIVT, SA test was conducted. Fig. 3 shows the number of infusion obtained and active and inactive lever responses by the mice self-administering saline, METH, or various dosages of PIPP and PIVT. In the number of infusion obtained, two-way ANOVA repeated measures displayed a significant effect of treatments [F (5, 252)=59.80, $p<0.001$], SA sessions [F (6, 252)=6.156, $p<0.001$], and interaction between the treatments and SA sessions [F (30, 252)=1.599, $p<0.001$] from the PIPP group. In a similar manner, a significant effect of treatments [F (4, 186)=94.54, $p<0.001$], SA sessions [F (6, 186)=8.674, $p<0.001$], and interaction between the two variables [F (24, 252)=1.917, $p<0.001$] were also observed in rats self-administering PIVT. However, only rats self-administering METH acquired significant number of infusion.

For the active lever responses, there was a significant variation in treatment groups [F (5, 252)=18.76, $p<0.001$] and SA sessions [F (6, 252)=12.96, $p<0.001$] in rats self-administering PIPP. A significant difference in treatment groups [F

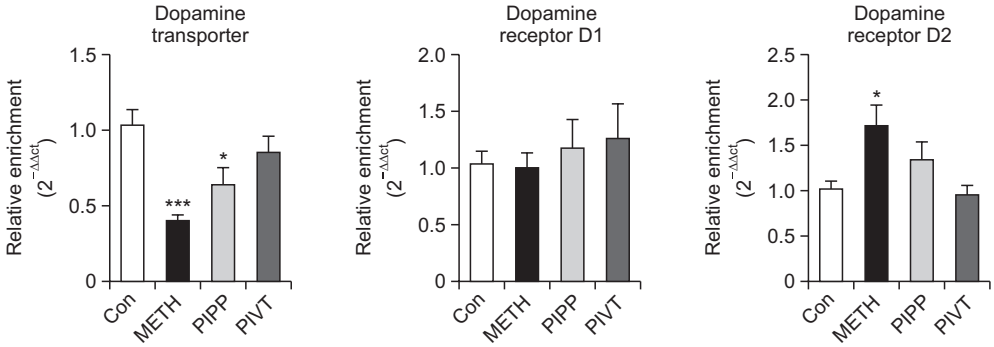

Fig. 5. The effects of PIPP and PIVT treatment for 7 days on dopamine transporter, dopamine receptor 1, and dopamine receptor 2 gene expression in the striatum of the mice. Values are mean ± SEM. *n*=6 animals per group. **p*<0.05, ****p*<0.001 significantly different from the saline group (Dunnett's posttest).

(4, 186)=15.46, *p*<0.001] and SA sessions [F (6, 186)=7.983, *p*<0.001] from the PIVT group. Only those rats that self-administered METH showed increase lever response. On the other hand, there was no significant difference in the inactive lever responses between treatment groups.

Locomotor sensitization

To assess the locomotor sensitization effects of PIPP and PIVT, the locomotor activity of the mice was monitored following repeated PIPP and PIVT administration and drug challenge (Fig. 4). Two-way ANOVA revealed a significant difference in treatment groups (distance moved [F (5, 216)=85.65, *p*<0.001], movement duration [F (5, 216)=82.61, *p*<0.001]) and treatment days (distance moved [F (4, 216)=12.76, *p*<0.001], movement duration [F (4, 216)=16.58, *p*<0.001]) and interaction between the treatment groups and treatment days (distance moved [F (20, 216)=10.26, *p*<0.001], movement duration [F (20, 216)=6.889, *p*<0.001]) in PIPP-treated mice. Similar results were also observed in mice treated with PIVT; significant variation in treatment groups (distance moved [F (4, 180)=113.1, *p*<0.001], movement duration [F (4, 180)=93.44, *p*<0.001]) and treatment days (distance moved [F (4, 180)=24.96, *p*<0.001], movement duration [F (4, 180)= 4.454, *p*<0.001]) and interaction between the treatment groups and treatment days (distance moved [F (16, 180)=14.14, *p*<0.001], movement duration [F (16, 180)=4.186, *p*<0.001]). However, further analysis showed that only METH-treated mice increased locomotor activity during the 7 days treatment and drug challenge.

qRT-PCR

To examine the effects of PIPP and PIVT on dopaminergic activity, dopamine-related genes (DAT, DRD1 and DRD2) in the striatum of mice treated with PIPP or PIVT through qRT-PCR analyses (Fig. 5). One-way ANOVA demonstrated significant effects of drugs [F (3, 23)=7.833, *p*<0.01] on DAT gene expression. Posttest revealed that PIPP and METH significantly decrease DAT expression in the striatum. On the other hand, DRD1 [F (3, 23)=0.3046, *p*>0.05] genes were significantly altered by the drug treatments. In DRD2, significant effects of drugs [F (3, 23)=4.207, *p*<0.05] were observed in the receptor's gene expression. Post-hoc comparisons showed that only METH increased DRD2 gene expression.

DISCUSSION

In this study, we synthesized two new synthetic cathinones with piperidine ring substitution on the nitrogen atom and evaluated their abuse potential through an array of animal models of drug addiction. The present results showed that PIPP induced CPP in mice, but not PIVT. However, neither PIPP nor PIVT was self-administered by the rats or produced locomotor sensitization in mice. qRT-PCR analysis demonstrated that PIPP, but not PIVT, significantly decreased DAT gene expression.

In our CPP tests, we have found that mice treated with 10 and 30 mg/kg of PIPP developed place preference for the drug, which suggests that PIPP has rewarding properties. This result corresponds with the findings of previous animal studies showing positive CPP for methcathinone or other synthetic cathinones (Lisek *et al*., 2012; Karlsson *et al*., 2014). Furthermore, it can be observed that the dose-effect curve for PIPP is inverted *U*-shaped function, with the low and high doses produced lesser CPP scores than the median dose. Similar inverted *U* dose-effect functions have also been reported for CPP with other psychostimulants like METH, cocaine, and amphetamine (Adriani and Laviola, 2003; Rodriguez-Alarcón *et al*., 2007; Zakharova *et al*., 2009). In relation to the CPP results, the qRT-PCR analysis also revealed that PIPP decreased the expression of DAT gene in the striatum. Although an increasing trend can be observed in DRD1 and DRD2, it did not reach statistical significance. METH-treated mice also demonstrated decreased DAT and increased DRD2 gene expression. These findings are in line with previous studies showing how repeated METH administration and other dopamine-acting drugs of abuse affect the expression of the genes above (Metzger *et al*., 2000; Zhu and Reith, 2008). Altered dopamine-related gene expressions are considered a manifestation of the dopaminergic activity of these drugs which may underlie their rewarding properties (Everitt and Robbins, 2013). Thus, it is possible that the rewarding effects induced by PIPP could be attributed to the decreased DAT gene expression in the striatum of the mice.

On the other hand, PIPP was not self-administered by the rats. This finding is rather interesting considering that PIPP was shown to produce CPP. Nevertheless, these discrepant results may highlight the importance of methodological variation when the rewarding effects of a drug are being evaluated

and that these two behavioral procedures represent different aspects of reward. In the CPP test, the subjective effects of the drug are present before the task; in the SA test, the subject is learning a task where responses produce near-immediate drug effects. Of these two assays, the latter is most likely to parallel the drug use in humans (Prus *et al*., 2009). For the limited information available regarding the disparity between the two procedures, we can only imply that PIPP may have the ability to produce CPP but lacks necessary persevering factor to support SA. In addition, PIPP failed to induce locomotor sensitization, even the highest dose (30 mg/kg) tested did not increase the locomotor of the mice. However, this result is similar to our previous study showing methoxetamine produced CPP albeit its inability to increase the locomotor activity of the rats (Botanas *et al*., 2015). Although it did not induce SA or locomotor sensitization, the CPP and qRT-PCR results could not be discounted. Thus, it may still be rational imply that PIPP may have potential for abuse.

On the other hand, mice treated with PIVT did not develop CPP or locomotor sensitization towards the drug. PIVT was not also self-administered by the rats. These results may indicate that PIVT has no rewarding and reinforcing effects in rodents. Moreover, qRT-PCR analyses revealed that repeated PIVT treatments did not significantly alter the expression of DAT, DRD1, and DRD2 gene in the striatum of the mice. Altogether, these data suggest that PIVT would likely have no or low potential for abuse.

In summary, the present findings showed that PIPP induced CPP, not PIVT. However, Neither PIPP nor PIVT supported SA or produced locomotor sensitization. On the other hand, PIPP decreased DAT gene expression, while PIVT was unable to alter dopamine-related genes in the striatum. These data indicate that PIPP has rewarding effects which may be attributed to the decreased DAT gene expression in the striatum. This also suggests that PIPP may have abuse potential. Additional studies are warranted to better understand the mechanism of action or pharmacokinetics of PIPP. The negative results of PIVT in the behavioral tests and qRT-PCR suggest that this drug would likely have no or low abuse potential. Nevertheless, the present study still advocates monitoring on synthetic cathinones with modifications similar to PIPP or PIVT. Finally, this study has provided information that might be useful in predicting the abuse potential of future synthetic cathinone entities with modifications on the nitrogen atom.

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