BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther, 578-585 (2017)

Evaluation of the Abuse Potential of Novel Amphetamine Derivatives with Modifications on the Amine (NBNA) and Phenyl (EDA, PMEA, 2-APN) Sites

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Abstract

Recently, there has been a rise in the number of amphetamine derivatives that serve as substitutes for controlled substances (e.g. amphetamine and methamphetamine) on the global illegal drug market. These substances are capable of producing rewarding effects similar to their parent drug. In anticipation of the future rise of new and similar psychoactive substances, we designed and synthesized four novel amphetamine derivatives with *N*-benzyl, *N*-benzylamphetamine HCl (NBNA) substituent on the amine region, 1,4-dioxane ring, ethylenedioxy-amphetamine HCl (EDA), methyl, para-methylamphetamine HCl (PMEA), and naphthalene, 2-(aminopropyl) naphthalene HCl (2-APN) substituents on the phenyl site. Then, we evaluated their abuse potential in the conditioned place preference (CPP) test in mice and self-administration (SA) test in rats. We also investigated the psychostimulant properties of the novel drugs using the locomotor sensitization test in mice. Moreover, we performed qRT-PCR analyses to explore the effects of the novel drugs on the expression of D1 and D2 dopamine receptor genes in the striatum. NBNA, but not EDA, PMEA, and 2-APN, induced CPP and SA in rodents. None of the test drugs have produced locomotor sensitization. qRT-PCR analyses demonstrated that NBNA increased the expression of striatal D1 dopamine receptor genes. These data indicate that NBNA yields rewarding effects, suggesting potential for abuse. Continual observation for the rise of related substances is thus strongly encouraged.

Key Words: Amphetamine derivatives, New Psychoactive Substances, Conditioned-place Preference, Self-administration, D1 & D2 Dopamine receptors, Abuse potential

INTRODUCTION

Over time, there has been a worldwide increase in the demand for and use of new psychoactive substances, specifically in the Asia-Pacific region (Ali, 2006; Kim *et al*., 2010; United Nations Office on Drugs and Crime, 2011, 2016; Tettey *et al*., 2013). Among these are classes of substances identified as amphetamine derivatives, which are commonly sold on the illegal drug market (e.g. smart shops, online) as 'legal highs' and/or party drugs (Drug Enforcement Administration, 2011). Amphetamine derivatives are synthetic substances that contain an amphetamine backbone, which include, but are not limited to, CNS stimulants (e.g. amphetamine, methamphetamine), hallucinogens (e.g. 3,4-methylenedioxy-methamphetamine [MDMA]), and entactogens (e.g., 3,4-methylenedioxy-amphetamine [MDA]) (Kalant, 2001; Berman *et al*., 2008, 2009). These substances exhibit profound psychostimulant properties, which render them liable to recreational abuse (Sitte and Freissmuth, 2015). Strict regulations (Drug Enforcement Administration, 2011) on specific substances (e.g. am-

Open Access **https://doi.org/10.4062/biomolther.2017.141**

Received Jul 17, 2017 **Revised** Jul 26, 2017 **Accepted** Aug 14, 2017
Published Online November 1, 2017

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phetamines) have led to the creation and synthesis of related compounds capable of producing similar pharmacological effects (Dean *et al*., 2013).

Several studies have indicated that modifications of the amphetamine base structure typically occur at the amine and phenyl sites via substitution of methyl and ethyl groups (Flomenbaum e*t al.*, 2006; Schatzberg and Nemeroff, 2009). Substances with these modifications have been reported to produce rewarding effects. For instance, methamphetamine (METH), a substance with *N*-methyl substituent at the amine site of amphetamine, produces psychostimulant effects and is highly addictive (Matsumoto *et al*., 2014). Moreover, it has also been reported that amphetamine derivatives affect dopamine levels in the brain, which contributes to their rewarding effects. Similarly, substances that affect the dopamine system are likely to be abused by humans (Lüscher and Malenka, 2011; Kang *et al*., 2016). Given these observations, it appears that the addition of substituents to the amine and phenyl structure may induce rewarding effects.

In anticipation of the future rise of new and similar psychoactive substances, we synthesized four novel amphetamine derivatives with substituents in the amine [N-benzylamphetamine HCl (NBNA)], and phenyl sites [ethylenedioxy-amphetamine HCl (EDA); para-methylamphetamine HCl (PMEA); 2-(aminopropyl)naphthalene HCl (2-APN)] of amphetamine (Fig. 1). We assessed the ability of these derivatives to produce rewarding and reinforcing effects with conditioned place preference and self-administration paradigms. We also performed a locomotor sensitization test over 7 days of drug treatment and during a drug challenge. Finally, we used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to examine the expression of the D1 and D2 dopamine receptor genes in the striatum in response to these derivatives. Methamphetamine (METH) was used as a positive control.

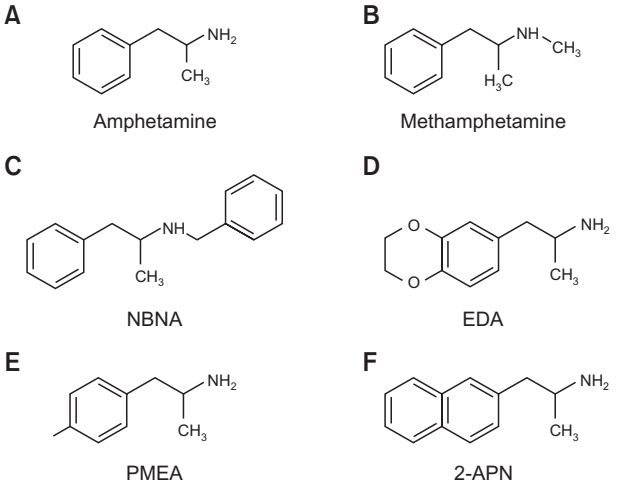

Fig. 1. The general chemical structure of (A) Amphetamine, (B) Methamphetamine, and novel amphetamine derivatives: (C) NBNA (N-benzylamphetamine HCl); (D) EDA (ethylenedioxy-amphetamine HCl); (E) PMEA (para-methylamphetamine HCl), and (F) 2-APN (2-(aminopropyl) naphthalene HCl).

MATERIALS AND METHODS

Animals

All animals were obtained from Hanlim Animal Laboratory Co. (Hwasung, Korea) and were housed in a temperature- and humidity-controlled animal room (temperature: 22 ± 2°C, relative humidity: 55 ± 5%) with a 12/12 h light/dark (07:00-19:00 h light) cycle. Male C57BL/6J mice were used in the CPP and locomotor sensitization tests. ICR mice were used for the qRT-PCR analysis. All mice were housed 6 per cage. Sprague-Dawley rats were caged individually and were used in the self-administration test. They were habituated in the animal room for 5 days prior to experiments. They had free access to food and water, except when the rats were engaged in lever training and actual SA sessions. The use of animals in this study was in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 85-23, 1985 revision) and the Animal Care and Use Guidelines of Sahmyook University.

Drugs

***N*-benzylamphetamine HCl (NBNA HCl):** NBNA HCl was synthesized by the reductive amination of phenylacetone with benzylamine and NABH4 as described previously, followed by treatment with HCl salt (Taniguchi e*t al.*, 2010). Its structure was confirmed by the following spectroscopic analyses: 1H NMR (400 MHz, D2O) δ 7.37-7.10 (m, 10H), 4.22-4.12 (m, 2H), 3.55-3.47 (m, 1H), 3.08 (dd, J=13.6 Hz, 5.6 Hz, 1H), 2.77 (dd, J=13.6 Hz, 8.8 Hz, 1H), 1.21 (d, J=6.5 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 136.2, 131.6, 129.8 (2C), 129.5, 129.2 (2C), 129.1 (2C), 128.8 (2C), 127.2, 55.8, 48.7, 39.0, 14.9; HR-MS calculated for C16H20ClN2 [M-Cl]+ was 226.1590, actual result was 226.1565.

Ethylenedioxy-amphetamine HCl (EDA HCl): EDA HCl was synthesized from 1,4-benzodioxan-6-carboxaldehyde as described previously (Robaa *et al*., 2011). Briefly, 1,4-benzodioxan-6-carboxaldehyde was condensed with nitroethane and then reduced with LiAlH4 to produce 1-(2,3-dihydro[1,4] dioxin-6-yl)propan-2-amine. The resulting compound was treated with HCl to produce EDA HCl. Its structure was confirmed by the following spectroscopic analyses: 1H NMR (400 MHz, CD3OD) δ 6.83 (d, J=8.2 Hz, 1H), 6.76 (d, J=2.1 Hz, 1H), 6.71 (dd, J=8.2 Hz, 1H), 4.24 (s, 4H), 3.51-3.42 (m, 1H), 2.85 (dd, J-13.7 Hz, 6.4 Hz, 1H), 2.71 (dd, J=13.7 Hz, 8.0 Hz, 1H) 1.27 (d, J=6.5 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 144.1, 143.2, 129.1, 121.9, 117.8, 117.4, 65.4, 64.4, 49.2, 39.9, 17.1; HRMS calculated for C11H16ClNO2 [M-Cl]+ was 194.1176, actual result was 194.1164.

***Para*-methylamphetamine HCl (PMEA HCl):** PMEA HCl was synthesized in three steps from p-tolualdehyde as described previously (Vallejos *et al*., 2005). Briefly, p-tolualdehyde was condensed with nitroethane and then reduced with LiAlH4 to produce 1-(p-tolyl)propan-2-amine. The resulting compound was treated with HCl to generate PMEA HCl. Its structure was confirmed by the following spectroscopic analyses: 1H NMR (400 MHz, CD3OD) δ 7.19 (d, J=8.1 Hz, 2H), 7.15 (d, J=8.1 Hz, 2H), 3.53-3.48 (m, 1H), 2.96 (dd, J=13.6 Hz, 6.2 Hz, 1H), 2.77 (dd, J=13.6 Hz, 8.3 Hz, 1H), 2.34 (s, 3H) 1.26 (d, J=6.7 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 136.9, 133.1, 129.4(2C), 139.1(2C), 49.2, 40.2, 20.0, 17.1; HRMS calculated for C10H16ClN [M-Cl]+ was 150.1277, actual result was 150.1195.

2-(Aminopropyl)naphthalene HCl (2-APN HCl): 2-APN HCl was synthesized from 2-naphthaldehyde as described previously (Vallejos e*t al.*, 2005). In brief, 2-naphthaldehyde was condensed with nitroethane and then reduced with LiAlH4 to give 1(naphthalene-2-yl)propan-2-amine. The resulting compound was treated with HCl to generate 2-APN HCl. Its structure was confirmed by the following spectroscopic analyses: 1H NMR (400 MHz, CD3OD) δ 7.90-7.85 (m, 3H), 7.77 (s, 1H), 7.52-7.49 (m, 2H), 7.42 (dd, J=8.4 Hz, 1.4 Hz, 1H), 3.69-3.64 (m, 1H), 3.17 (dd, J=13.6 Hz, 6.3 Hz, 1H), 3.00 (dd, J=13.6 Hz, 8.1 Hz, 1H), 1.33 (d, J=6.5 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 133.9, 133.7, 133.0, 128.6, 128.0, 127.6, 127.5, 127.0, 126.2, 125.8, 49.0, 40.8, 17.3; HRMS calculated for C13H16ClN [M-Cl]+ was 186.1277, actual result was 186.1218.

Methamphetamine was purchased from Sigma (St. Louis, MO, USA). All drugs were diluted in 0.9% sterile saline solution and administered intraperitoneally (i.p.) (CPP test and locomotor sensitization) or intravenously (SA test). All dosages of drugs (METH, NBNA, EDA, PMEA and 2-APN) used in the present study were based on previously published findings evaluating the rewarding and reinforcing properties of amphetamine derivatives (Marona-Lewicka e*t al.*, 1996; Cain e*t al.*, 2008; Funada e*t al.*, 2014).

Conditioned-place preference test

Apparatus: The CPP apparatus consisted of two large compartments measuring 17.4×12.7×12.7 cm^3. Each compartment provided distinct visual and tactile cues, with one white compartment containing a stainless steel mesh floor (6.352 mm), and another black compartment with a stainless steel grid floor (rods 3.2 mm in diameter positioned 7.9 mm apart). Each compartment also contained Plexiglass covers and illuminating lights. A guillotine door provided access to both compartments. Animal movements were detected by infrared beams and were recorded, quantified, and analyzed with a computer program.

Procedure: The test consisted of three phases: (1) habituation and pre-conditioning, (2) conditioning, and (3) post-conditioning. During the habituation phase, mice were given free access to both compartments for 20 min on two consecutive days. The pre-conditioning phase was begun the following day, where the time spent on each side was recorded. Subjects were assigned to groups based on the data obtained from the pre-conditioning phase. Mice that spent over 840 s in one of the compartments were excluded from the subsequent phases. During the conditioning phase, subjects received an i.p. injection of NBNA, EDA, 2-APN, PMEA (1, 10, 30 mg/kg), METH (1 mg/kg), or saline, and were randomly placed in one of the compartments for 45 min. On alternate days, mice received saline injections and were confined in the compartment opposite of the drug-paired compartment. Immediately following the last conditioning day, the post-conditioning phase began in which mice were drug-free and allowed to access both compartments for 20 minutes, similar to the pre-conditioning phase.

Locomotor sensitization

Apparatus: The locomotor activity of the mice was assessed in a square black Plexiglas container with an open-field arena (42×42×42 cm^3). A computer system (Ethovison, Noldus, Netherlands) was utilized to record the total distance moved (cm) and the movement duration(s) of each mouse.

Procedure: This behavioral assay consisted of four phases: habituation, drug treatment, drug abstinence, and drug challenge. For the first two days, mice were habituated to the apparatus for 30 min. On the third day, locomotor activity was recorded and used as a baseline parameter. Thereafter, 1, 3, or 10 mg/kg NBNA, EDA, PMEA, or 2-APN, 1 mg/kg METH, or saline was administered to the mice for 7 days. Mice were then challenged with the same drug and dose after 7 days of abstinence. Locomotor activity was assessed for 30 min immediately following the first, third, and seventh day of both drug treatment and abstinence, as well as on the challenge day.

Self-administration test

Apparatus: To conduct the SA Test, standard operant chambers (Coulborn Instruments, Allentown, PA, USA) were kept inside sound-attenuating boxes with built-in ventilation fans. Each operant chamber contained a pellet dispenser, left and right response levers (4.5 cm long), a stimulus light source situated above the left lever, and a centrally located house light (2.5 W, 24 V) on top of the chamber. Downward pressure (25 g) on a lever resulted in an automated consequence. Adjacent to each operant chamber was a mechanically operated syringe pump that transported solutions at a flow rate of 0.01 ml/s through Teflon tubes that were attached to the animal's IV-catheter, which was then connected to a swivel system that allowed free movements for rats. Built-in Graphic State Notation software (Coulborn Instruments) allowed automatic control over the experimental parameters and the collection of data.

Procedure: Initial training on drug-paired lever pressing was conducted for three consecutive days (30 min/day) for a contingent food pellet reward on a continuous schedule of reinforcement. Rats that obtained >80 pellets on the last day of training were selected and prepped for surgery. Essential perioperative techniques were carried out as previously described (de la Peña e*t al.*, 2013). During the recovery phase, rats were housed individually over a 5-day recovery period. After the recovery period, rats were placed on a consumable daily diet of pellets (approximately 20 g) and were subjected to a 2 h-daily SA session under a fixed-ratio (FR) 1 schedule for 7 consecutive days. During the SA sessions, both levers (right/left) were available. A press on the left lever (active lever) resulted in a delivery of 0.1 ml of NBNA (0.1, 0.3, or 1 mg/kg/infusion), EDA (0.1, 0.3, or 1 mg/kg/infusion), PMEA (0.1, 0.3, 1 mg/kg/infusion), 2-APN (0.1, 0.3, 1 mg/kg/infusion) or saline. Simultaneously, the house light was switched off, and the stimulus light was illuminated and remained lit for 20 s after the end of the infusion (the time-out period). Lever presses during periods of "time-out" were recorded but did not have any resultant effects. Right lever presses (inactive) were recorded but not reinforced. The catheter tubing system was checked for patency by the instillation of 0.1 ml of thiopental sodium (10 mg/kg) one day before and on the last day of the SA test. Rats that did not lose muscle tone within 3-5 s were excluded from the experiment.

Tissue collection, RNA extraction, and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Mice treated with 10 mg/kg NBNA, EDA, PMEA & 2-APN,

and saline for 7 days were sacrificed (n=6 animals per group). Brains were isolated and transferred to ice-cold saline in preparation for qRT-PCR analysis. The striatum was carefully dissected and placed in a deep freezer (-80°C) until usage. Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to guidelines set by the manufacturer. A Hybrid-RTM Kit (Geneall Biotechnology, Seoul, Korea) was used for further RNA purification. The total RNA concentration was determined with a Colibri Microvolume Spectrometer (Titertek-Berthold, Pforzheim, Germany).

Quantitative real time-PCR (qRT-PCR) was utilized to identify and measure the expression level of dopamine-related genes, including the D1 and D2 dopamine receptors, in the mouse brain. One microgram of total RNA was reversely transcribed into cDNA using AccuPower CycleScript RT Premix according to the manufacturer's guidelines (Bioneer, Seoul, Korea). The cDNA amplification was performed with custom-made sequence-specific primers (Cosmogenetech, Seoul, Korea) (see Supplementary Table 1) and was detected with SYBR Green (Solgent, Korea). The qRT-PCR analysis was performed in triplicate and the values were normalized to the mRNA levels of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Relative expression levels were calculated using the $2^{-\Delta\Delta}$Ct method.

Data analysis

All data are represented as mean and standard error of the mean (SEM). For the CPP tests (Fig. 2), results are expressed as the difference between the durations spent in the drug-paired compartment during the pre-and post-conditioning periods. One-way analysis of variance (ANOVA) was used to compare between group differences, followed by Dunnett's post-hoc test to compare individual groups to the control group. In the locomotor sensitization test (Fig. 3), data during treatment and abstinence (days) were analyzed using

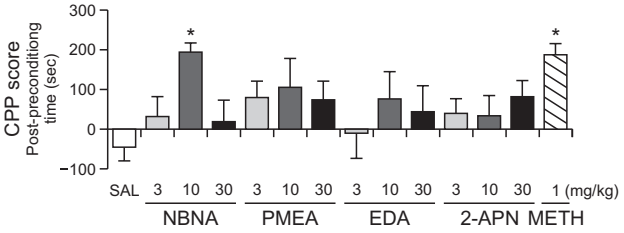

Fig. 2. The effects of NBNA, PMEA, EDA, 2-APN and METH on the CPP test in mice. Each bar represents the mean ± SEM of the difference in the time spent in the drug-paired or saline-paired side during the post- minus the preconditioning phases. *n*=7-8 animals per group. **p*<0.05, significantly different from the SAL group (Dunnett's posttest).

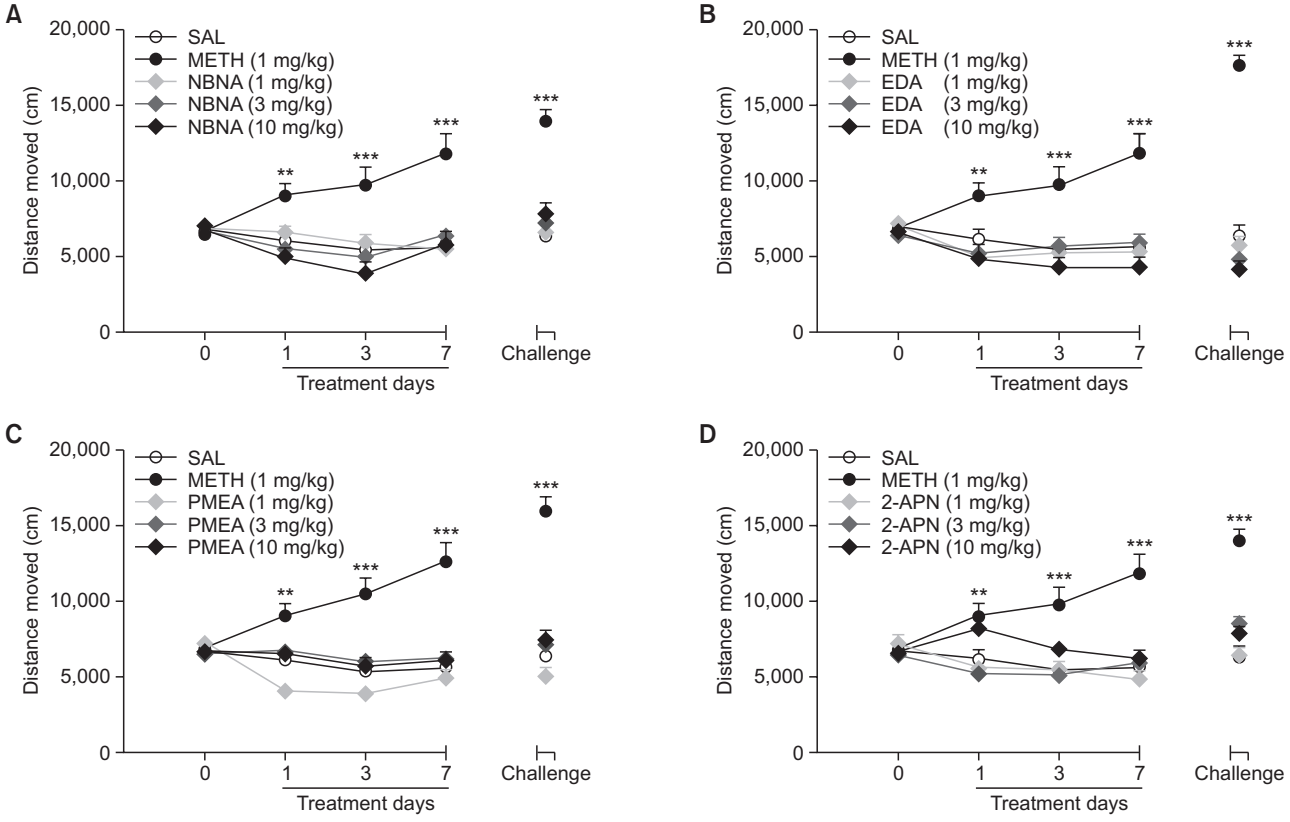

Fig. 3. The effects of NBNA, EDA, PMEA, 2-APN and METH on the locomotor activity during 7 days of treatment and on the day of challenge. Mice were treated with vehicle, NBNA, EDA, PMEA, 2-APN (1, 3, 10 mg/kg) or METH (1 mg/kg) repeatedly for 7 days, and then challenge with the same drug and dose following a 7-day withdrawal. Locomotor activity was assessed before drug treatment, on the 1st, 3rd, and 7th day of treatment and on the day of challenge. Values are (mean ± SEM). n=8-10 animals per group. ***p*<0.01, ****p*<0.001 significantly different from the SAL group (Bonferroni's posttest).

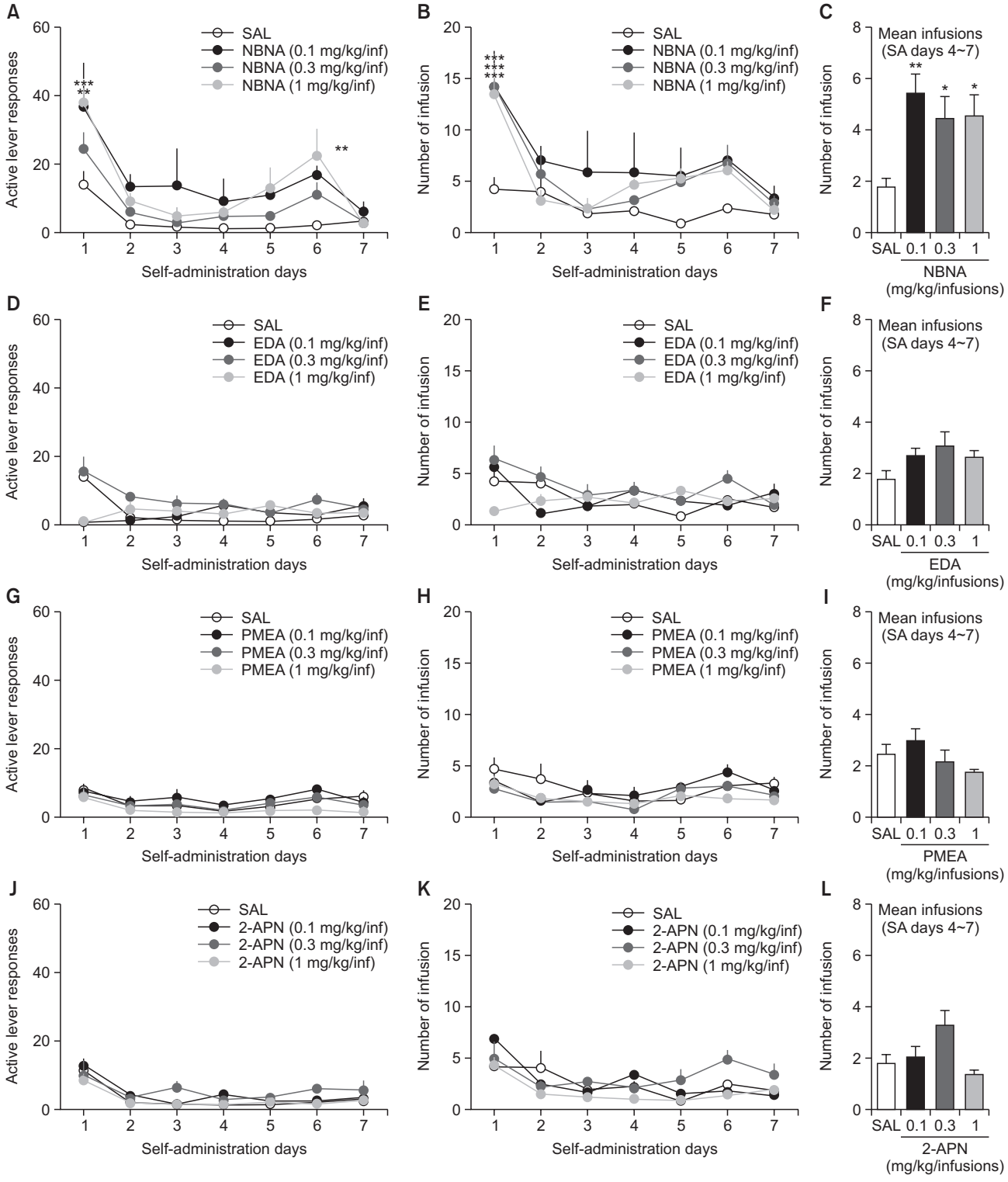

Fig. 4. The effects of NBNA, EDA, PMEA, 2-APN on the SA in rats. Active lever responses made (A, D, G, J) and number of infusions (B, E, H, K) obtained during the 2-H, 7 days SA sessions under the FR1 schedule. The mean number of infusion during stable days (4th to 7th day) of SA was also presented (C, F, I, L). Values are mean ± SEM. n=6-8 animals per group. *p<0.05, **p<0.01, ***p<0.001 relative to SAL group [Active lever presses and infusions (Bonferroni's posttest) and Mean infusions (T-test)].

two-way ANOVA repeated measures, with treatments as the between-subjects factor, and test days as the within-subjects factor. Bonferroni's test was used for analyses of multiple comparisons. For the SA test, active lever responses and the number of infusions during the 2-h, 7-day sessions under a FR1 schedule were analyzed using repeated measures two-way ANOVA. Post-hoc comparisons were performed with Bonferroni's test. The mean infusion during stable days (days 4-7) was also presented and analyzed with unpaired, two tailed *t*-test. In Fig. 5, qRT-PCR results were analyzed using one-way ANOVA to determine the effects of treatments on the expression of D1 and D2 dopamine receptor genes in the striatum. Dunnett's post-test was utilized to compare results between the saline group and the treatment groups.

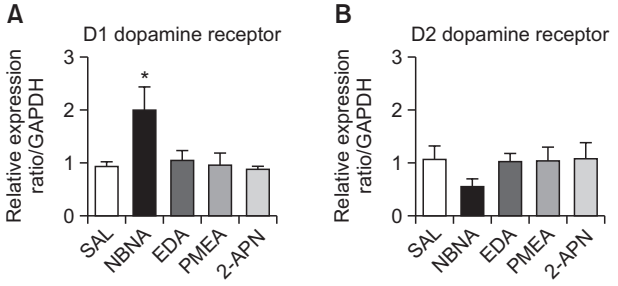

Fig. 5. The effects of NBNA, EDA, PMEA, and 2-APN treatment for 7 days on dopamine receptors D1 and D2 gene expression in the striatum of the mice. Values are mean ± SEM. n=6 animals per group. *p<0.05, significantly different from the SAL group (Dunnett's posttest).

RESULTS

Conditioned place preference test

Fig. 2 shows the CPP scores of mice treated with saline, METH and the test drugs (i.e. NBNA, EDA, PMEA & 2-APN). A one-way ANOVA revealed a significant difference between the treatment groups [F (13, 131)=1.841; p<0.05]. Post-hoc analyses revealed that mice conditioned with 10 mg/kg NBNA and 1 mg/kg METH had significantly higher CPP scores than the saline group.

Locomotor sensitization test

Fig. 3 shows the locomotor activity (distance moved) of mice during the 7-day treatment and on the day of challenge. A two-way ANOVA revealed that treatment with METH significantly increased the distance moved (METH vs. NBNA vs. SALINE: F (4, 180)=10.34, p<0.001 [Fig. 3A]; METH vs. EDA vs. SALINE: F (4, 180)=5.728, p<0.001 [Fig. 3B]; METH vs. PMEA vs. SALINE: F (4, 180)=9.795, p<0.001 [Fig. 3C]; and METH vs. 2-APN vs. SALINE: F (4, 180)=10.24, p<0.001 [Fig. 3D]) of the mice.

Self-administration test

Fig. 4 shows the number of infusions obtained and the active and inactive lever responses by the rats that self-administered saline and various dosages of NBNA, EDA, PMEA, and 2-APN. A repeated measures two-way ANOVA revealed a significant difference in treatment [F (3, 132)=9.130, p<0.001] and SA days [F (6, 132)=15.81, p<0.001] when NBNA was compared to saline. Bonferroni post-tests revealed an increase in lever pressing during the first day of SA with exposure to 0.1 mg/kg/infusion (p<0.01) and 1 mg/kg/infusion (p<0.001) (Fig. 4A). A significant difference was also observed on the sixth day (p<0.01) of exposure to 1 mg/kg/infusion. A significant difference in the number of infusions by treatment [F (3, 132)=5.871, p<0.01] and SA days [F (6, 132)=14.98, p<0.001] was also observed. Post-hoc tests revealed that there was an increase in the number of infusions during the first day of the SA test across all drug dosages [0.3 mg/kg/infusion (p<0.001), 0.1 mg/kg/infusion (p<0.001) and 1 mg/kg/infusion (p<0.001)] (Fig. 4B). Moreover, unpaired t-tests revealed that during the stable SA days (days 4-7), there was a significant increase in infusions at dosages of 0.1 mg/kg/infusion (p<0.01), 0.3 mg/kg/infusion (p<0.05), and 1 mg/kg/infusion (p<0.05) as compared to the saline group (Fig. 4C). Self-administration of EDA led to a significant difference in treatment [F (3, 132)=4.479, p<0.05], and post-hoc tests revealed that EDA increased the number of lever presses during the first day of SA when administered at dosages of 0.1 mg/kg/infusion (p<0.001) and 1 mg/kg/infusion (p<0.001). There were no differences, however in the number of infusions, and a t-test of mean infusions from days 4-7 also revealed no differences (p>0.05). There were no significant differences between rats self-administering PMEA and 2-APN in the number of lever presses or mean infusions (p>0.05), and t-tests revealed no differences in mean infusions when compared to the saline group (p>0.05).

qRT-PCR

Fig. 5 illustrates the effects of test drugs on D1 and D2 dopamine receptor gene expression in the striatum. A one-way ANOVA showed a significant difference between treatment groups [F (4, 25)=3.857; p<0.05]. Post-hoc revealed that NBNA significantly increased (p<0.05) the expression of D1 dopamine receptor genes as compared to saline. None of the treatments significantly altered the gene expression of D2 dopamine receptor.

DISCUSSION

In the present study, we assessed the ability of four novel amphetamine derivatives to induce rewarding and pyschostimulating effects in rodents and affect the expression of dopamine-related genes in the striatum. The results showed that only NBNA produced CPP in mice and SA in rats. NBNA also significantly increased the expression of striatal D1 dopamine receptor genes. None of the test drugs induced locomotor sensitization in mice.

In the current experiment, mice treated with 10 mg/kg NBNA developed a place preference towards the drug. This result is consistent to that of a previous study (Marona-Lewicka e*t al.*, 1996) in which *N-methyl-1,3-benzodioxolylbutanamine* (MBDB), a substance with modification in the amine site, led to the development of CPP. Interestingly, the dose of NBNA that produced CPP (10 mg/kg) is also similar to that of MBDB (Marona-Lewicka e*t al.*, 1996). Moreover, NBNA was self-administered by rats, although modest, as evidenced by an elevated mean number of infusions during stable SA days (days 4-7) (Fig. 4C). These results suggest that NBNA has rewarding effects in mice. In addition, we found that NBNA increased striatal D1 dopamine receptor gene expression (Fig.

5). Altered gene expression is one mechanism by which drugs of abuse can alter behavior, and D1 dopamine receptors are key genetic players. Thanos *et al*. (2001) showed that an increase in the availability of dopamine during psychostimulant use stimulates the postsynaptic dopamine receptors, particularly D1 dopamine receptors. Furthermore, an increase in D1 receptor activity has been implicated in the development of addiction and has contributed to the expression of drug "reward" (McPherson and Lawrence, 2007). Taken together, the present results suggest that NBNA has rewarding properties which may be attributed to its ability to increase D1 dopamine receptor in the striatum.

However, the locomotor sensitization test revealed that NBNA failed to alter the motor activity of mice. This result is in contrast to the well-characterized locomotor sensitization effects of amphetamine and methamphetamine (Moore e*t al.*, 1995; Salomon e*t al.*, 2006). However, other studies have also reported that some amphetamine derivatives do not produce locomotor stimulating effects (Halberstadt e*t al.*, 2013). These results suggest that, despite their chemical similarities, amphetamine derivatives have varying pharmacological effects.

In connection with this reasoning, the amphetamine derivatives EDA, PMEA, and 2-APN failed to induce CPP and locomotor sensitization in mice and were not self-administered by rats. These results may indicate that these substances do not have rewarding and reinforcing effects. Accordingly, qRT-PCR analyses revealed that these drugs did not alter the expression of D1 and D2 dopamine receptor genes in the striatum. Altogether, the data suggest that EDA, PMEA, and 2-APN have no abuse potential.

In conclusion, we have found that amphetamine derivative NBNA, but not EDA, PMEA, and 2-APN, produced rewarding effects in rodents. Similarly, only NBNA increased the expression of the D1 dopamine receptor gene in the striatum. These data indicate that NBNA has the potential for abuse. The findings of the present study provide important insights that might be useful in predicting the abuse potential of novel and future amphetamine derivatives.

CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Food and Drug Safety (MFDS) of Korea (14182MFDS979) and the National Research Foundation of Korea (NRF-2017R1D1A1A02018695).

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