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Short communication

Anxiolytic-like effects of olanzapine, risperidone and fluoxetine in the elevated plus-maze test in rats

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Abstract:
In the present study was examined the effect of treatment with olanzapine or risperidone, given separately or in combination with fluoxetine, in the elevated plus-maze test (an animal model of anxiety) in male Wistar rats. The obtained results showed that treatment with olanzapine (1 mg/kg), risperidone (0.1 and 0.3 mg/kg) or fluoxetine (5 and 10 mg/kg) induced an anxiolytic-like effect in the elevated plus-maze test. Olanzapine, risperidone and fluoxetine, tested in doses effective in the model of anxiolytic-like actions, did not affect motor coordination, while olanzapine (3 mg/kg) and risperidone (0.3 mg/kg) produced a significant reduction of exploratory activity in the open field test. In a combination study, the anxiolytic-like effect of olanzapine or risperidone was significantly antagonized by co-treatment with fluoxetine. Additionally, co-treatment with olanzapine or risperidone and fluoxetine disturbed the motor coordination of rats in a rota-rod test. These findings indicate that olanzapine, risperidone and fluoxetine *per se* may be clinically effective in treating anxiety disorders, but their effects may be attenuated when they are used in combination with other medications.

Key words:
olanzapine, risperidone, fluoxetine, elevated plus-maze test, rats

Introduction

Several preclinical and clinical reports have suggested a beneficial effect of the addition of a low dose of an atypical antipsychotic drug, e.g., olanzapine or risperidone, to the ongoing treatment with antidepressant drugs, especially selective serotonin reuptake inhibitors, on the treatment of drug-resistant depression [e.g., 1, 5, 10, 19, 23]. Furthermore, atypical antipsychotics are also used in the treatment of anxiety-related disorders, giving inconclusive results, though [4]. Some clinical data suggest that clozapine, olanzapine, quetiapine and risperidone improve symptoms of an obsessive-compulsive disorder and a panic disorder, while other data show a worsening effect of each of these drugs on the above-mentioned disorders [2]. In the case of other anxiety disorders such as, e.g., a post-traumatic stress disorder, some studies indicate that atypical antipsychotic drugs improve certain symptoms [6, 12, 18], while others fail to reach a similar conclusion [3, 7]. Furthermore, the effectiveness of some antipsychotic drugs in a combined therapy for treatment-resistant anxiety disorders has also been reported [8, 22, 24].

Preclinical data concerning the anxiolytic-like activity of atypical antipsychotics are also inconclusive. Some of them report an anxiolytic-like, an anxiogenic-like or no effect at all in various anxiety-like tests [e.g., 9, 11, 13, 14, 25].

In order to understand the mechanism of clinical efficacy of a combination therapy with an atypical antipsychotic drug and an antidepressant for treatment-resistant anxiety disorders, in the present study we examined the effect of olanzapine or risperidone, given separately or in combination with fluoxetine in the elevated plus-maze test (an animal model of generalized anxiety) in rats. The effect of co-treatment with the above-mentioned antipsychotics (olanzapine or risperidone) and fluoxetine in the elevated plus-maze test of rats had not been previously studied. We also used an open field and a rota-rod tests to evaluate the exploratory activity and motor coordination of rats following treatment with olanzapine or risperidone, given separately or in combination with fluoxetine.

Materials and Methods

Animals

The experiments were carried out on male Wistar rats (250–270 g) (Charles River Laboratories, Sulzfeld, Germany). The animals were housed by 4 per cage (57 × 35 × 20 cm) in a colony room kept at 22 ± 1°C with a 40–50% humidity, on a 12-h light-dark cycle (the light on at 7 a.m.). The rats had free access to food and water before the experiments. All the experiments were conduced during the light phase in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC). All the experimental protocols were approved by the Local Bioethics Commission for Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Drug administration

Fluoxetine hydrochloride (Pliva, Kraków, Poland) was dissolved in distilled water, and olanzapine (Sigma-Aldrich, St. Louis, USA) or risperidone (Tocris, Bristol, UK) were suspended in a 1% aqueous solution of Tween 80. All the drugs were injected intraperitoneally (*ip*) at a volume of 2 ml/kg. Fluoxetine (2.5, 5 and 10 mg/kg) was given at 60 min, and olanzapine (0.3, 1 and 3 mg/kg) or risperidone (0.05, 0.1 and 0.3 mg/kg) at 30 min before the elevated plus-maze, open field and rota-rod tests. Each experimental group consisted of 8 rats.

Elevated plus-maze test

The maze and the testing procedure of the elevated plus-maze test were described by Pellow and File [17]: wooden plus-maze apparatus, elevated to a height of 50 cm, consisted of two open arms (50 × 10 cm; walls 38 cm high), arranged so that the two identical arms of each type were opposite each other. The plus-maze was placed in a darkened room, and the centre of the apparatus was illumined with a 25 W electric bulb hanging 100 cm above. Each rat was placed in the center of the plus-maze, facing one of the closed arms immediately after a 5-min adaptation period in a wooden box (60 × 60 × 35 cm) to enhance its overall activity in the plus-maze. During a 5-min test period, two experimenters who were sitting in the same room ca. 1 m away from the open arms recorded the number of open and closed arms entries, as well as the time spent in either type of the arms. An entry was rated once an animal was in the arms with all its four feet [16]. The maze was thoroughly cleaned after each trial.

Exploratory activity in the open field test

For the experiments, we used a black circular platform (1 m in diameter) without walls divided into six symmetrical sectors and elevated 50 cm above the floor. The laboratory room was dark and only the centre of the open field was illuminated with a 75 W bulb placed 75 cm above the platform. At the beginning of the test, the animals were gently placed in the centre of the platform and were allowed to explore. Their exploratory activity in the open field, i.e., the time of walking, the number of sector lines crossings (ambulations), episodes of peeping under the edge of the area and rearing, were assessed for 5 min [20].

Rota-rod test

The rats were preselected 1 h before the test on a rotating rod (BD-10, COTM Białystok, Poland; 6 cm in diameter, 6 rpm). Those staying on the rotating rod for 2 min (approximately 95% of the animals) were

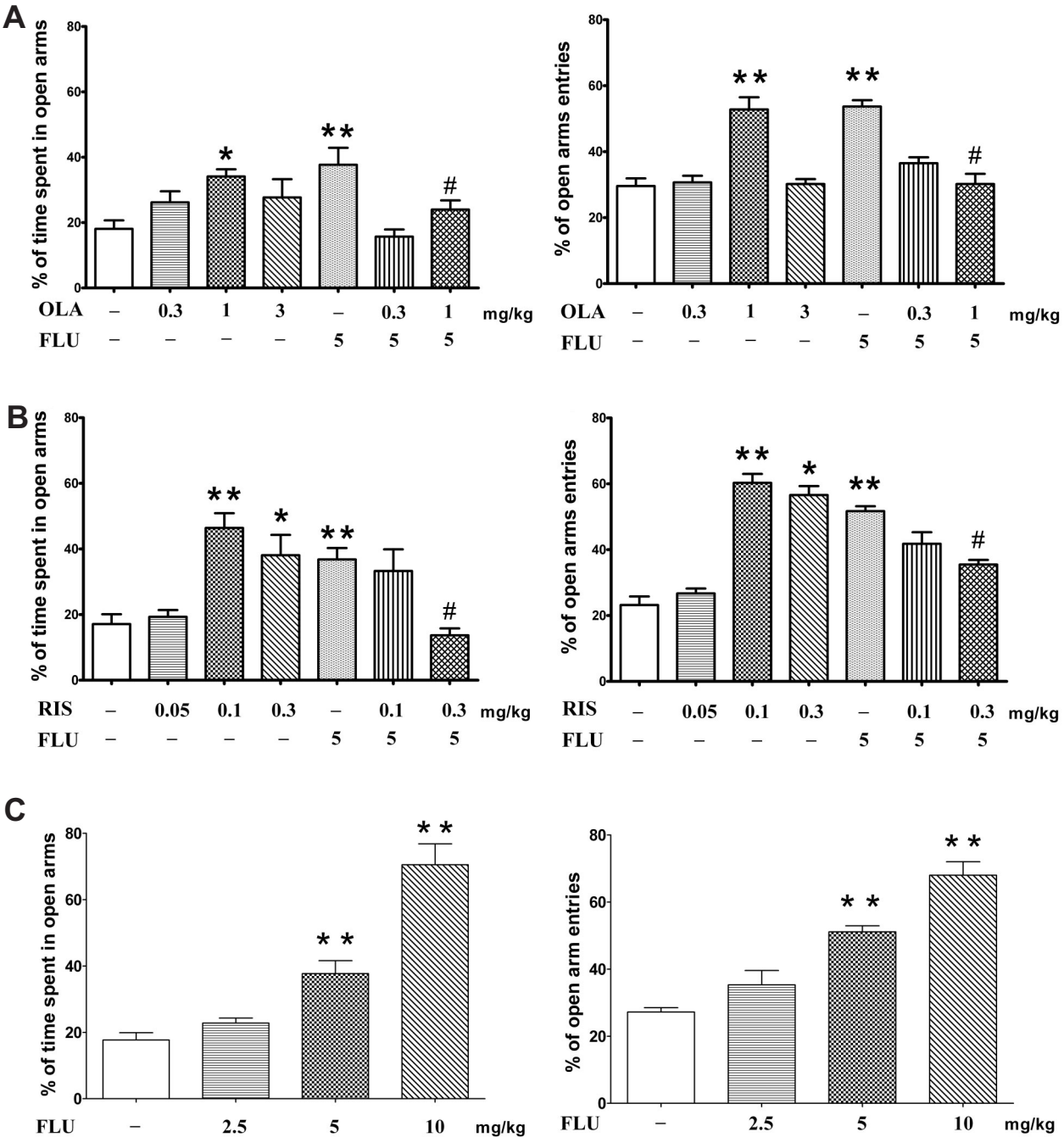

Fig. 1. The effect of olanzapine (OLA; 0.3, 1 and 3 mg/kg, *ip*) (**A**), risperidone (RIS; 0.05, 0.1 and 0.3 mg/kg, *ip*) (**B**) and fluoxetine (FLU; 2.5, 5 and 10 mg/kg, *ip*) (**C**), given alone or in combination with OLA (1 mg/kg) or RIS (0.1 and 0.3 mg/kg, *ip*) and FLU (5 mg/kg, *ip*), in the elevated plus-maze test in rats. FLU was given at 60 min, and OLA or RIS at 30 min before the test. The results are shown as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons with Dunnett's test. * $p < 0.05$, ** $p < 0.001$ *vs.* vehicle-treated group; # $p < 0.001$ *vs.* OLA- or RIS-treated group

placed again on the same rotating rod after drug administration and were observed for 2 min. The number of animals that fall from the rota-rod within 2 min was recorded. Fluoxetine was given at 60 min, and olanzapine or risperidone at 30 min before the test (like in the elevated plus-maze test).

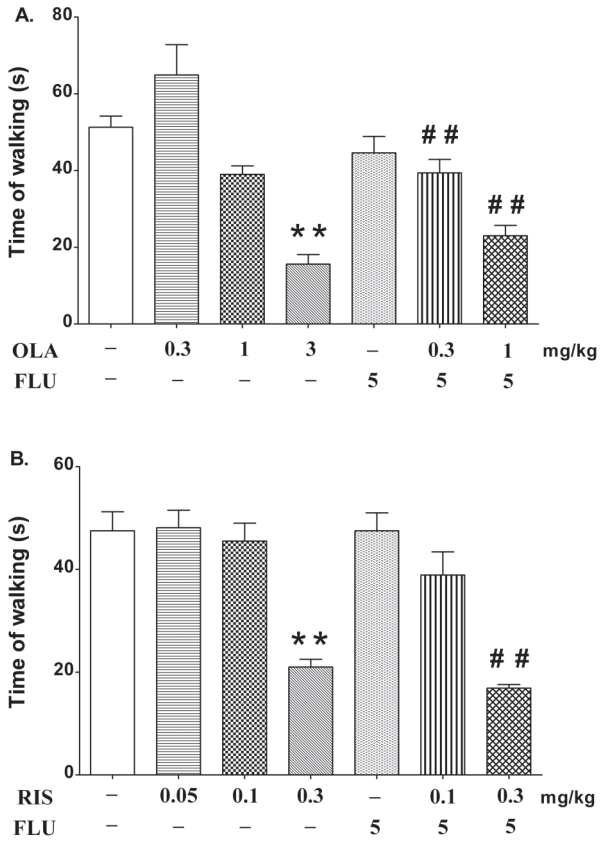

Fig. 2. The effect of olanzapine (OLA; 0.3, 1 and 3 mg/kg, *ip*) (**A**), risperidone (RIS; 0.05, 0.1 and 0.3 mg/kg, *ip*) (**B**) given alone or in combination with OLA (1 mg/kg) or RIS (0.1 and 0.3 mg/kg, *ip*) and FLU (5 mg/kg, *ip*), on exploratory activity (time of walking) in the open field test in rats. FLU was given at 60 min, and OLA or RIS at 30 min before the test. The results are shown as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons with Dunnett's test. * $p < 0.001$ *vs.* vehicle-treated group; # $p < 0.001$ *vs.* OLA- or RIS-treated group

Statistical analysis

The data were evaluated by a one-way analysis of variance (ANOVA), followed, when appropriate, by individual comparisons with the control using Dunnett's test.

Results and Discussion

An anxiolytic-like effects of the atypical antipsychotic drugs olanzapine or risperidone, and the selective serotonin reuptake inhibitor fluoxetine were evaluated in the elevated plus-maze test. In that test, the total number of entries (open + closed arms entries), recorded in control rats during a 5-min test session, was ca. 6 in the present set of experiments and was regarded as 100%. In control rats, 23.2, 29.6 and 27.2% of the entries were made into the open arms, and 17.1, 18.1 and 17.7% of the total time (265 s) spent in the arms (either type) was spent in the open arms. Olanzapine in a dose of 1 mg/kg (but not 0.3 and 3 mg/kg) significantly (up to 34.1%; $F(3,28) = 3.13$; $p < 0.05$) increased the percentage of the time spent in the open arms and the percentage of entries into the open arms (up to 52.8%; $F(3,28) = 20.21$; $p < 0.001$ (Fig. 1A). Risperidone administered in a dose of 0.05 mg/kg, did not change the number of open arms entries or the time spent in the open arms. When given in higher doses (0.1 or 0.3 mg/kg), that drug significantly increased the time spent in the open arms (up to 46.4 or 38.1%, respectively; $F(3,28) = 11.02$; $p < 0.001$), and the percentage of entries into the open arms (up to 60.3 or 56.6%, respectively; $F(3,28) = 65.53$; $p < 0.001$ (Fig. 1B). Fluoxetine administered in the lower dose (2.5 mg/kg) did not induce any anxiolytic-like activity in the elevated plus-maze test, while its higher doses (5 or 10 mg/kg) significantly increased the time spent in the open arms (up to 37.7 or 70.5%, respectively; $F(3,36) = 36.36$; $p < 0.001$), and the percentage of entries into the open arms (up to 51.1 or 68.0%, respectively; $F(3,36 = 11.29$; $p < 0.001$ (Fig. 1C). Our earlier studies indicated that diazepam, i.e., the positive standard, administered in a dose of 1.25 mg/kg was ineffective in the elevated plus-maze test; however, when given in higher doses (2.5 and 5 mg/kg), it exhibited anxiolytic-like activity and significantly increased the percentage of the time spent in the open arms (up to 47.2 and 70.4%, respectively) and the percentage of entries into the open arms (up to 73.8 and 76.2%, respectively) [21]. The above data show that the effect of olanzapine (1 mg/kg) or risperidone (0.1 and 0.3 mg/kg) in this test is similar to that produced by diazepam in a lower dose (2.5 mg/kg), while that of fluoxetine (5 and 10 mg/kg) resembles the effect evoked by the higher doses (2.5 and 5 mg/kg) of diazepam. Olanzapine, risperidone and fluoxetine in all the doses used did not change the total time spent in the arms (either type) or the total number of entries (data not shown).

An involvement of atypical antipsychotic drugs in psychiatric disorders, such as anxiety, has been suggested by other authors [11, 15, 25]. Anxiolytic-like

effects of risperidone or olanzapine were observed in the multiple measures of fear in rats. Risperidone, especially in a dose of 1 mg/kg, significantly decreased the number of avoidance responses, 22 kHz ultrasonic vocalization, the avoidance conditioning-induced hyperthermia and startle reactivity, but did not affect defecation or the time spent in the open arms. Olanzapine (2 mg/kg) significantly decreased the number of avoidance responses, 22 kHz vocalization and the number of defecations, but it did not inhibit startle reactivity or the time spent in the open arms. Haloperidol and citalopram did not display any anxiolytic-like properties in these tests [25]. Using a conditioned fear stress paradigm in mice (a model of anxiety), showed that risperidone in the low dose (0.01 mg/kg), fluvoxamine (1.25–10 mg/kg) and milnacipran (0.5–4 mg/kg) evoked anxiolytic-like activity. In a combination study, the anxiolytic-like effect of risperidone (0.01 mg/kg) was significantly reduced by fluvoxamine (1.25 and 2.5 mg/kg) or milnacipran (0.5– 2 mg/kg) [11]. Similarly, our present study indicates that the anxiolytic-like effect of olanzapine (1 mg/kg) or risperidone (0.1 and 0.3 mg/kg) in the elevated plus-maze test is significantly reduced by co-treatment with fluoxetine (5 mg/kg) (Fig. 1).

Administration of olanzapine (0.3 and 1 mg/kg) did not evoke any significant reduction of the exploratory activity of rats, as evaluated in the open field test. When given in the higher dose (3 mg/kg), that drug significantly reduced exploratory activity (the time of walking, ambulation and peeping + rearing episodes). Combined treatment with olanzapine (1 mg/kg, but not 0.3 mg/kg) and fluoxetine (5 mg/kg) evoked a significant reduction of exploratory activity (the time of walking; $F(5,42) = 10.48$; $p < 0.001$ (Fig. 2A). Risperidone in a dose of 0.3 mg/kg (but not 0.1 mg/kg) significantly reduced the exploratory activity of rats. Co-treatment with risperidone 0.3 mg/kg (but not at 0.1 mg/kg) and fluoxetine (5 mg/kg) evoked a statistically significant reduction of exploratory activity (the time of walking; $F(5,42) = 18.54$; $p < 0.001$) (Fig. 2B), while no dose of fluoxetine (2.5, 5 and 10 mg/kg) changed the exploratory activity of rats (the time of walking; $F(3,28) = 2.29$; ns) (data not shown).

Moreover, olanzapine, risperidone and fluoxetine, used in doses effective in the elevated plus-maze test, do not disturb motor coordination measured in the rota-rod test (data not shown), while co-treatment with fluoxetine (5 mg/kg) and olanzapine (1 mg/kg) or risperidone (0.3 mg/kg) induces disturbed motor coordination (the falling from the rota-rot within 2 min observation concerned 75% (6/8) of the rats for fluoxetine + olanzapine, and 87.5% (7/8) of the rats for fluoxetine + risperidone, respectively). Since the anxiolytic-like effect of olanzapine or risperidone in the elevated plus-maze test was reduced by co-treatment with fluoxetine an important role of the motor coordination disturbance in mediating their action has been suggested. Further studies are necessary to clarify these effects.

In conclusion, the above results indicate that atypical antipsychotic drugs (olanzapine or risperidone), as well as the antidepressant fluoxetine are useful for the treatment of patients suffering from anxiety disorders, whereas a combined therapy with these drugs may not always produce desirable therapeutic effects.

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