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Cardiotoxic effects of [3-[2-(diethylamino)ethyl]-1*H*-indol-4-yl] acetate and 3-[2-[ethyl(methyl)amino]ethyl]-1*H*-indol-4-ol

Kyung Sik Yoon, Jin-Moo Lee, Young-Hoon Kim, Soo Kyung Suh, Hye Jin Cha*

National Institute of Drug and Safety Evaluation, Ministry of Food and Drug Safety, Osong, Cheongju, Republic of Korea

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ABSTRACT

Two synthetic tryptamines, namely [3-[2-(diethylamino)ethyl]-1*H*-indol-4-yl] acetate (4-AcO-DET) and 3-[2-[ethyl(methyl)amino]ethyl]-1*H*-indol-4-ol (4-HO-MET), are abused by individuals seeking recreational hallucinogens. These new psychoactive substances (NPSs) can cause serious health problems because their adverse effects are mostly unknown. In the present study, we evaluated the cardiotoxicity of 4-AcO-DET and 4-HO-MET using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, electrocardiography (ECG), and the human ether-a-go-go-related gene (hERG) assay. In addition, we analyzed the expression level of p21 (CDC42/RAC)-activated kinase 1 (PAK1), which is known to play various roles in the cardiovascular system. In the MTT assay, 4-AcO-DET- and 4-HO-MET-treated H9c2 cells proliferated in a concentration-dependent manner. Moreover, both substances increased QT intervals (as determined using ECG) in Sprague–Dawley rats and inhibited potassium channels (as verified by the hERG assay) in Chinese hamster ovary cells. However, there was no change in PAK1 expression. Collectively, the results indicated that 4-AcO-DET and 4-HO-MET might cause adverse effects on the cardiovascular system. Further studies are required to confirm the relationship between PAK1 expression and cardiotoxicity. The findings of the present study would provide science-based evidence for scheduling the two NPSs.

1. Introduction

The United Nations Office on Drugs and Crime (UNODC) first defined designer drugs in the early 2000s as “novel chemical substances with psychoactive properties, designed on the basis of the chemical structure of a given parent drug and synthesized specifically for sale on the illicit market and to circumvent regulations on controlled substances” (United Nations Office on Drugs and Crime, 2003). Later, as “designer drugs” gained tremendous global popularity, the UNODC introduced a new term, “new psychoactive substances” (NPSs), and defined it as “substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat” (UNODC). Because numerous NPSs have been introduced to and have vanished from underground markets since their emergence, they have become serious health and social threats (Ledberg, 2015; Yoon et al., 2019a). The NPS phenomenon is particularly concerning because these substances are gaining popularity among youths, possibly because of their relatively low price and high availability (Badila et al., 2015; Bae et al., 2018).

Among different NPS categories, tryptamines, which belong to serotonergic hallucinogens, are known as 5-HT_{2A} receptor agonists, and they alter sensory perception and mood (Araujo et al., 2015). The basic chemical structure of tryptamines comprises tryptophan (Araujo et al.,

Abbreviations: 4-AcO-DET, [3-[2-(Diethylamino)ethyl]-1*H*-indol-4-yl] acetate; 4-HO-MET, 3-[2-[Ethyl(methyl)amino]ethyl]-1*H*-indol-4-ol; 4-HO-DMT, 3-[2-(Dimethylamino)ethyl]-1*H*-indol-4-ol; AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; ANOVA, Analysis of variance; ATCC, American Type Culture Collection; CHO, Chinese hamster ovary; DMEM, Dulbecco’s modified Eagle’s medium; ECG, Electrocardiogram, electrocardiography; HI FBS, Heat-inactivatedfetal bovine serum; hERG, Human ether-a-go-go-related gene; HRP, Horseradish peroxidase; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; I_{kr}, Rectifier potassium channel; MFDS, Ministry of Food and Drug Safety; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NPS, New psychoactive substance; PAK1, P21 (CDC42/RAC)-activated kinase 1; S.E., Standard error; SD, Sprague–Dawley; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; UNODC, United Nations Office on Drugs and Crime

* Corresponding author at: Pharmacological Research Division, National Institute of Drug and Safety Evaluation, Ministry of Food and Drug Safety, Osong Health Technology Administration Complex, 187, Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, 28159, Republic of Korea.

E-mail addresses: kof9696@korea.kr (K.S. Yoon), elzem@korea.kr (J.-M. Lee), k1631@korea.kr (Y.-H. Kim), suhsk@korea.kr (S.K. Suh), chahj1@korea.kr (H.J. Cha).

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2015; Fantegrossi et al., 2008). Tryptamines have an indole ring structure connected to an amino group by two-carbon side chain (Tittarelli et al., 2015), and the general alteration in the molecule that results in the hallucinogenic properties of these substances occurs in the terminal amine or through substitution at the fourth or fifth position of the ring (Countyourculture, 2012; Jebadurai, 2012).

In general, tryptamines exist in both natural and artificial forms. Natural tryptamines have a long history of use; for example, they have been used as an ingredient in '*Ayahuasca*,' a hallucinogenic brew used in the religious rituals of certain tribes (Ujvary, 2014). Synthetic tryptamines pose health threats similar to those of other NPS classes. As the NPS phenomenon spread globally, several researchers have developed synthetic tryptamines by altering the parent structure of tryptamine to attract users who sought recreational hallucinogens (Araujo et al., 2015). There is one comprehensive review on tryptamine derivatives describing their chemistry and pharmacological and toxicological properties in a general context (Araujo et al., 2015). According to the article, tryptamine derivatives exert their hallucinogenic effects by binding to the 5-HT_{2A} receptors, and several typical behaviors were observed in rodents treated with tryptamines, such as neophobia and agoraphobia. The other adverse effects observed in experimental animals include attention deficiency (Compton et al., 2011), impairment of certain types of cognition, and loss of control of body temperature (Williams et al., 2007). Because most synthetic tryptamines are prohibited for human use, there are limited systemic human studies. The adverse effects of synthetic tryptamines in humans are only deducible from subjective effects described in anecdotal user reports. According to those reports, the substances induce unwanted effects such as restlessness, anxiety, tension, vomiting, palpitation, muscle pain, bruxism, headache, frightening or distressing hallucinations, severe fear, abdominal discomfort, and respiratory discomfort or distress (Greene, 2013). Even worse, some tryptamines cause serious intoxication and fatal consequences (Erowid, 2019a, 2019b; Tittarelli et al., 2015). Among the various toxicities and adverse effects associated with synthetic tryptamines, cardiotoxicity is one of the most critical, because it is life threatening. Regarding the cardiotoxicity of synthetic tryptamines, myocardial infarction, hypertension, migraine, and vasoconstriction, have been reported (Anwar et al., 2013; Wust et al., 2017). However, the majority of reports on the adverse effects of NPSs have focused on neuropsychiatric aspects, such as dependence or abuse potentials and neurotoxicity. To comprehensively elucidate the adverse effects of an NPS, both neuropsychiatric and toxicological aspects should be evaluated.

In the present study, we evaluated the potential cardiotoxic effect of two tryptamines, namely [3-[2-(diethylamino)ethyl]-1*H*-indol-4-yl] acetate (4-AcO-DET) and 3-[2-[ethyl(methyl)amino]ethyl]-1*H*-indol-4-ol (4-HO-MET), using *in vitro* and *in vivo* methods.

2. Materials and methods

2.1. Animals

All experimental procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were authorized by the Institutional Animal Care and Use Committee of Ministry of Food and Drug Safety (MFDS-17-74; Cheongju, Korea) (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Male SD rats (6 week old) for ECG and timed-pregnant SD rats for primary cardiomyocyte culture were obtained from the MFDS, a member of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, Osong, Republic of Korea). The rats for ECG were allocated to one of the following groups: the control (vehicle) and test substance groups (2.0 mg/kg 4-AcO-DET or 1.5 mg/kg 4-HO-MET). All rats had free access to solid diet and tap water, and they were allowed to acclimatize for 1 week prior to *in vivo* experiments. All efforts were made to minimize distress and prevent suffering in the rats. The rearing temperature was maintained at 21–24 °C with a relative humidity of 40–60% and a 12-h light/dark cycle (lights on from 08:00 to 20:00 h).

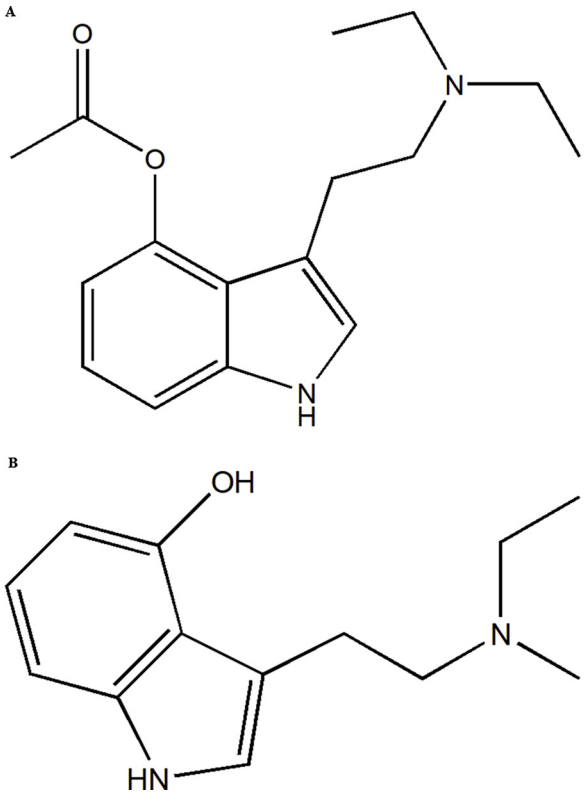

Fig. 1. Chemical structure of (A) 4-AcO-DET and (B) 4-HO-MET.

2.2. Materials

4-AcO-DET (Fig. 1A) and 4-HO-MET (Fig. 1B) were purchased from Cayman Chemical (Ann Arbor, MI, USA). H9c2 (CRL-1446) cell line was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA), unless indicated otherwise.

2.3. Cytotoxicity in H9c2 cells

H9c2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (HI FBS; Gibco) and 1% antibiotics/antimycotics (Invitrogen, Carlsbad, CA, USA), and the plate was incubated at 37 °C and 5% CO_2 for 24 h using the standard culture methods. After sufficient cell culturing, H9c2 cells were counted and then distributed into a 96-well plate (100 μL/well; 1×10^4 cells/well). After overnight incubation, the medium was replaced with fresh medium containing solvent (DMSO:Tween80:saline, 1:1:18) or varying concentrations of test substance [4-AcO-DET or 4-HO-MET (0.1–100 μM)]. The final concentration of solvent did not exceed 0.5% (v/v), which was found to be nontoxic to the cell line. Subsequently, cell viability was measured using an MTT assay kit (Roche, Basel, Schweiz). The optical density of the reaction mixture was measured at 570 nm using a microplate reader (SpectraMAX M5; Molecular Devices, Sunnyvale, CA, USA). Cell viability was calculated as a percentage of the value of the control group.

2.4. QT interval measurement in electrocardiogram of SD rats

SD male rats were intraperitoneally injected with sodium pentobarbital (50 mg/kg), and after anesthetization, the anesthetic state was

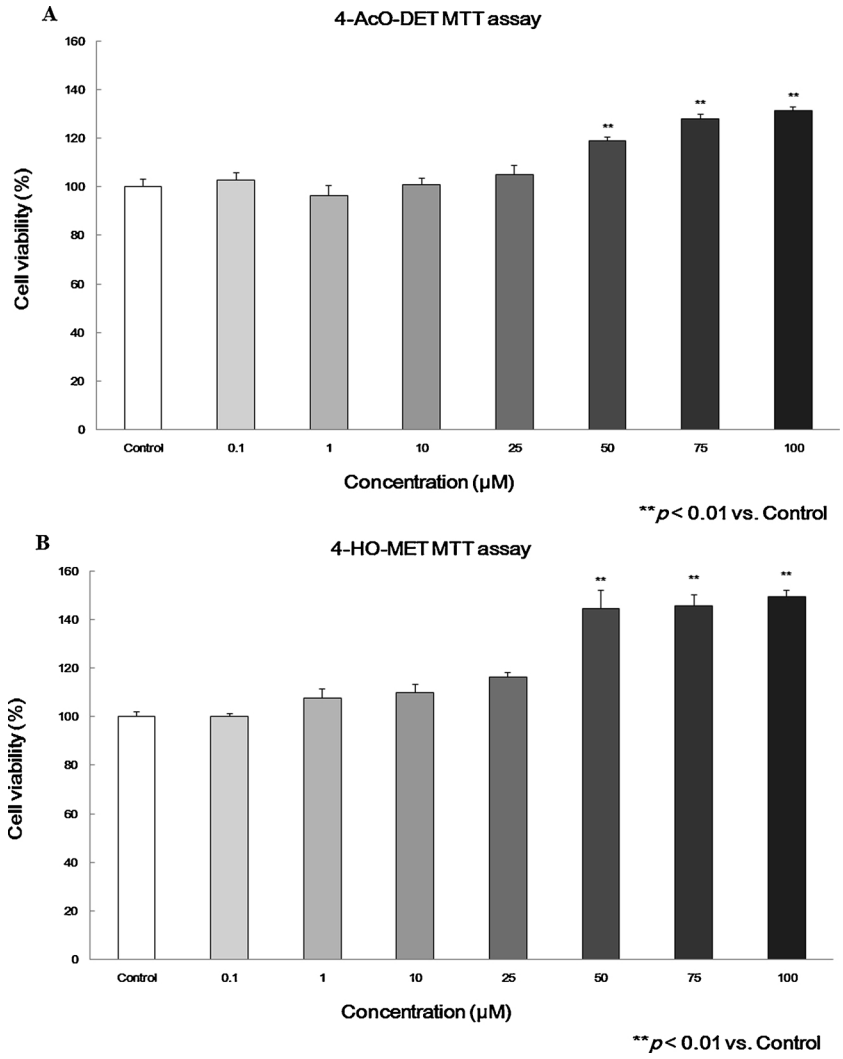

Fig. 2. Effects of (A) 4-AcO-DET and (B) 4-HO-MET on H9c2 cell viability, as determined using the MTT assay. Cells were exposed to the indicated concentrations of test substances for 24 h, and then their viability was measured using the MTT assay. Data are presented as mean ± S.E. (n = 6). Cell viability was expressed as a percentage relative to that of the control. Statistical differences were determined using one-way ANOVA and Bonferroni's *post-hoc* test with $^{**}P < 0.01$ *vs.* control.

maintained for more than 1 h by inhalation of 1% isoflurane (100% O_2). The anesthetized rats were the restrained in a supine position, and their body temperature was maintained by placing a rubber water bag filled with warm water under each rat. After shaving the chest of the rats using an electric clipper, ECG was performed with needle electrodes placed on the shaved region of the rats at three optimized positions (*viz.*, the left upper thorax, right upper thorax, and left lower thorax) in a noninvasive method to obtain the maximal amplitude recording to ensure accurate measurement of QTc interval. ECG signals were recorded using Animal Bio Amp and PowerLab 8/35. The LabChart software (ADInstruments, Colorado Springs, CO, USA) was used to analyze the recorded ECG signals and calculate QRS complex, isoelectric level, P-wave, and T-wave as follows. After measuring the basal level (10 min before injection of test substances), vehicle control (DMSO:Tween80:saline, 1:1:18) or test substance (2.0 mg/kg 4-AcO-DET or 1.5 mg/kg 4-HO-MET) was intravenously (i.v.) injected to the rats at an administration rate of 1 mL·kg^{-1} min^{-1}. When measuring ECG signals, the total blood volume was related to the body weight of the rat as follows: 0.06 × body weight (g) + 0.77 (mL) (Lee and Blaufox, 1985), and it was used to set the concentration of the test substance injected. The QT intervals at each minute were then averaged and corrected using Bazett's equation: $QTc = QT/(RR)^{1/2}$. Delta QTc interval (ΔQTc) was derived using the basal level of QTc interval (QTc_{basal}, an average of continuous QTc interval for 10 min before injection of the substances) and each QTc interval (QTc_{each}) ($\Delta QTc = QTc_{each} - QTc_{basal}$).

2.5. Recording of the hERG currents

To assess hERG potassium channel inhibition caused by 4-AcO-DET and 4-HO-MET, the hERG assay was performed according to a previously described method (Yoon et al., 2019a,b). Briefly, the hERG assay was performed using Chinese hamster ovary (CHO) cells, and the drug concentration that inhibited the ionic currents by 50% was calculated using Hill equation: $f = X^H/(IC_{50}{}^H + X^H)$; where, H = Hill coefficient, IC_{50} = 50% inhibitory concentration, X = concentration, f = inhibition ratio (Kirsch et al., 2004; Lee et al., 2016; Redfern et al., 2003; Shin et al., 2006).

2.6. PAK1 protein expression in H9c2 cells

H9c2 cells were maintained in DMEM with 10% HI FBS and 1% antibiotics/antimycotics in an atmosphere of 95% air and 5% CO_2. The cells were plated in a six-well plate (1.0×10^6 cells/well) and

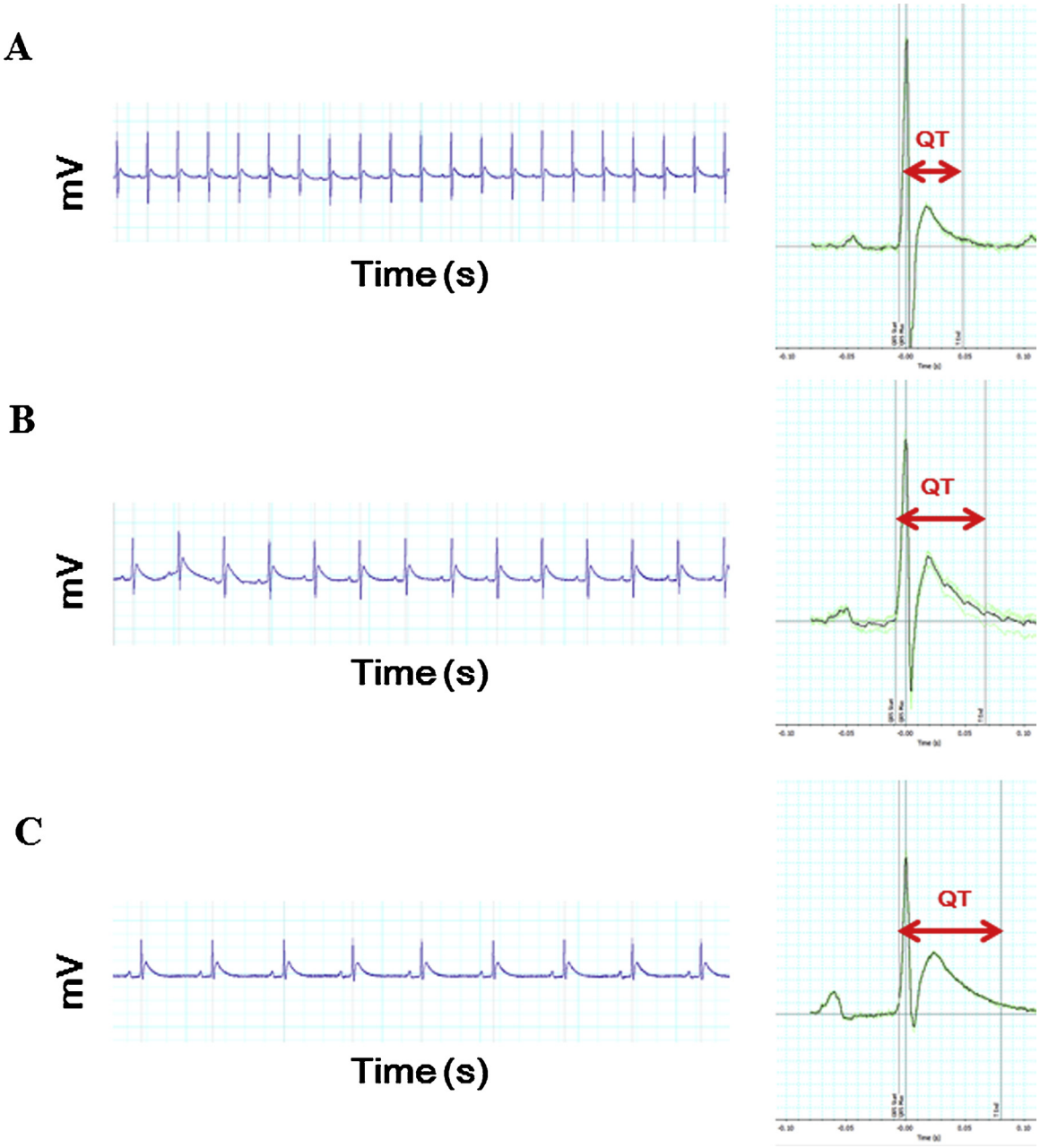

Fig. 3. Effects of 4-AcO-DET and 4-HO-MET on the QT intervals of the heart of SD rats. Electrocardiograms (ECGs) of rats administered (A) vehicle, (B) 4-AcO-DET (2.0 mg/kg), or (C) 4-HO-MET (1.5 mg/kg) were recorded before and after injection. Representative ECG recordings showed electrocardiographic patterns and variations between the vehicle control and substance-treated groups.

incubated overnight. The cell culture medium was replaced with fresh medium containing solvent (DMSO:Tween80:saline, 1:1:18) or test substances at 100 μM concentration (4-AcO-DET or 4-HO-MET). The final concentration of solvent did not exceed 0.5% (v/v), which was found to be nontoxic to the cell line. After incubation, H9c2 cells were lysed in RIPA buffer containing a protease inhibitor (Thermo Fisher Scientific, Waltham, MA, USA) at 4 °C. The cell lysate was used for western blotting. The extracted proteins from H9c2 cells treated with test substances were used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (10%; Bio-Rad, Hercules, CA, USA). The separated proteins were transferred onto a polyvinylidene fluoride membrane (Invitrogen). After blocking, the membrane was incubated with PAK1 primary antibody (rabbit, 1:250; Sigma–Aldrich) at 4 °C overnight. After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (1:1,000; Sigma–Aldrich) at 20–25 °C for 1 h. The expression level of the PAK1 protein was detected using an ECL Plus detection system (GE Healthcare, Piscataway, NJ, USA) with an ChemiDoc™ MP imaging system (Bio-Rad). Images were captured using the Image Lab 4.1 gel documentation program (Bio-Rad).

2.7. PAK1 protein expression in primary cardiomyocytes

Rat primary cardiomyocytes were obtained using a Pierce™ Primary Cardiomyocyte Isolation Kit (Thermo Fisher Scientific) and maintained in DMEM with 10% HI FBS and 1% antibiotics/antimycotics in an atmosphere of 95% air and 5% CO_2. The cells were plated in a six-well plate (2.5×10^6 cells/well) and incubated overnight. The medium was replaced every three days according to the manufacturer's instructions for approximately 7–10 days, and the cells were treated with fresh

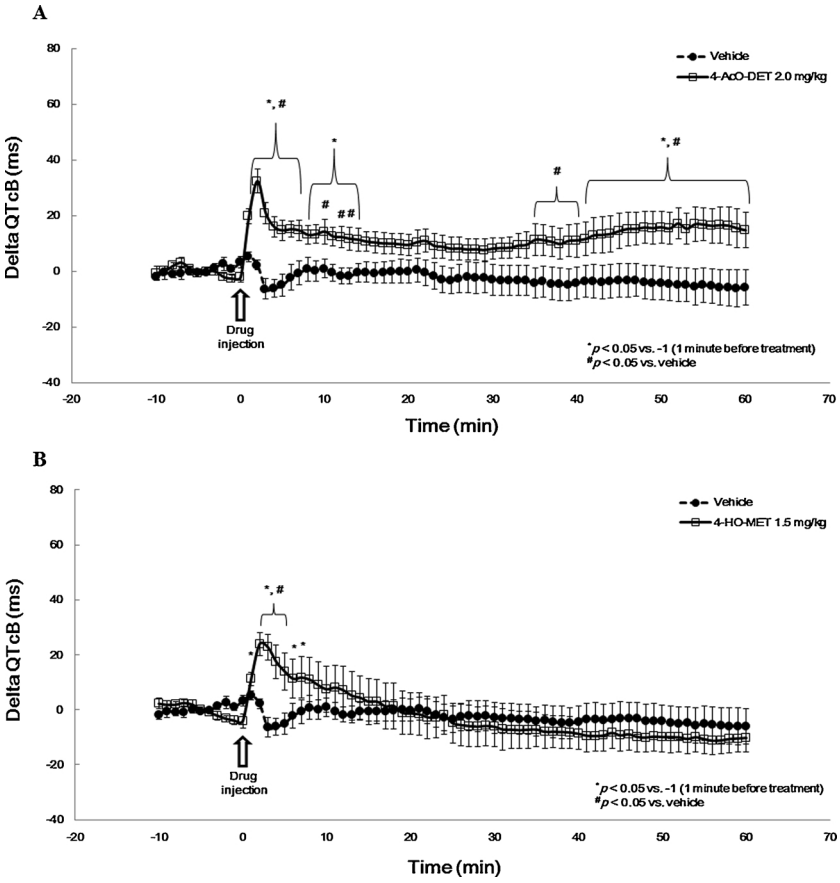

Fig. 4. Effects of (A) 4-AcO-DET and (B) 4-HO-MET on the QTcB of the hearts of SD rats. In the graph, the drug injection time is 0 min. Data are presented as mean ± S.E. (n = 5). Statistical differences were determined using two-way repeated-measure ANOVA and Bonferroni's *post-hoc* test with $^{*}P < 0.05$ *vs.* −1 min values and $^{\#}P < 0.05$ *vs.* vehicle control group.

medium containing 100 μM solvent (DMSO:Tween80:saline, 1:1:18) or test substance (4-AcO-DET or 4-HO-MET) after full culture. The final concentration of solvent did not exceed 0.5% (v/v), which was found to be nontoxic to the cells. Western blotting analysis was carried out according to the procedure described in Section 2.6.

2.8. Statistical analysis

SigmaStat (https://systatsoftware.com/, Systat Software Inc., San Jose, CA, USA) was employed to analyze the *in vitro* and *in vivo* results. All data are presented as mean ± standard error of the mean (S.E.). Statistically significant differences between the vehicle- and drug-treated groups were analyzed using Student's *t*-test. Furthermore, one-way or two-way repeated-measure analysis of variance (ANOVA) and Bonferroni's *post-hoc* test were used for equal variance data, followed by Dunnett's test for non-equal variance data. The level of significance was set at a *P*-value of < 0.05.

3. Results

3.1. MTT assay in H9c2 cell line

H9c2 cells proliferated in a concentration-dependent manner in the presence of 50, 75, or 100 μM 4-AcO-DET and 4-HO-MET, compared with those in the vehicle control group (Fig. 2). In particular, cell viability increased by more than 120% in the presence of 4-AcO-DET at concentrations above 75 μM and by 140% in the presence of 4-HO-MET at concentrations above 50 μM, compared to that in the vehicle control group. However, no significant change in cell viability was observed in the presence of 0.1 μM–25 μM 4-AcO-DET and 4-HO-MET.

3.2. ECG measurement in SD rats

To investigate the effects of 4-AcO-DET and 4-HO-MET on cardiac rhythm, ECG was performed in rats. Compared with that at baseline, ΔQTc was significantly prolonged by 2.0 mg/kg 4-AcO-DET and 1.5 mg/kg 4-HO-MET (Fig. 3). In addition, compared with that in the vehicle group, ΔQTc was prolonged by 4-AcO-DET at 1–7, 10, 12–13, and 35–60 min after injection and by 4-HO-MET at 2–5 min after injection (Fig. 4). Specifically, the mice in the test groups, which were injected with the two synthetic tryptamines, showed increased average of QT intervals, compared with those in the vehicle-treated group, and the differences between the test and negative control groups were the greatest during the first 5 min. However, ΔRR in the test groups was not significantly different from that in the vehicle group (Fig. 5).

3.3. hERG assay

Inhibition of the potassium channels, which is known as a key factor involved in the prolongation of QT intervals, was evaluated using the hERG assay in CHO cells. Both 4-AcO-DET and 4-HO-MET inhibited the hERG channel in a concentration-dependent manner (Fig. 6). Percentage of hERG channel inhibition was measured at three different concentrations, and the dose-response curve was drawn in sigmoidal shape. IC_{50} values were calculated using the dose-response curve, and the IC_{50} values of 4-AcO-DET and 4-HO-MET were 5.8 ± 0.5 and 14.4 ± 1.5 μM, respectively (Fig. 7).

3.4. Western blotting of cardiomyocytes

Changes in the expression of PAK1 after treatment with 4-AcO-DET and 4-HO-MET were evaluated in two types of cardiomyocytes, H9c2 cells and rat primary cardiomyocytes. No significant change in PAK1

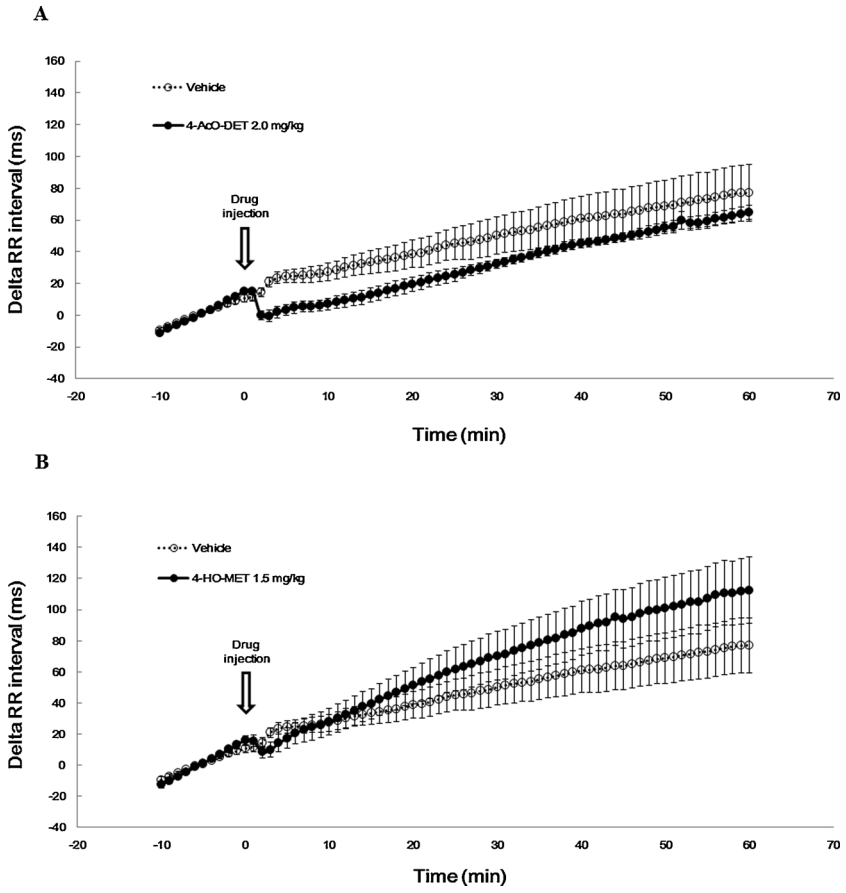

Fig. 5. Effects of (A) 4-AcO-DET and (B) 4-HO-MET on the RR intervals of the hearts of SD rats. In the graph, the drug injection time is 0 min. Data are presented as mean ± S.E. (n = 5). Statistical differences were determined using the two-way repeated-measure ANOVA and Bonferroni's *post-hoc* test.

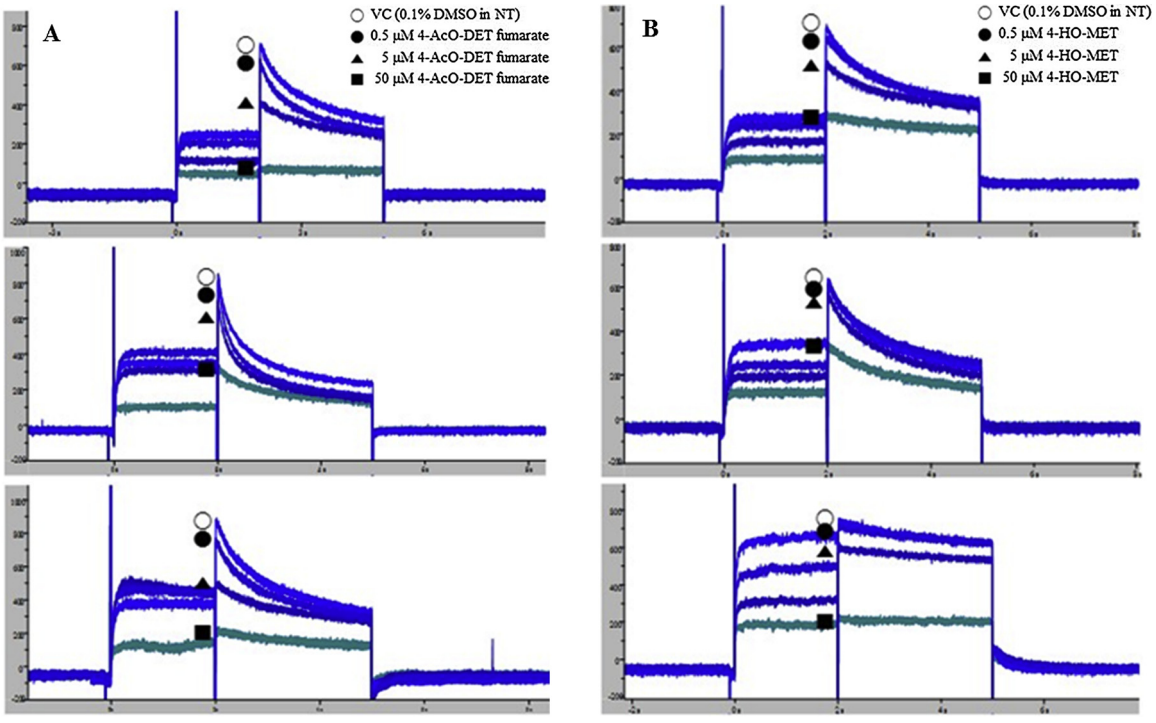

Fig. 6. Effects of (A) 4-AcO-DET and (B) 4-HO-MET on the human ether-a-go-go-related gene (hERG) potassium channel. 4-AcO-DET and 4-HO-MET inhibited the hERG potassium channel in a concentration-dependent manner. (Negative control group (vehicle) (○), 0.5 μM, (●), 5 μM (▲), and 50 μM (■)) (n = 3).

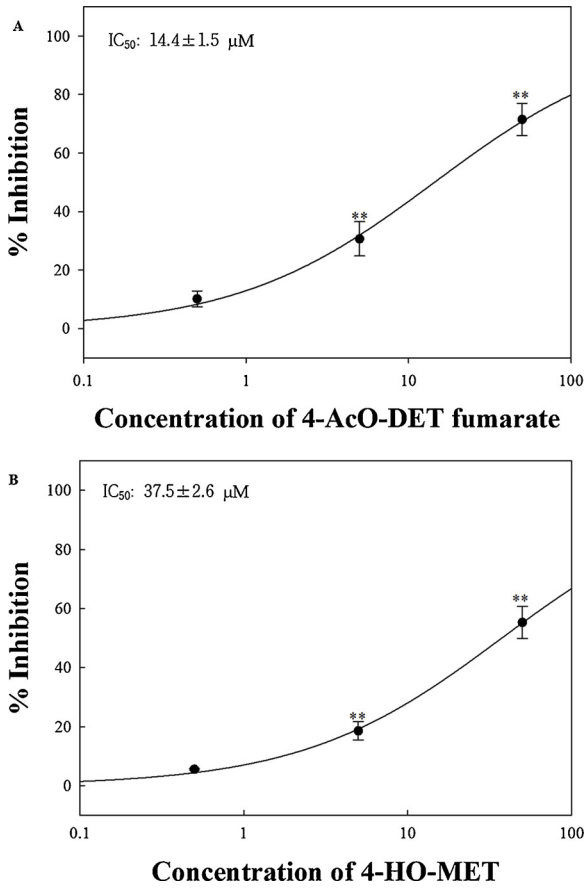

Fig. 7. IC_{50} of (A) 4-AcO-DET and (B) 4-HO-MET on human ether-a-go-go-related gene (hERG) potassium channel inhibition. Data points are presented as mean ± S.E. (n = 3). Statistical differences were determined using one-way ANOVA and Dunnett's *post-hoc* test with $^{**}P < 0.01$ *vs*. control group.

expression was observed in both H9c2 cells and rat primary cardiomyocytes (Fig. 8).

4. Discussion

4-AcO-DET (ethacetin) and 4-HO-MET (methylcybin or metocin) are *N*-substituted homologs of 3-[2-(dimethylamino)ethyl]-1*H*-indol-4-ol (4-HO-DMT; psilocin), a naturally occurring psychedelic substance, and they are known to primarily act by binding to the $5\text{-}HT_{2A}$ receptors (Araujo et al., 2015). There is little experiment-based information on their pharmacology and toxicity. According to some anecdotal reports, the two compounds seem to share adverse effects with other tryptamines (PsychonautWiki et al., 2019a,b). Regarding the cardiovascular effects of the two tryptamines, 4-AcO-DET is likely to cause abnormal heartbeat and raise blood pressure, and 4-HO-MET might increase heart rate (Jebadurai, 2012; Kjellgren and Soussan, 2011). However, as mentioned above, these effects depend on the subjective effects of users, and this kind of information cannot be used as an evidence for scheduling these substances by law.

In the present study, we determined the adverse effects of 4-AcO-DET and 4-HO-MET on the cardiovascular system. Firstly, *in vitro* MTT assay revealed that 4-AcO-DET and 4-HO-MET stimulated the proliferation of H9c2 cells in a concentration-dependent manner. However, the two substances reduced the viability of neuroblasts (PC12 cells; data not shown) and did not affect that of hepatocytes (HepG2 cells; data not shown), indicating that 4-AcO-DET and 4-HO-MET specifically proliferated heart-originated myoblasts. Interestingly, unlike other toxic substances, the two synthetic tryptamines increased cell viability, compared with that in the negative control group. A possible explanation would be that the two synthetic tryptamines might accelerate cell maturation, resulting in the stimulation of cell proliferation. A previous study has shown that 5-MeO-DMT, a representative psychedelic tryptamine, increases the proliferation of granule cells in the dentate gyrus, suggesting that the substance stimulates neurogenesis (da Cruz et al., 2018). The results of the present study are in line with this finding, that is, tryptamines also increased cell proliferation, although the cell types were different. Further studies are needed to elucidate the exact roles of tryptamines in the cell cycle.

Secondly, in the present study, QT intervals in rats were monitored using ECG to investigate whether the two tryptamines induce QT prolongation, which is a key factor of cardiac arrhythmia, ventricular fibrillation, and ventricular tachycardia (O'Laughlin et al., 2016; Shah, 2010; Song et al., 2011). Both substances induced significant prolongation of QT intervals. In the ECG measurement, interpretation of signals is important and, therefore, various formulae for normalization have been suggested. Among the normalization methods, Bazett's and Fridericia's corrections are the most widely used (International Conference on Harmonisation of Requirements for Registration of Pharmaceuticals for Human Use, 2005). Generally, Friderica's equation seems to be more reliable for normalizing differences in heart rates among and within subjects (ICH, 2017). However, the best correction approach is still a matter of controversy (International Conference on Harmonisation of Requirements for Registration of Pharmaceuticals for Human Use, 2005). In the present study, Bazett's equation was applied to normalize ECG signals because the purpose of this experiment was to compare the substance- and vehicle-treated groups. Thirdly, it has been acknowledged that inhibition of the cardiac potassium channels results in delayed repolarization, which is responsible for QT prolongation (Kirsch et al., 2004; Yap and Camm, 2003). In addition, inhibition of the rapidly activating delayed rectifier potassium channels (I_{Kr}), encoded by the hERG, is the most common cause of drug-induced QT interval prolongation and blockade of the I_{Kr} current, resulting in QT interval prolongation (Kirsch et al., 2004; Nogawa and Kawai, 2014; Priest et al., 2008; Redfern et al., 2003; Yap and Camm, 2003; Yun et al., 2016a). In the present study, the hERG channels were inhibited in a concentration-dependent manner by treatments with 4-AcO-DET and 4-HO-MET. Considering previous findings regarding the relationship between the hERG channels and QT intervals, inhibition of the hERG potassium channels might be the cause of QT prolongation in the 4-AcO-DET- and 4-HO-MET-administered animals.

Finally, PAK1 has been known to be related to various cardiac functions, including cardiac remodeling, cardiac channel activity, arrhythmia, cardiac hypertrophy, and contractility (Behjati et al., 2014; Davis et al., 2015; Egom et al., 2010; Liu et al., 2011; Wang et al., 2014a, b). No change in PAK1 expression was observed after treatment with 4-AcO-DET and 4-HO-MET. The results of the present study are comparable with those of a previous study on phenethylamines (Yoon et al., 2019a, b). Phenethylamines reduce cell viability and down-regulate PAK1 expression, whereas tryptamines did not affect cell viability and PAK1 expression. This indicated that PAK1 might be associated with cardiotoxicity through regulation of the cell cycle.

Although the present study would be useful at the present situation of the NPS phenomenon in that it provided scientific information on the cardiotoxicity of two synthetic tryptamines, there are limitations as follows. Firstly, because the present study was performed in rodents and not in humans, caution should be taken when interpreting the results. It would be better to consider the clinical effects together with the presented results. Secondly, the relationship between PAK1 expression and cardiotoxicity was not completely investigated in the present study because only two substances were evaluated. Further studies using greater number of NPSs are needed to elucidate the function of PAK1 in cardiotoxicity. Lastly, in order to investigate systematic molecular changes regarding the cardiac effects induced by novel compounds, selection of differentially expressed genes (DEGs) should be preceded. Further studies would be necessary in this regard.

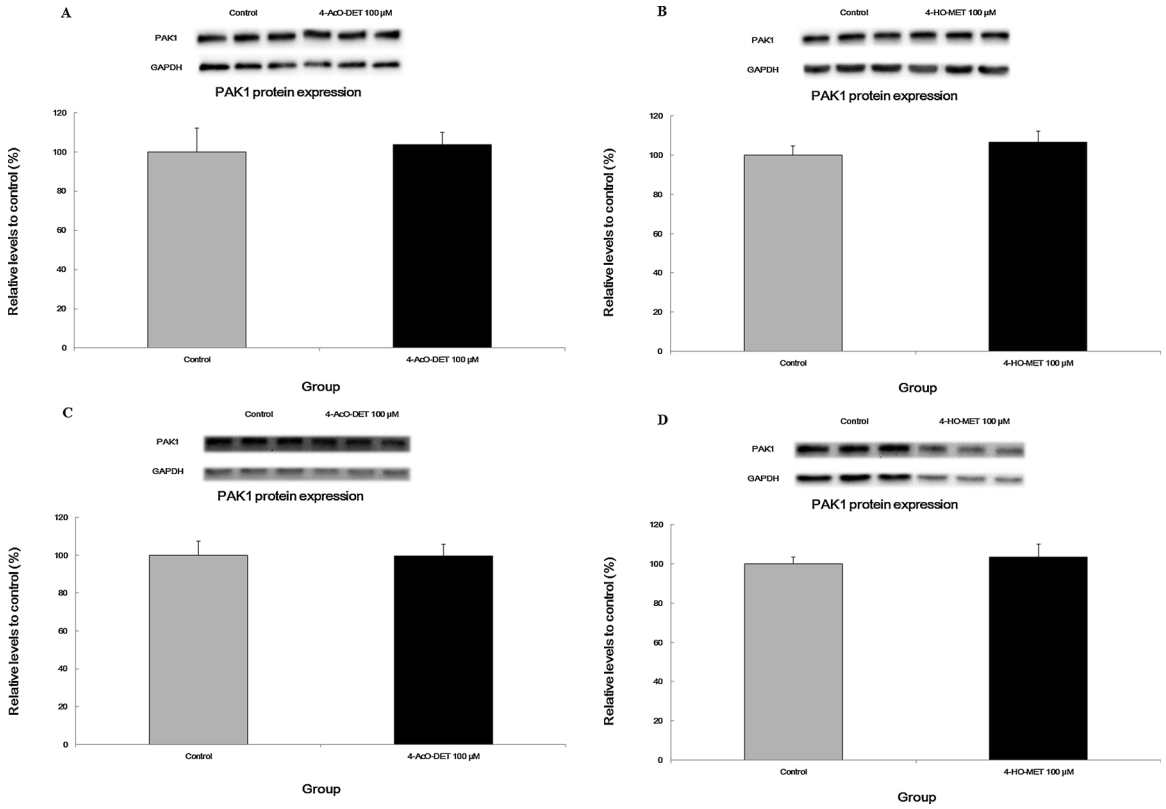

Fig. 8. Expression of p21 (CDC42/RAC)-activated kinase 1 in H9c2 cells treated with (A) 4-AcO-DET and (B) 4-HO-MET and in rat primary cardiomyocytes treated with (C) 4-AcO-DET and (D) 4-HO-MET. Data are presented as mean ± S.E. (n = 3). Statistical differences were determined using one-way ANOVA and Dunnett's *post-hoc* test.

5. Conclusions

Collectively, the findings indicated that 4-AcO-DET and 4-HO-MET exerted potential cardiotoxic effects because they prolonged QT intervals in rats and inhibited the hERG potassium channels in CHO cells. Further studies are needed to investigate the possible relationship between cell viability and PAK1 expression. The findings of the present study would be useful for scheduling the two tryptamines on the international or national level.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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