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Original Article

Effects of acute or chronic administration of novel 3,4-dimethoxyphenylethylamine derivates on anxiety-like behavior

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Abstract: Novel anxiolytic medications are necessary to broaden treatment therapy. Thus, the aim of the present study was to compare the clinically effective anxiolytic, diazepam with the novel 3,4-dimethoxyphenylethylamine derivates. The novel 3,4-dimethoxyphenylethylamine derivates (PK, 0.1, 1.0, 10.0 mg/kg, i.p.) and diazepam (1.0 mg/kg) were injected acutely or chronically in animals subjected to the black-white model and the open field test. The acute administration of PK-2122 (0.1 mg/kg, i.p.) exerted anxiogenic-like effect, while in the middle or high doses PK-2122 exerted anxiolytic-like effect compared with the control group ($p<0.05$). The repeated treatment with PK-2111 was followed by anxiolytic-like effect in doses of 0.1 or 1.0 mg/kg which was more significant compared not only with control group, but with comparison to group treated with diazepam ($p<0.05$). Chronic treatment with PK-2123 or PK-2122 in all tested doses produced anxiolytic-like effect ($p<0.05$), compared with control group and diazepam group. These results demonstrate that PK-2126, but not PK-2122, is dose independent and may be effective in experimental model of anxiety in rats when administered acutely or repeatedly.

Keywords: Anxiety, black and white model, open field test, dopamine

Introduction

Anxiety disorders are a common and debilitating mental illnesses. Current pharmacological treatments have limitations including resistance, delayed efficacy and side effects [1, 2]. Better understanding the novel neurotransmitter pathways and the interplay between these systems is broadening the scope of anxiolytic drug treatments [1].

The dopamine (DA) pathway is activated by stress, and this response has as a corollary on the induction of stress-related behaviors such as anxiety [3]. A number of clinical observations led to the hypothesis that DA dysfunction may play a role in social anxiety disorder [4]. Tentative evidence of involvement of DA in anxiety disorders comes from a study measuring the catecholamine concentrations in patients [3]. Human studies of DA and its metabolites have reported reduced cerebrospinal fluid (CSF) homovanillic acid (HVA) levels in patients with panic disorder with comorbid social anxiety compared with panic disorder patients, as well as correlations between low CSF HVA and measures of introversion [5]. Although the use of animal models to study the role of DA in social anxiety has inherent problems because of the interactional nature of the disorder, some studies have suggested that reduced DA function is associated with increased anxiety [3, 6, 7]. A strain of timid mice with reduced DA levels is available, and DA depletion results in increased anxiety [5]. The DA receptors have been implicated in anxiety mechanisms by preclinical evidence from behavioral studies [1, 8, 9]. However information about the role of DA in

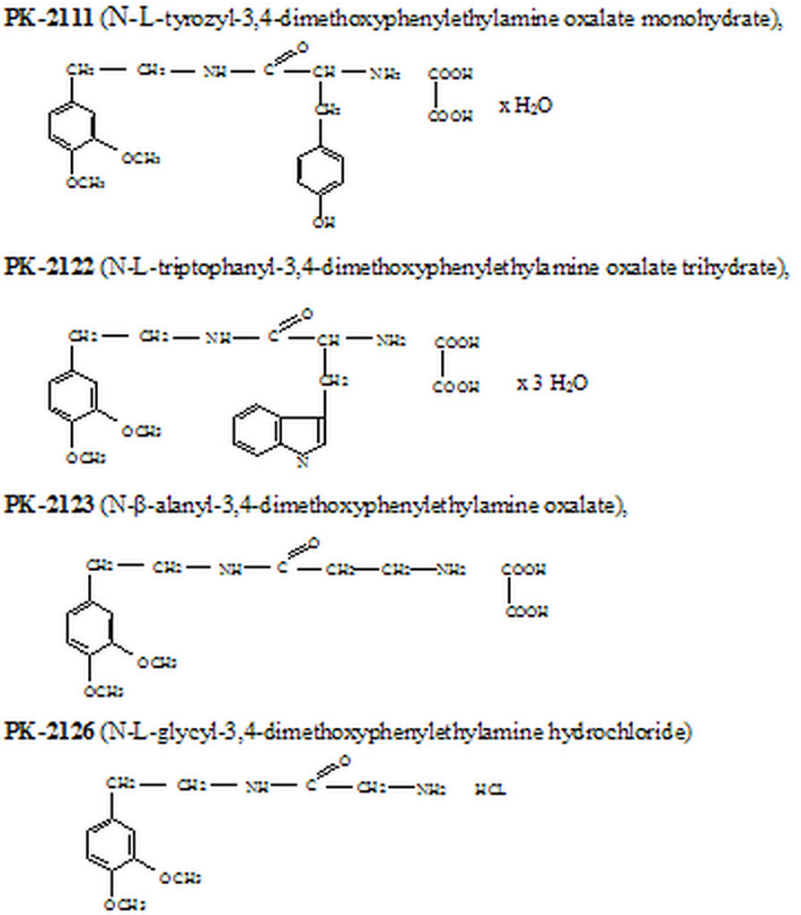

Figure 1. Structure of novel 3,4-dimethoxyphenylethylamine derivates.

anxiety remains scarce. Therefore, novel psychotropic drugs based on the structure of dopamine for the treatment and prevention of a number of affective disorders are vital to the ongoing treatment of such disorders. In this regard, the department of Neuropharmacology of Institute for Experimental Medicine (St. Petersburg, Russia) synthesized novel dopaminergic substances; -3,4-dimethoxyphenylethylamine derivates.

Thus, the aim of the present study was to explore the anxiolytic-like effects of these novel 3,4-dimethoxyphenylethylamine derivates after acute or chronic treatment in rats. In the present experiments, a comparison between acute and chronic treatment was made in order to evaluate the possible change of drug efficacy depending on a repeated administration and on those of a clinically effective anxiolytic, diazepam.

Methods

Animals

Male rats (Wistar strain, purchased from Rappolovo, Russia, 180-220 g) were used. For at least one week prior to the experiments, the rats were housed six to a cage under standard environmental conditions: constant temperature of 23 ± 1°C, 60% humidity, 12-h light/dark cycle (light on 8:00 a.m.), food and water *ad libitum*. All experiments were carried out in accordance with the guide for care and use of Laboratory Animals published by the National Institute of Health (National Research council, publication No 85-23, revised in 1996), and the Animal Welfare Assurance Renewal for Pavlov Institute of Physiology. The rationale, design and methods of this study have been approved by the Ethical Committee for Animal Research, Pavlov Institute of Physiology.

All animals were gently handled by experienced keepers from the facility each day for a week prior to experimental procedures. Any environmental or physical stress was avoided in order to habituate the rats to manual handling. Animals were randomly assigned to experimental groups and were used only once in the behavioral experiments.

3,4-Dimethoxyphenylethylamine derivates and treatment

The following 3,4-dimethoxyphenylethylamine derivates were tested in this study (**Figure 1**): PK-2111 (N-L-tyrozyl-3,4-dimethoxyphenylethylamine oxalate monohydrate), PK-2122 (N-L-triptophanyl-3,4-dimethoxyphenylethylamine oxalate trihydrate), PK-2123 (N-β-alanyl-3,4-dimethoxyphenylethylamine oxalate) and PK-

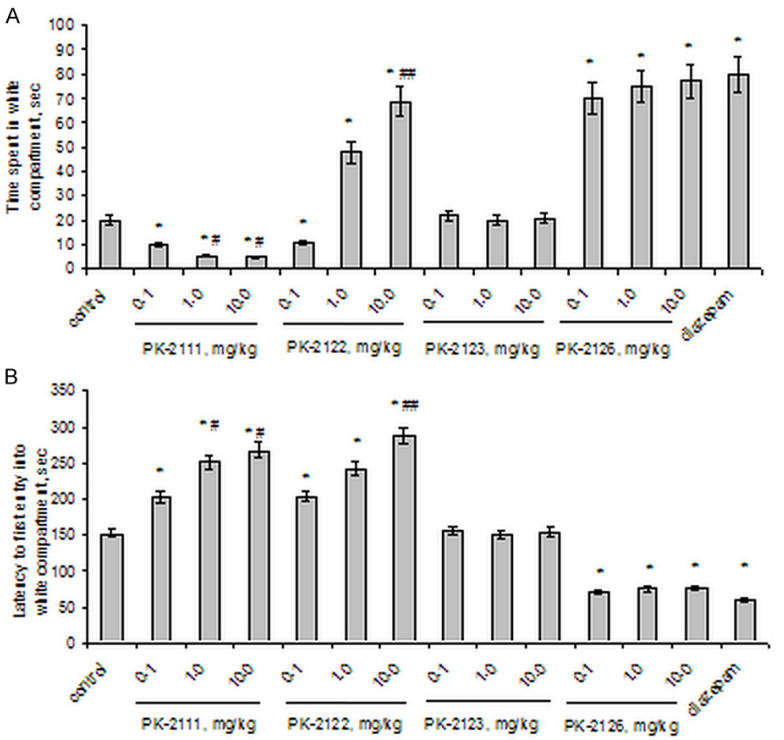

Figure 2. Effects of novel 3,4-dimethoxyphenylethylamine derivates after acute administration on the time spent into white compartment and the latency to first entry of experimental groups in the BWM. Columns represent the time spent in white compartment (A) mean ± SEM (in sec) and latency to first entry into white compartment (B) mean ± SEM (in sec). Each group comprised a minimum of seven rats. *-P < 0.05 vs control group of rats, **-P < 0.05 vs rats treated with diazepam, #-P < 0.05 vs 0.1 mg/kg dose level group, ##--P < 0.05 vs 1.0 mg/kg dose level group.

2126 (N-L-glycyl-3,4-dimethoxyphenylethylamine hydrochloride).

Animals were randomly assigned to any experimental groups and were used only once in the behavioral experiments. For each behavioral test the rats were assigned to 14 independent groups (n = 7 in each group). The control group received only the vehicle in which 3,4-dimethoxyphenylethylamine derivates were dissolved (0.9% NaCl solution), another 12 groups received PK-2111 or PK-2122 or PK-2123 or PK-2126 in several doses (0.1, 1.0, 10.0 mg/kg) and the last group received 1.0 mg/kg of diazepam (Nycomed, Denmark), which is effective to exert an anxiolytic-like effect in the black and white model (BWM) [10]. The investigated substances and diazepam were freshly diluted in physiological saline and injected intraperitoneally (i.p.) acutely (1 h prior to behavioral testing) or chronically (for 21 days, the last injection being made 1 h prior to behavioral testing) in animals subjected to BWM and open field test (OFT).

Behavioral tests

Black and white model (BWM): The BWM has been broadly validated as a useful tool for anxiety study and for the screening of anxiolytic and anxiogenic drugs [11]. The dimensions of BWM were 80 × 40 × 40 cm [10]. The box was further divided in two equal chambers (40 × 40 × 40 cm) by a barrier possessing a doorway (10 × 10 cm) that allows the rats to cross freely from one chamber to another. The black compartment was not illuminated, whereas the white compartment was completely illuminated by a 40 W white light. A video camera was installed above the illuminated compartment to record rat activity. Later, two independent observers measured the behavioral variables. On the day of test, the rats were brought to the experimental room at 18:00 h (initiating dark phase) and left for 1 h to acclimatize to the novel surroundings. The BWM was initiated at 19:00 h, 1 h after initiating the dark phase. After this duration, each rat was placed into the middle of the black compartment facing the doorway, and the behavioral activity measured for 5 min. The variables were: a) latency to the first entry into the white compartment (the time taken after initial placement of a rat in the black compartment until it crossed completely to the white compartment); b) time spent in the white compartment (the sum of all time periods in the white compartment), and c) frequency of entries into the white compartment (the total number of entries to the white compartment) as previously described [10]. A video camera was installed above the model to record rat activity in the maze. Two independent observes measured the behavioral variables. Additionally, the frequency and time spent in the exploration toward white compartment was evaluated, assuming exploration

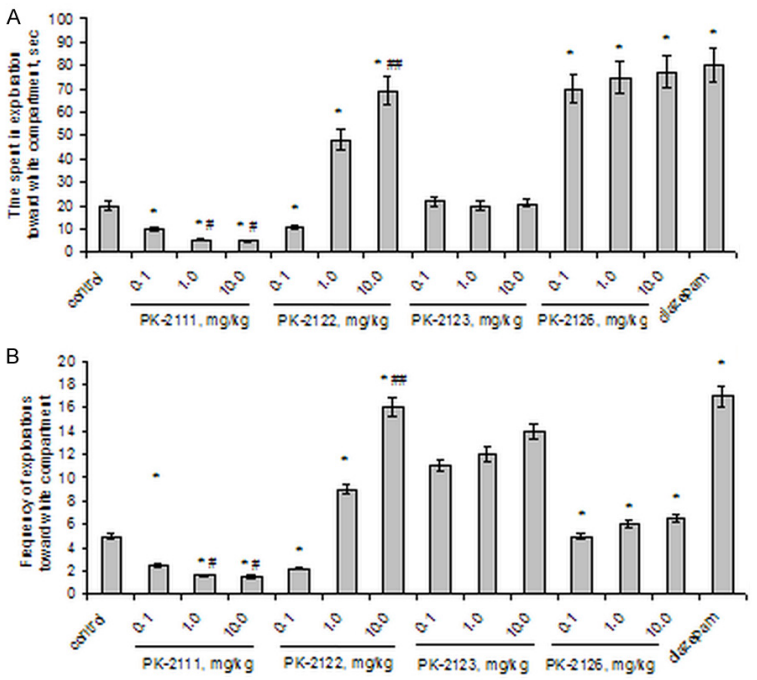

Figure 3. Effects of novel 3,4-dimethoxyphenylethylamine derivates after acute administration on the time spent into white compartment and the latency to first entry of experimental groups in the BWM. Columns represent the time spent in white compartment (A) mean ± SEM (in sec) and latency to first entry into white compartment (B) mean ± SEM (in sec). Each group comprised a minimum of seven rats. *-P < 0.05 vs control group of rats, **-P < 0.05 vs rats treated with diazepam, #-P < 0.05 vs 0.1 mg/kg dose level group, ##–P < 0.05 vs 1.0 mg/kg dose level group.

when the rat was leaning out of the white compartment (that is, until the head and half of the body was into the white compartment without crossing completely to the illuminated compartment). After BWM test, the rat was immediately submitted to the Open Field Test (OFT).

OFT: To evaluate the effect of new substances on spontaneous locomotor activity, grooming, and rearing, each rat was individually submitted to a 5-min period to the OFT. An opaque plexiglass cage (44 × 33 cm) with walls 20-cm in height was used. The floor was divided into 12 squares (11 × 11 cm). A video camera was installed above the model to recode the rat activity in the maze. Two independent observes measured the behavioral variables. The general locomotor activity of the rats associated with treatments was evaluated, which could interfere with the behavioral activity of the rats in the BWM. No other measures were evaluated.

At the beginning of the test, the rat was gently placed in a corner of the cage, and such variables measured: the number of squares crossed by the rat (crossing), assuming crossing when an animal passed from one square to another with its rear legs; frequency of rearing, assuming rearing when the rat acquired a vertical posture with respect to the cage floor; frequency of grooming included paw licking, nose/face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind the ears), body grooming/scratching (body fur licking and scratching the body with the hind paws), leg licking, and tail/genitals grooming (licking of the genital area and tail) as previously described by others [12].

After each test session, the BWM and OFT cage were carefully cleaned and deodorized with a cleaning solution (ammonia 0.5%, ethanol 15%, extran 10%, isopropyl alcohol 5%, antiseptic with aromatizing 19%, and distillated water 50.5%).

Statistical analysis

All data were analyzed using one-way ANOVA and the post-hoc Dunnett's test for multiple comparisons to control. The paired Student's *t*-test was used for intra-group comparisons. A *P*-value of 0.05 or less was considered as indicative of a significant difference. All values were expressed as mean ± S.E.M.

Results

Effects of acute administration of novel 3,4-dimethoxyphenylethylamine derivates on anxiety-like behavior in the BWM

No differences in the frequency of entries into the white compartment among the control group, diazepam treatment and different doses of novel dopaminergic substances after acute administration [F(5,72) = 1.92, P = 0.11, NS] were observed. However, a significant difference in the time spent into the white compartment [F(5,72) = 21.95, P < 0.001], in the latency to the first entry into the white compartment

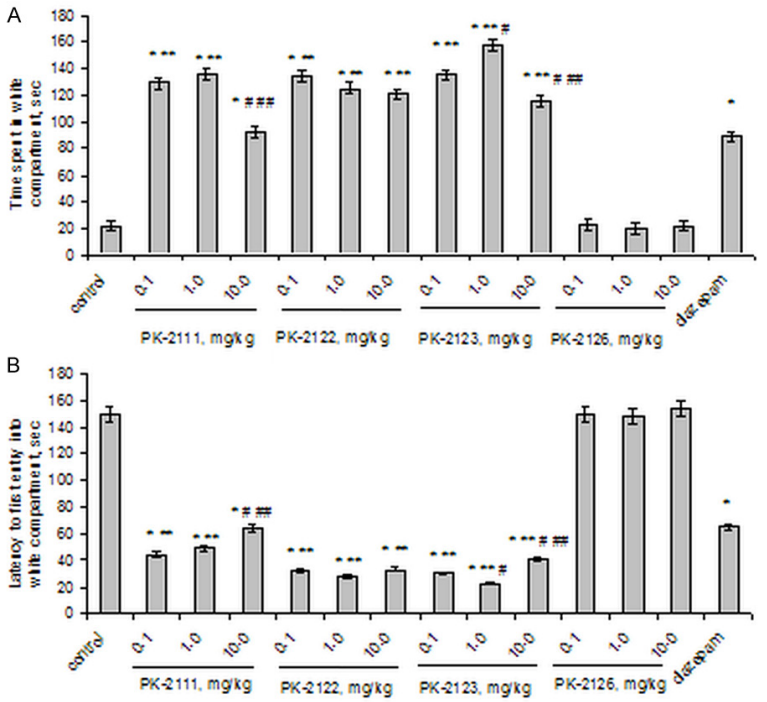

Figure 4. Effects of novel 3,4-dimethoxyphenylethylamine derivates after repeated administration on the time spent in white compartment and the latency to first entry of experimental groups in the BWM. Columns represent the time spent in white compartment (A) mean ± SEM (in sec) and latency to first entry into white compartment (B) mean ± SEM (in sec). Each group comprised a minimum of seven rats. *-P < 0.05 vs control group of rats, **-P < 0.05 vs rats treated with diazepam, #-P < 0.05 vs 0.1 mg/kg dose level group, ##–P < 0.05 vs 1.0 mg/kg dose level group.

[F(5,72) = 15.22, P < 0.003], in the time spent in exploration toward white compartment [F(5,72) = 9.40, P < 0.001] and in the frequency of explorations toward white compartment [F(5,72) = 14.52, P < 0.001] were observed among the different treatments. Diazepam (1.0 mg/kg) significantly increased the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment, but reduced the latency to the first entry into the white compartment compared to the control group (P < 0.05, **Figures 2A**, **2B**, **3A** and **3B**).

The time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment were reduced after PK-2111 administration in all tested doses compared with the control group (P < 0.05, **Figures 2A**, **2B**, **3A** and **3B**). Moreover, a decrease of the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment after PK-2111 administration in the middle dose (1.0 mg/kg) or in the high dose (10.0 mg/kg) was more significant compared to 0.1 mg/kg dose level of this group. Concurrently, the latency to the first entry into the white compartment after all doses of PK-2111 was increased compared to the control group (P < 0.05, **Figure 2A**). The increase of the latency to the first entry into the white compartment after the middle dose (1.0 mg/kg) or the high dose (10.0 mg/kg) of PK-2111 was significantly increased compared to 0.1 mg/kg dose level of this group.

The time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment were reduced after PK-2122 0.1 mg/kg compared to the control group (P < 0.05, **Figures 2A**, **2B**, **3A** and **3B**). However, administration of PK-2122 in the middle dose (1.0 mg/kg) or in the high dose (10.0 mg/kg) significantly increased the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment compared to the control group (P < 0.05, **Figures 2A**, **2B**, **3A** and **3B**). An increase of the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment after PK-2122 administration in the largest dose (10.0 mg/kg) was significantly increased compared to 1.0 mg/kg dose level of this group. Also, the latency to the first entry into the white compartment was increased after of PK-2122 administration in the small dose (0.1 mg/kg) compared to the control group (P < 0.05, **Figure 2A**). On the contrary, administration of PK-2122 in the middle dose (1.0 mg/kg) or in the high dose (10.0 mg/

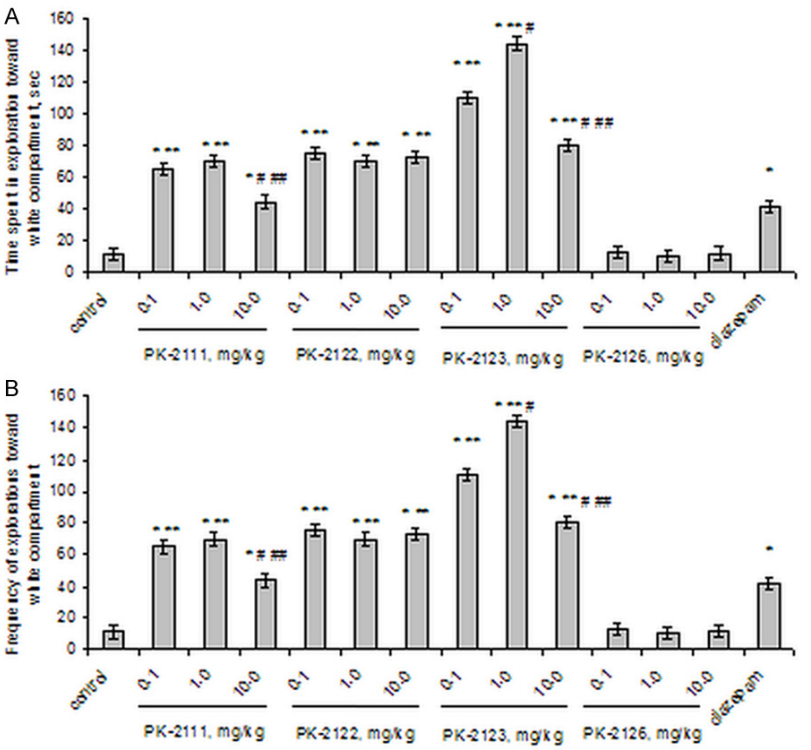

Figure 5. Effects of novel 3,4-dimethoxyphenylethylamine derivates after repeated administration on the time spent in white compartment and the latency to first entry of experimental groups in the BWM. Columns represent the time spent in white compartment (A) mean ± SEM (in sec) and latency to first entry into white compartment (B) mean ± SEM (in sec). Each group comprised a minimum of seven rats. *-P < 0.05 vs control group of rats, **-P < 0.05 vs rats treated with diazepam, #-P < 0.05 vs 0.1 mg/kg dose level group, ##--P < 0.05 vs 1.0 mg/kg dose level group.

kg) significantly reduced the latency to the first entry into the white compartment compared to the control group (P < 0.05, **Figure 2A**). Moreover, a decrease of the latency to the first entry into the white compartment after PK-2122 administration in the high dose was significantly increased compared to 1.0 mg/kg dose level of this group.

All doses of PK-2126 increased the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment and decreased the frequency of explorations toward white compartment compared to the control group (P < 0.05, **Figures 2A**, **2B**, **3A** and **3B**).

However, all tested doses of PK-2123 failed to change the time spent into the white compartment, the latency to the first entry into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment compared to the control group.

Effects of chronic administration of novel 3,4-dimethoxyphenylethylamine derivates on anxiety-like behavior in the BWM

No significant differences in the frequency of entries into the white compartment among the control group, diazepam treatment and different doses of novel dopaminergic substances after repeated administration were observed [F(5,72) =23.22, P = 0.3, NS].

Significant differences in the time spent into the white compartment [F (5,72) = 7.20, P < 0.001], in the latency to the first entry into the white compartment [F(5,72) = 14.95, P<0.003], in the time spent in exploration toward white compartment [F(5,72) = 2.97, P < 0.001] and in the frequency of explorations toward white compartment [F(5,72) = 15.11, P < 0.001] among the different treatments were observed. Also, diazepam (1.0 mg/kg) significantly increased the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment, but reduced the latency to the first entry into the white compartment compared to the control group (P < 0.05, **Figures 4A**, **4B**, **5A** and **5B**).

After repeated administration of PK-2111 0.1 and 1.0 mg/kg significantly increased the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment compared to the control group and group receiving diazepam (P < 0.05, **Figures 4A**, **4B**, **5A** and **5B**). The repeated administration of PK-2111 in the high dose significantly increased the time spent into the white compartment, the time spent in explora-

Table 1. Effects of novel 3,4-dimethoxyphenylethylamine derivates after acute administration on behavior in the open field test for 5 min

Groups	Crossing	Rearing	Grooming
Control	42.0 ± 3.6	13.5 ± 1,8	2.4 ± 0.4
PK-2111 0.1 mg/kg	39.5 ± 2.6	6.7 ± 0.6*	1.0 ± 0.2*
PK-2111 1.0 mg/kg	46.2 ± 3.2	7.0 ± 0.6*	1.2 ± 0.2*
PK-2111 10.0 mg/kg	40.7 ± 2.8	12.4 ± 1.2**	3.0 ± 0.2**
PK-2122 0.1 mg/kg	68.4 ± 5.6*,**	6.0 ± 0.8*	1.0 ± 0.2*
PK-2122 1.0 mg/kg	44.6 ± 4.4	20.6 ± 2.8*,**,#	7.6 ± 0.2*,**,#
PK-2122 10.0 mg/kg	76.8 ± 3.8*,**,#	6.2 ± 0.8*	1.1 ± 0.4*
PK-2123 0.1 mg/kg	39.5 ± 2.6	6.7 ± 0.6*	1.0 ± 0.2*
PK-2123 1.0 mg/kg	46.2 ± 3.2	7.0 ± 0.6*	1.2 ± 0.2*
PK-2123 10.0 mg/kg	40.7 ± 2.8	12.4 ± 1.2**	3.0 ± 0.2**
PK-2126 0.1 mg/kg	68.4 ± 5.6*,**	6.0 ± 0.8*	1.0 ± 0.2*
PK-2126 1.0 mg/kg	44.6 ± 4.4	20.6 ± 2.8*,**,#	7.6 ± 0.2*,**,#
PK-2126 10.0 mg/kg	76.8 ± 3.8*,**,#	6.2 ± 0.8*	1.1 ± 0.4*
Diazepam 1.0 mg/kg	41.2 ± 3.8	21.3 ± 1.4*	4.1 ± 0.6*

Each group comprised a minimum of seven rats. *-P < 0.05 vs control group of rats, **-P < 0.05 vs rats treated with diazepam, #-P < 0.05 vs 0.1 mg/kg dose level group, ##–P < 0.05 vs 1.0 mg/kg dose level group.

tion toward white compartment and the frequency of explorations toward white compartment compared to the control group. Moreover, an increase of the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment after PK-2111 administration in the high dose (10.0 mg/kg) was less effective compared to 0.1 mg/kg or 1.0 mg/kg dose levels of this group.

Rats treated with PK-2122 in all tested doses significantly spent more time in the white compartment compared to the control and diazepam group (P < 0.05, **Figure 4A**). Also, these rats demonstrated significantly increased time spent in exploration toward white compartment and the frequency of explorations toward white compartment compared to the control and diazepam group (P < 0.05, **Figure 5A** and **5B**). Moreover, the rats treated with PK-2122 in all tested doses had significantly reduced latency period to the first entry to the white compartment compared to the control and diazepam group (P < 0.05, **Figure 4B**).

In contrast, chronic administration of PK-2126 in all tested doses failed to modify the time spent into the white compartment, the latency of the first entry into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment compared to the control group (post-hoc, P > 0.05, **Figures 4A**, **4B**, **5A** and **5B**).

PK-2123 in all tested doses significantly increased the time spent into the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment compared to the control and diazepam group (P < 0.05, **Figures 4A**, **4B**, **5A** and **5B**). The increase of the time spent in the white compartment, the time spent in exploration toward white compartment and the frequency of explorations toward white compartment after PK-2123 administration in the middle dose (1.0 mg/kg) were significantly higher compared to 0.1 mg/kg dose level of this group, and the same parameters after PK-2123 administration in the high dose (10.0 mg/kg) were significantly less compared to 0.1 mg/kg or 1.0 mg/kg dose levels of this group. Also, PK-2123 significantly decreased the latency to the first entry to the white compartment compared to the control group and group receiving diazepam (P < 0.05, **Figure 4B**). The decrease of the latency to the first entry to the white compartment after PK-2123 administration in the middle dose (1.0 mg/kg) was significantly higher compared to 0.1 mg/kg dose level of this group, and the same parameter after PK-2123 administration in the high dose (10.0 mg/kg) was significantly less compared to 0.1 mg/kg or 1.0 mg/kg dose levels of this group.

Effects of acute administration of novel 3,4-dimethoxyphenylethylamine derivates on behavior in the OFT

No significant differences in the crossing of the rats treated with different substances were observed [F(5,72) = 2.23, P > 0.09]. However, significant differences in the rearing and grooming between drug treatments were observed ([F(5,72) = 11.56, P < 0.05], [F(5,72) = 8.01, P < 0.002], respectively) and rats treated with

Table 2. Effects of novel 3,4-dimethoxyphenylethylamine derivates after repeated administration on behavior in the open field test for 5 min

Groups	Crossing	Frequency of Rearing	Frequency of Grooming
Control	39.1 ± 2.4	13.1 ± 1.8	2.2 ± 0.4
PK-2111 0.1 mg/kg	40.5 ± 2.6	33.4 ± 1.2*,**	5.4 ± 0.2*,**
PK-2111 1.0 mg/kg	43.2 ± 3.2	31.8 ± 1.6*,**	5.2 ± 0.2*,**
PK-2111 10.0 mg/kg	46.7 ± 2.8	22.4 ± 1.2*,#,##	3.7 ± 0.2*,#,##
PK-2122 0.1 mg/kg	43.2 ± 3.2	32.5 ± 1.2*,**	5.0 ± 0.4*,**
PK-2122 1.0 mg/kg	39.6 ± 4.4	29.6 ± 1.6*,**	4.9 ± 0.2*,**
PK-2122 10.0 mg/kg	43.2 ± 3.2	32.3 ± 1.7*,**	5.6 ± 0.2*,**
PK-2123 0.1 mg/kg	39.5 ± 2.6	34.7 ± 1.7*,**	6.3 ± 0.8*,**
PK-2123 1.0 mg/kg	47.2 ± 3.2	46.5 ± 2.1*,**,#	7.6 ± 0.8*,**,#
PK-2123 10.0 mg/kg	38.7 ± 2.8	29.3 ± 0.8*,**,#,##	4.8 ± 0.4*,**,#,##
PK-2126 0.1 mg/kg	42.9 ± 1.4	13.5 ± 1.6	2.4 ± 0.2
PK-2126 1.0 mg/kg	39.3 ± 2.4	12.7 ± 1.6	2.6 ± 0.2
PK-2126 10.0 mg/kg	40.1 ± 3.2	13.8 ± 1.8	2.3 ± 0.4
Diazepam 1.0 mg/kg	43.4 ± 3.8	22.8 ± 1.8*	3.9 ± 0.4*

Each group comprised a minimum of seven rats. *-P < 0.05 vs control group of rats, **-P < 0.05 vs rats treated with diazepam, #-P < 0.05 vs 0.1 mg/kg dose level group, ##--P < 0.05 vs 1.0 mg/kg dose level group.

diazepam (1.0 mg/kg) had significantly increased the frequency of rearing and the frequency of grooming compared to the control rats (P < 0.05, **Table 1**).

All tested doses of PK-2111 reduced the frequency of rearing and the frequency of grooming compared to the control group (P < 0.05, **Table 1**). The decrease of the frequency of rearing and the frequency of grooming after PK-2111 administration in the middle dose (1.0 mg/kg) or in the high dose (10.0 mg/kg) was significantly increased compared to 0.1 mg/kg dose level of this group.

Low dose administration of PK-2122 (0.1 mg/kg) reduced the frequency of rearing and the frequency of grooming compared to the control group (P < 0.05, **Table 1**). However, administration of PK-2122 in the middle dose or in the high dose significantly increased the frequency of rearing and the frequency of grooming compared to the control group (P < 0.05, **Table 1**). Moreover, an increase of the frequency of rearing and the frequency of grooming after PK-2122 administration in the high dose (10.0 mg/kg) was more significantly compared to 1.0 mg/kg dose level of this group.

All doses of PK-2126 significantly increased the frequency of rearing and the frequency of grooming compared to control (P < 0.05, **Table 1**).

Neither 0.1 mg/kg or 1.0 mg/kg, or 1.0 mg/kg doses of PK-2123 modified the frequencies of rearing and grooming in the OFT compared to the control group (P > 0.05, **Table 1**).

Effects of chronic administration of novel 3,4-dimethoxyphenylethylamine derivates on behavior in the OFT

No significant differences in the crossing of the rats treated with different substances after the repeated administration were observed [F(5,72) = 1.48, P > 0.2]. Yet, a significant difference in the frequency of rearing and frequency of grooming between drug treatments after the repeated administration was affirmed ([F(5,72) = 4.17, P < 0.05], [F(5,72) = 9.40, P < 0.002], respectively) and rats treated with diazepam (1.0 mg/kg) had significantly increased frequency of rearing and the frequency of grooming compared to the control rats (P < 0.05, **Table 2**).

After repeated administration of PK-2111 0.1 and 1.0 mg/kg, the frequency of rearing and the frequency of grooming were significantly increased compared to the control and diazepam group (P < 0.05, **Table 2**). The repeated administration of PK-2111 in the high dose also significantly increased the frequency of rearing and the frequency of grooming compared to control. Moreover, an increase of the frequency of rearing and the frequency of grooming after PK-2111 administration in the high dose was less effective compared to 0.1 mg/kg or 1.0 mg/kg dose levels of this group.

The frequency of rearing and the frequency of grooming after repeated administration of PK-2123 were significantly increased compared to the control and diazepam groups (P < 0.05, **Table 2**). The increase of frequency of rearing and the frequency of grooming after PK-2123 administration in the middle dose were significantly higher compared to 0.1 mg/kg dose level of this group, and the same parameters after PK-2123 administration in

the high dose were significantly less compared to 0.1 mg/kg or 1.0 mg/kg dose levels of this group.

Repeated administration of PK-2122 in all doses significantly increased the frequency of rearing and the frequency of grooming compared to the control and diazepam groups ($P < 0.05$, **Table 2**).

Neither 0.1 mg/kg or 1.0 mg/kg, or 1.0 mg/kg doses of PK-2126 modified the frequencies of rearing and grooming in the OFT compared to control ($P > 0.05$, **Table 2**).

Discussion

The Dopamine (DA) pathway is activated by stress, and this response has as a corollary in the induction of stress-related behaviors such as anxiety [3]. A number of clinical observations led to the hypothesis that DA dysfunction may play a role in social anxiety disorder [4]. The search and development of new psychotropic drugs based on the structure of DA for the treatment and prevention of affective disorders are of great interest, and in this regard, a series of novel substances on basis of DA structure has been developed: PK-2111, PK-2122, PK-2123, PK-2126.

In this study, the anxiolytic-like effects of such compounds in rats in the BWM and the OFT were explored, and were compared with diazepam.

The BWM has been useful to screen substances with anxiolytic or anxiogenic potency [11, 13]. An anxious animal increases the latency to the first entry to the white compartment and reduces the time spent in this compartment [11, 14]. On the contrary, animals treated with anxiolytic drugs (diazepam or alprazolam) spent more time in the white compartment [14, 15], which suggests an anxiolytic-like effect. In this study, the rats treated with diazepam (1.0 mg/kg) had a short latency to the first entry to the white compartment and spent more time in it, compared to control. Bradley et al. [16] reported that acute or chronic administration of diazepam (1.0 mg/kg, i.p.) increased the exploration toward the white compartment, decreased the latency to the first entry to this compartment, and increased the time spent in it, which is in accordance with the effect produced by anxiolytic substances in the BWM [15]. In the BWM, it is possible to detect false anxiolytic effects when drugs increase general locomotor activity. For example, methamphetamine enhances transitions between black-white compartments and increases the time spent in the white compartment; however, it was associated with a general locomotor hyperactivity [11, 14]. Therefore, this behavioral change in the BMW is not related with an anxiolytic-like effect; rather, it is a psychostimulant effect [14, 15]. In this study, in rats treated with diazepam, the reduced latency to entry, the increased time spent into the white compartment, and the increased exploration toward it were not related with a general locomotor hyperactivity, because diazepam did not induce any increase in crossing in the OFT. The findings are supported by previous reports that anxiolytic drugs such as diazepam increases the time spent in the white compartment in the BWM, without producing significant changes in locomotion in the OFT [17, 18]. In some cases, grooming abnormally increases in low-stressed rats [19, 20], and contrarily, in animals submitted to high-stress such as electric footshock, grooming is diminished significantly [21]. This reduction of grooming is prevented by anxiolytic drugs returning it to control [22]. In the study presented here, rats treated with diazepam spent more time in grooming in the OFT as compared to control. In this connection, a brightly illuminated compartment (in the BWM) produces anxiety in the animals [11]. Therefore, when rats treated with saline are evaluated in the OFT (after BWM test) they reduce grooming probably as a consequence of stress generated in the BWM. The rats treated with diazepam prevented the reduction of grooming in the OFT which is consistent with the effect of anxiolytic drugs (alprazolam and diazepam) on grooming in stressed rats [22]. Additionally, rats treated with diazepam had an increased rearing as compared with the control group which is interpreted as indicator of exploration; an effect enhanced by anxiolytic substances in the OFT [23].

In this study, we found that the behavioral effects of the novel 3,4-dimethoxyphenylethylamine derivates on anxiety-like behavior are depended on type of treatment (acute or repeated). The results obtained from the BWM and the OFT tests showed that the acute administration of all tested doses (0.1, 1.0 or 10 mg/kg) of PK-2126 induced anxiolytic-like effect in

rats. The acute administration of 0.1, 1.0 or 10 mg/kg doses of PK-2111 was followed by an anxiogenic-like effect compared with control. Also, the anxiogenic-like effect of the middle and the high doses of PK-2111 was more effective that with low dose of PK-2111. In contrast, acute administration of PK-2123 in all doses failed to produce any effect on anxiety-like behavior in the BWM and the behavioral reactions in the OFT. Indeed, an interesting result of the present experiments are the different effects for small dose (0.1 mg/kg) and for middle or high doses (1.0 and 10.0 mg/kg) of PK-2122 when it acutely administered on the behavioral parameters in the BWM and the OFT tests. In dose of 0.1 mg/kg, PK-2122 exerted anxiogenic-like effect, while in doses of 1.0 or 10.0 mg/kg, PK-2122 exerted anxiolytic-like effect compared with the control group. In fact, anxiolytic-like effect of PK-2122 in dose of 10.0 mg/kg dose was higher than it in a dose of 1.0 mg/kg.

The repeated treatment with PK-2111 was followed by an anxiolytic-like effect in doses of 0.1 or 1.0 mg/kg which was more profound compared to both control and diazepam group. Also, chronic treatment of PK-2111 in the high dose (10.0 mg/kg) induced anxiolytic-like effect compared with the control group, and this effect was less effective than it in a doses of 0.1 and 1.0 mg/kg. Chronic treatment with PK-2123 was followed by an anxiolytic-like effect in all doses which was significant compared with control group and group treated with diazepam. The decrease of anxiety-like behavior after PK-2123 administration in the middle dose was significantly higher compared to 0.1 mg/kg dose, and the same parameter was significantly less compared to 0.1 mg/kg or 10.0 mg/kg doses. Also, chronic treatment of PK-2123 in the high dose induced anxiolytic-like effect compared with the control group, and this effect was less effective than it in another doses. The results received from the BWM and the OFT tests showed that the chronic PK-2122 administration in all tested doses induced anxiolytic-like effect in rats. In contrast, chronic PK-2126 administration in all doses failed to produce any effect on anxiety-like behavior in the BWM and the behavioral reactions in the OFT.

Taken together these finding support the effects of the novel 3,4-dimethoxyphenylethylamine derivates after acute treatment on anxiety-like behavior are inverse after repeated treatment. The results can be summarized as follows: a) acute administration of PK-2126 exerted anxiolytic-like effect in the BWM and increased the rearing and grooming in a manner similar to diazepam, while repeated administration of PK-2126 failed to modify anxiety-like behavior in the BWM and behavioral reactions in the OFT; b) acute administration of PK-2123 failed to modify an anxiety-like behavior in the BWM and behavioral reactions in the OFT, while the repeated administration of PK-2123 exerted anxiolytic-like effect in the BWM, and increased the rearing and grooming which are exceeded the effect of diazepam; c) acute administration of PK-2111 induced anxiogenic-like effect in the BWM and decreased the rearing and grooming in the OFT, while repeated administration of PK-2111 produced anxiolytic-like effect in the BWM and increased the rearing and grooming which exceeded the effect of diazepam; d) acute administration of PK-2122 in small dose induced anxiogenic-like effect in the BWM, and decreased the rearing and grooming in the OFT; and in the middle and high doses of PK-2122 induced anxiolytic-like effect in the BWM and increased the rearing and grooming in the OFT, while repeated administration of PK-2122 in all doses produced anxiolytic-like effect in the BWM and increased the rearing and grooming which are exceeded the effect of diazepam.

Further studies exploring the participation of D_1- and D_2-receptors in the anxiolytic-like and anxiogenic-like effects of novel 3,4-dimethoxyphenylethylamine derivates should be undertaken.

Conclusions

In conclusion, these results suggest that acute or repeated administration of PK-2126 and PK-2122 may be effective in experimental model of anxiety in rats Further research is needed to elucidate the mechanisms by which these compounds affect the dopaminergic system, and if there is potential for these compounds in the clinicalarena.

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Disclosure of conflict of interest

None.

Abbreviations

BWM, black and white model; CSF, cerebrospinal fluid; DA, dopamine; HVA, homovanillic; OFT, open field test.

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