INTRODUCTION

Major depressive disorder (MDD) is a chronic mental illness with a life time prevalence of 5 – 12% in adult men and 9 – 26% in adult women (1). Although initial antidepressant (AD) therapy significantly reduces symptoms of depression in many patients, only 50 – 60% of persons suffering from MDD respond to the treatment. Moreover, ca. 30 – 40% of MDD patients never achieve symptom resolution by means of a standard AD therapy (2, 3). The problem of AD-resistant depression has been the subject of a number of comprehensive studies, with no apparent therapeutic success, though. Several clinical reports postulated a beneficial effect of the addition of a low dose of risperidone (an atypical antipsychotic) to the ongoing treatment with ADs (in particular, selective serotonin reuptake inhibitors (SSRI), such as citalopram, fluoxetine, fluvoxamine or paroxetine) (1, 4-7).

Risperidone at therapeutic doses, like other atypical antipsychotic drugs, is known to produce minimal extrapyramidal side-effects (catalepsy) and sedation compared to classic antipsychotics (e.g., haloperidol) (24). This drug is more potent in the binding to serotonin 5-HT$_2A$ than to dopamine D$_2$ receptors. Affinities of risperidone for serotoner gic 5-HT$_2A$, dopaminer gic D$_2$, adrener gic α$_1$, α$_2A$, and histaminer gic H1 receptors (Ki value) are 0.81, 6.4, 2.5, 10, and 33 nM, respectively (25). Our previous studies and reports of other authors indicated that risperidone applied at a low dose enhanced the antidepressant-like activity of citalopram in the forced swim test, and that 5-HT$_1A$ receptors may play some role in these effects. Moreover, a low dose of risperidone may also enhance the anxiolytic-like action of the studied antidepressants.

KEY WORDS: antidepressants, selective serotonin reuptake inhibitors, escitalopram, mirtazapine, risperidone, forced swim test, elevated plus-maze test
the antidepressant-like activity of ADs in the forced swim test (FST) in mice (26-28).

In the present study, we examined the effect of the AD escitalopram (ESC a SSRI) (29, 30) and a low dose of risperidone, given separately or jointly, in the FST (an animal test widely used for screening of potential antidepressant-like activity). Furthermore, we used 5-HT1A receptor antagonists to determine the role of those receptors in the antidepressant-like effect induced by co-treatment with ESC and risperidone in this test.

In order to understand the mechanism of clinical efficacy of the combination therapy with an atypical antipsychotic drug and an AD for treatment-resistant anxiety disorders, in the present study, we evaluated the effect of risperidone, given separately or in combination with ESC in the elevated plus-maze test (it is a conflict model which is used for evaluate an anxiety-like behavior in rodents). We also used the antidepressant mirtazapine (MIR) (31) to compare its effects with ESC in the elevated plus-maze test. It was earlier demonstrated that the combination treatment with MIR and risperidone enhanced cognitive and reduced negative symptoms in schizophrenic patients (32, 33). Admittedly, MIR has a broad spectrum of affinities for serotoninergic, dopaminergic D2, adrenergic α1, α2A, and histaminergic H1 receptors that could and probably contribute to its beneficial effects in patients and preclinical models. Affinities of MIR (Ki value) for 5-HT1A, D2, α1, α2A, and H1 receptors are 69, > 5454, 608, 20, and 1.6 nM, respectively (25). Especially the partial agonistic properties of MIR for 5-HT1A receptors may contribute to the antipsychotic properties of this drug (34-36). The effect of co-treatment with the above-mentioned antipsychotic (risperidone) and ESC in the FST, and risperidone with ESC or MIR in the elevated plus-maze test of rats has not been previously studied.

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 4 per cage (57 x 35 x 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 experiments were conducted during the light phase and the rats had free access to food and water before the experiments.

All the experiments were carried out according to the procedures approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Cracow.

Drug administration

Escitalopram oxalate (ESC) and WAY 100635 (Tocris Bioscience, Bristol, UK) were dissolved in a 0.9% NaCl, while risperidone and mirtazapine (MIR, Tocris Bioscience, Bristol, UK) were dissolved in 0.1 M tartaric acid and the solution was adjusted to pH 6 – 7 with 0.1 N NaOH. In the FST, all drugs were injected three times in a volume of 2 ml/kg. The first dose of ESC (1, 2.5, 5 or 10 mg/kg, i.p.) was injected 30 min after pre-test (15 min following the return of rats back to their home cages) and 23.5 h before the FST. The second and the third dose of ESC were given at 5 and 1 h before the retest sessions. WAY 100635 (0.1 mg/kg, s.c.) was given 10 min before ESC (1 mg/kg) and RIS (0.05 or 0.1 mg/kg, i.p.) at 30 min after ESC treatment. In the elevated plus-maze test, ESC (1, 2.5, 5 and 10 mg/kg, i.p.) or MIR (1, 2.5, 5 and 10 mg/kg, i.p.) were injected once at 60 min, and risperidone (0.05, 0.1 mg/kg, i.p.) at 30 min before the test.

Forced swim test (FST) in rats

On the first day of the FST (pre-test), the rats were placed individually in a cylinder (50 cm high x 23 cm in diameter) filled to a 30-cm depth with water (25 ± 1°C) for 15 min and then were removed from the water, dried with towels and placed in a warmer enclosure for 15 min, and then returned back to their home cages, as described previously (37, 38). The cylinders were emptied and cleaned between rats. Twenty-four hours following the first exposure to the forced swimming, the rats were retested for 5 min under identical conditions. Retest sessions were evaluated by two observers unaware of the treatment condition, who measured the swimming, climbing and immobility time. A rat was rated to be immobile if it was making only movements necessary to keep its head above water; swimming behavior was considered if a rat was actively making swimming movements that caused it to move within the centre of cylinder and swim below the surface of water (diving); climbing behavior was recorded if a rat was making forceful thrashing movements with its forelimbs against the walls of cylinder. The data are expressed as the mean time (± S.E.M.) of swimming, climbing and immobility, within the 5-min observation period. Each group consisted of 6 rats.

Exploratory activity of rats in the open field test

Exploratory activity was assessed in the elevated open field test. A black circular platform without walls having 1 m in diameter was divided into six symmetrical sectors and was 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 animal was placed gently in the centre of the platform and was allowed to explore. The exploratory activity in the open field, i.e. the time of walking, the number of sector line crossings (ambulation), episodes of peeping under the edge of the area and rearing, were assessed for 5 min RIS and ESC were given three times before the test (like in the FST) (39) or once before the test (like in the elevated plus-maze test) (40). Each group consisted of 6 rats.

Elevated plus-maze test

The maze and the testing procedure of the elevated plus-maze test were described by Pellow and File (41): wooden plus-maze apparatus, elevated to a height of 50 cm, consisted of two open arms (50 cm x 10 cm; walls 38 cm high), arranged so that the two identical arms of each type were opposite to each other. The plus-maze was placed in a darkened room, and the centre of the apparatus was illuminated with a 25 W electric bulb hanging 100 cm above. Each rat was placed in the centre of the plus-maze, facing one of the closed arms immediately after a 5-min adaptation period in a wooden box (60 cm x 60 cm x 35 cm) to enhance its the 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 entered in the arms with all its four feet. The maze was thoroughly cleaned after each trial. Each group consisted of 8 rats.

Statistical analysis

All of the results are shown as the mean ± S.E.M.; the data were analyzed by one-way analysis of variance (ANOVA), followed, when appropriate, by individual comparisons with the control using Dunnett's test when ESC was given alone in the
forced swim test (FST) (Fig. 1A) or when ESC or MIR were given alone in the elevated plus-maze test (Figs. 2A and 3A). In case of co-treatment with ESC or MIR and RIS statistically significant differences between the studied group were calculated using a two-way ANOVA following by the Newman-Keuls test (Figs. 1B, 1C, 2B, 3B, 3C, 4A, 4B). P < 0.05 was considered statistically significant.

RESULTS

Forced swim test (FST) in rat

The effects of ESC (1, 2.5 and 5 mg/kg) given alone on swimming, climbing and immobility time of rats in the FST are shown in Fig. 1A. One-way ANOVA showed that ESC at doses of 2.5 and 5 mg/kg (but not 1 mg/kg) in a significant manner increased the swimming time [\(F(3,20) = 11.20, P < 0.001\)] and only at a dose of 5 mg/kg decreased the immobility time [\(F(3,20) = 5.06, P < 0.01\)] but did not change the climbing time of those rats [\(F(3,20) = 0.90, \text{ns}\)].

Two-way ANOVA showed that co-treatment with ESC (1 mg/kg) and risperidone (0.05 mg/kg) evoked antidepressant-like effect in this test by increasing the swimming time, namely, the analysis revealed effects of ESC (1 mg/kg) [\(F(1,20) = 25.2885, P < 0.000064\)], and RIS (0.05 mg/kg) [\(F(1,20) = 35.4312, P < 0.000008\)], and an interaction between ESC and risperidone [\(F(1,20) = 19.4745, P < 0.000268\)], or by decreasing the immobility time of rats with a significant, effect of ESC (1 mg/kg) [\(F(1,20) = 8.216, P < 0.009544\)], and RIS (0.05 mg/kg) [\(F(1,20) = 158.2712, P < 0.000000\)], and an interaction between ESC (1 mg/kg) and risperidone (0.05 mg/kg) [\(F(1,20) = 12.166, P < 0.001\) vs. vehicle-treated group (Fig. 1B vs. vehicle-treated group (Fig. 1A)). P < 0.001 vs. vehicle-treated group (Fig. 1A). In case of co-treatment with ESC and RIS statistically significant differences between the studied group were calculated using a two-way ANOVA following by the Newman-Keuls test (Fig. 1B and 1C). *P < 0.001 vs. vehicle-treated group; *P < 0.001 vs. ESC- or RIS-treated group; \(^{a}P < 0.01\) vs. ESC + RIS-treated group.
P < 0.0002319]. WAY 100635 (0.1 mg/kg) abolished this antidepressant-like effect (on the swimming time and immobility time) in those animals [F(4,25) = 8.02, P < 0.001 and F(4,25) = 17.41, P < 0.001, respectively]. The climbing time was decreased in both studied groups, namely, the analysis demonstrated no effect of ESC (1 mg/kg) [F(1,20) = 3.3270, P < 0.083128], no effect of RIS (0.05 mg/kg) [F(1,20) = 0.3697, P < 0.550026], and an interaction between ESC plus RIS [F(1,20) = 6.7899, P < 0.006915] or a significant effect of co-treatment with WAY 100635 plus ESC plus RIS [F(4, 25) = 7.00, P < 0.001] (Fig. 1B).

Two-way ANOVA showed that co-treatment with ESC (1 mg/kg) and risperidone at a higher dose (0.1 mg/kg) also induced antidepressant-like effect as manifested by the increased swimming time with a significant, effect of ESC (1 mg/kg) [F(1,20) = 24.4888, P < 0.000076], and an interaction between ESC (1 mg/kg) and risperidone (0.1 mg/kg) [F(1,20) = 27.95, P < 0.000036], no effect of RIS (0.1 mg/kg) [F(1,20) = 0.3697, P < 0.000077], or by the decreased immobility time with an, effect of ESC (1 mg/kg) [F(1,20) = 3.3270, P < 0.006261], and an interaction between ESC (1 mg/kg) and risperidone (0.1 mg/kg) [F(1,20) = 14.3582, P < 0.001] (Way 100635 (0.1 mg/kg) abolished this antidepressant-like effect on the swimming time and immobility time in rats [F(4,25) = 18.88, P < 0.001] and [F(4,25) = 16.51, P < 0.001, respectively]. The climbing time was decreased in both studied groups, i.e. ESC plus RIS, and the analysis showed no effect of ESC (1 mg/kg) [F(1,20) = 0.4388, P < 0.515244], effect of RIS (0.1 mg/kg) [F(1,20) = 14.3582, P < 0.001], and an interaction between ESC and risperidone [F(1,20) = 8.0389, P < 0.010225], and a significant effect of WAY 100636 plus ESC plus RIS [F(4,25) = 9.52, P < 0.001] (Fig. 1C).

**Exploratory activity of rats in the open field test**

ESC given alone three times before the test (like in the FST) at the used doses (1, 2.5, 5 and 10 mg/kg) and risperidone (0.05 or 0.1 mg/kg) or combined treatment with ESC (1 mg/kg) and risperidone (0.05 or 0.1 mg/kg) did not evoke any significant change in the exploratory activity of rats, as evaluated in the open field test (data not shown).

**Elevated plus-maze test**

The anxiolytic-like effects of the antidepressant ESC or MIR and the atypical antipsychotic risperidone were evaluated in the elevated plus-maze test. In that test, the total number of entries (open + closed arm 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, 36.5, 28.4, 26.7, 32.6 and 29.7% of the entries were made into the open arms, and 12.4, 9.6, 7.7, 18.3 and 10.3% of the total time (268 s) spent in the arms (either type) was spent in the open arms.

One-way ANOVA showed that ESC at a dose of 5 mg/kg only (but not at 1, 2.5 and 10 mg/kg) significantly [F(4,35) = 37.83, P < 0.001] increased the percentage of the time spent in the open arms, and the percentage of entries into the open arms [F(4,35) = 6.27, P < 0.001] (Fig. 2A).

Two-way ANOVA showed that co-treatment with ESC (2.5 mg/kg) and risperidone at a dose of 0.05 mg/kg induced also anxiolytic-like activity by, significantly increasing the time spent in

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**Fig. 2.** The effect of escitalopram (ESC; 1, 2.5, 5 and 10 mg/kg) given alone (A), or the combination of ESC (2.5 or 5 mg/kg) with risperidone (RIS; 0.05 or 0.1 mg/kg) (B) in the elevated plus-maze test in rats. ESC was given at 60 min, and RIS at 30 min before the test. The results are shown as the mean ± S.E.M. of 8 animals/group. Statistical significance was evaluated using a one-way analysis of variance (ANOVA), followed by individual comparison using Dunnet’s test. *P < 0.001 vs. vehicle-treated group (Fig. 2A). In case of co-treatment with ESC and RIS statistically significant differences between the studied group were calculated using a two-way ANOVA following by the Newman-Keuls test (Fig. 2B). **P < 0.001 vs. vehicle-treated group; *P < 0.01 vs. ESC- or RIS-treated group; ^P < 0.01 vs. RIS (0.1 mg/kg)-treated group.
Fig. 3. The effect of mirtazapine (MIR; 2.5, 5 and 10 mg/kg) given alone (A), or the combination of MIR (1 or 2.5 mg/kg) with risperidone (RIS; 0.05 mg/kg) (B) or RIS (0.1 mg/kg) (C) in the elevated plus-maze test in rats. MIR was given at 60 min, and RIS at 30 min before the test. The results are shown as the mean ± S.E.M. of 8 animals/group. Statistical significance was evaluated using a one-way analysis of variance (ANOVA), followed by individual comparison using Dunnet's test (Fig. 3A). *P < 0.001 vs. vehicle-treated group. In case of co-treatment with MIR and RIS statistically significant differences between the studied group were calculated using a two-way ANOVA following by the Newman-Keuls test (Fig. 3B and 3C). *P < 0.01 vs. vehicle-treated group; *P < 0.01 vs. MIR- or RIS-treated group.
the open arms; namely, the analysis revealed effects of risperidone (0.05 mg/kg) \( [F(1,28) = 61.373, P = 0.00000] \), and ESC (2.5 mg/kg) \( [F(1,28) = 8.721, P < 0.01] \), and an interaction between ESC (2.5 mg/kg) and risperidone (0.05 mg/kg) \( [F(2,42) = 16.203, P < 0.001] \), and by elevating the percentage of entries into the open arms with the significant effect of risperidone (0.05 mg/kg) \( [F(1,28) = 20.786, P = 0.0001] \), and ESC (2.5 mg/kg) \( [F(1,28) = 13.692, P < 0.001] \), and an interaction between ESC (2.5 mg/kg) and risperidone (0.05 mg/kg) \( [F(1,28) = 3.484, P = 0.07] \) (Fig. 2B).

Two-way ANOVA showed that risperidone (0.05 mg/kg) did not increase the effect of an effective dose of ESC (5 mg/kg) on the anxiolytic-like activity, namely, with regard to the time spent in the open arms, there was no effect of risperidone (0.05 mg/kg) \( [F(1,28) = 0.770, ns] \), but there was a significant effect of ESC (5 mg/kg) \( [F(1,28) = 24.171, P < 0.00001] \), and an interaction between ESC (5 mg/kg) and risperidone (0.05 mg/kg) \( [F(1,28) = 6.638, P < 0.02] \), and with regard to the percentage of entries into the open arms, the analysis demonstrated, no effect of effect of risperidone (0.05 mg/kg) \( [F(1,28) = 0.741, ns] \), a significant effect of ESC (5 mg/kg) \( [F(1,28) = 30.502, P < 0.00000] \) and an interaction between ESC (5 mg/kg) and risperidone (0.05 mg/kg) \( [F(1,28) = 3.484, P = 0.06] \) (Fig. 2B).

In contrast, two-way ANOVA showed that ESC (2.5 mg/kg) reduced the anxiolytic-like effect of risperidone (0.1 mg/kg). With regard to the time spent in the open arms, there were effects of risperidone (0.1 mg/kg) \( [F(1,28) = 40.495, P = 0.000000] \), and ESC (2.5 mg/kg) \( [F(1,28) = 15.268, P < 0.0005] \), and there was a significant interaction between ESC (2.5 mg/kg) and risperidone (0.1 mg/kg) \( [F(1,28) = 8.133, P < 0.01] \), and with regard to the percentage of entries into the open arms, the analysis indicated an, effect of risperidone (0.1 mg/kg) \( [F(1,28) = 22.701, P = 0.00005] \), no effect of ESC (2.5 mg/kg) \( [F(1,28) = 1.549, ns] \), and a significant interaction between ESC (2.5 mg/kg) and risperidone (0.1 mg/kg) \( [F(1,28) = 8.598, P = 0.01] \) (Fig. 2B).

Two-way ANOVA showed that co-treatment with an effective dose of risperidone (0.1 mg/kg) and ESC (5 mg/kg) reduced the anxiolytic-like activity, compared with the action of this drug given alone. Specifically, with regard to the time spent in the open arms, there was no effect of risperidone (0.1 mg/kg) \( [F(1,28) = 1.994, ns] \), no effect of ESC (5 mg/kg) \( [F(1,28) = 0.483, ns] \), but there was a significant interaction between ESC (5 mg/kg) and risperidone (0.1 mg/kg) \( [F(1,28) = 23.842, P < 0.0001] \), and with regard to the percentage of entries into the open arms, the analysis demonstrated no effect of risperidone (0.1 mg/kg) \( [F(1,28) = 0.399, ns] \), no effect of ESC (5 mg/kg) \( [F(1,28) = 0.0313, ns] \), and a significant interaction between ESC (5 mg/kg) and risperidone (0.1 mg/kg) \( [F(1,28) = 24.183, P = 0.0001] \) (Fig. 2B).

In the following experiment, one-way ANOVA showed that MIR administered at 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 ( \( [F(3,28) = 12.89, P < 0.001] \) and the percentage of entries into the open arms \( [F(3,28) = 13.81, P < 0.001] \) (Fig. 3A).

Two-way ANOVA showed that co-treatment with the MIR (1 mg/kg) and risperidone at a dose of 0.05 mg/kg induced anxiolytic-like activity, by significantly increasing the time spent in the open arms with, effects of risperidone (0.05 mg/kg) \( [F(1,28) = 9.264, P < 0.01] \), and MIR (1 mg/kg) \( [F(1,28) = 15.526, P < 0.001] \), and a significant interaction between MIR
Co-treatment with a higher dose of MIR (2.5 mg/kg) and risperidone (0.05 mg/kg) induced also anxiolytic-like activity by significantly increasing the time spent in the open arms, with significant effects of risperidone (0.05 mg/kg) \( F(1,28) = 106.324, P < 0.0000 \), and MIR (2.5 mg/kg) \( F(1,28) = 151.188, P < 0.0000 \), and an interaction between MIR (1 mg/kg) and risperidone (0.05 mg/kg) \( F(1,28) = 28.882, P = 0.01 \) (Fig. 3B).

The present data showed that MIR (2.5 mg/kg) also did not increase anxiolytic-like activity risperidone at a higher dose (0.1 mg/kg) by the time spent in the open arms; namely, the analysis showed, an effect of risperidone (0.1 mg/kg) \( F(1,28) = 83.486, P < 0.0000 \), no effect of MIR (0.1 mg/kg) \( F(1,28) = 3.516, ns \), and no interaction between MIR (1 mg/kg) and risperidone (0.1 mg/kg) \( F(1,28) = 2.083, ns \), and the percentage of entries into the open arms, effect of risperidone (0.1 mg/kg) \( F(1,28) = 54.966, P < 0.0000 \), no effect of MIR (1 mg/kg) \( F(1,28) = 1.871, ns \), and no interaction between MIR (1 mg/kg) and risperidone (0.1 mg/kg) \( F(1,28) = 0.954, ns \) (Fig. 3B).

Furthermore, the present data showed that (like in the elevated plus-maze test) co-treatment with ESC (2.5 and 5 mg/kg) or MIR (2.5 mg/kg) and risperidone at a higher dose (0.1 mg/kg) induced a statistically significant reduction of the exploratory activity (only the time of walking by ca. 30 – 40% as compared to control group), namely, two-way ANOVA showed, no effect of ESC (2.5 mg/kg) \( F(1,20) = 6.8206, P < 0.020958 \), no effect of RIS (0.1 mg/kg) \( F(1,20) = 5.7930, P < 0.025872 \), and a significant interaction between ESC (2.5 mg/kg) and risperidone (0.1 mg/kg) \( F(1,20) = 6.0344, P < 0.008303 \), in addition, there was no effect of a higher dose of ESC (5 mg/kg) \( F(1,20) = 8.5959, P < 0.23294 \), but there was a significant interaction between ESC (5 mg/kg) and risperidone (0.1 mg/kg) \( F(1,20) = 9.2765, P < 0.006833 \) (Fig. 4A). Moreover, we observed no effect of MIR (1 mg/kg) \( F(1,20) = 0.113, P < 0.740614 \), no effect of RIS (0.1 mg/kg) \( F(1,20) = 0.451, P < 0.509681 \), and no interaction between MIR (1 mg/kg) and risperidone (0.1 mg/kg) \( F(1,20) = 0.028, P < 0.868398 \), further, there was no effect of a higher dose of MIR (2.5 mg/kg) \( F(1,20) = 5.682, P < 0.05959 \), but there was significant interaction between MIR (2.5 mg/kg) and risperidone (0.1 mg/kg) \( F(1,20) = 11.701, P < 0.002709 \) (Fig. 4B).

**DISCUSSION**

The results of the present study indicated that ESC evoked antidepressant-like effect in the FST in rats by increasing the swimming time and decreasing the immobility time. Moreover, the atypical antipsychotic drug risperidone alone at either dose used did not produce any antidepressant-like effect in that test, whereas it enhanced the action of ESC by increasing the swimming time and decreasing the immobility time in those rats. WAY 100635 (a 5-HT\(_{1A}\) receptor antagonist) abolished the antidepressant-like effect induced by co-treatment with ESC and risperidone. It is commonly known that false positive action in the FST can be induced by different dopamine stimulants used at doses that increase locomotor activity (42, 43). The present experiments showed that none of the tested drugs, i.e. neither ESC nor risperidone given alone or in combination enhanced exploratory activity of rats as measured in the open field test, what indicated that potentiation of the antidepressant-like effect of ESC by risperidone in the FST did not reflect the increase in general activity, in addition, our data suggested that 5-HT\(_{1A}\) receptors might be involved in the effect.

The contribution of 5-HT\(_{1A}\) and noradrenergic receptors to the activity of ADs in the FST in rats has been widely examined. For example, it was earlier shown that SSRI (fluoxetine and paroxetine) or 5-HT\(_{1A}\) receptor agonist (8-OH-DPAT and gepirone) increased the swimming time and decreased the immobility time, while a selective noradrenaline reuptake inhibitor (desipramine or maprotyline) increased climbing and decreased the immobility time in the FST in rats, what indicated that the enhancement of 5-HT transmission might mediate swimming behavior, while enhancement of noradrenaline neurotransmission might mediate climbing behavior in the FST (37).

Similarly, our earlier experiment also showed that risperidone enhanced the swimming time and decreased the immobility time of fluoxetine (SSRI) in the FST in rats, and WAY 100635 reduced the antidepressant-like effect induced by co-treatment with fluoxetine and risperidone, what indicated that enhancement of 5-HT\(_{1A}\) transmission might mediate this action. Furthermore, risperidone enhanced the swimming and climbing time, and decreased the immobility time of MIR (this drug enhances noradrenergic and 5-HT\(_{1A}\)-mediated serotonergic neurotransmission by antagonizing central \(\alpha2\)-auto- and hetero-adrenergic receptors) (31) and WAY 100635 abolished the antidepressant-like effect induced by co-treatment with mirtazapine and risperidone. Since risperidone enhanced the swimming and climbing time, and decreased the immobility time of MIR in the FST, an important role of 5-HT\(_{1A}\) and noradrenergic receptors in mediating their action has been suggested (39).

The present results are in line with all the above-cited studies which indicated that risperidone enhanced the swimming time and decreased the immobility time of ESC (SSRI) in the FST, what suggested that enhancement of 5-HT transmission might contribute to this action.

In addition, clinical studies report lower serum zinc in depressed patients, suggesting a strong link between zinc and mood disorders. Also copper as an antagonistic element to zinc seems to play a role in depression, where elevated concentration is observed. The behavioral data indicated that chronic (but not acute) treatment with ADs, escitalopram or reboxetine normalized but imipramine increased this zinc/copper ratio. Observation in this study normalization of serum zinc/copper ratio in depression-like conditions by chronic ADs suggest that this ratio may be consider as a marker of depression or treatment efficacy (44). It was also demonstrated that chronic unpredictable mild stress (CUMS) induced testicular dysfunctions and oxidative stress. While treatment of CUMS rats with fluoxetine decreases the depressive behavior, it causes further worsening of testicular dysfunctions and oxidative stress. Administration of resveratrol (a phytoalexin with antioxidant properties) improves testicular dysfunctions and oxidative stress.
that are caused by CUMS and further worsened by fluoxetine treatment (45).

Moreover, it is suggest that atypical antipsychotic drugs, all of which are relatively more potent as 5-HT2A than dopamine D2 antagonists, may improve negative and cognitive dysfunctions in schizophrenia, in part, via increasing cortical dopamine release (46). Our earlier study showed that combined treatment of an ineffective dose of risperidone with MIR reduced the head switch reaction induced by DOI (a 5-HT2A agonist) what suggested that this combination of MIR and risperidone might enhance the antipsychotic-like effect of risperidone in the animal test modeling some positive symptoms of schizophrenia (47). Moreover, co-treatment with an ineffective dose of risperidone and MIR or ESC abolished the deficits evoked by MK-801 in the social interaction test in rats what suggested that antidepressants especially MIR, and to a smaller degree ESC might enhance the antipsychotic-like effect of a low dose of risperidone in the animal test used to evaluate some negative symptoms of schizophrenia (48). It is known that risperidone is more potent in binding to serotonin 5-HT2A receptor than MIR (about 85 times), affinities of those drugs for 5-HT2A receptors (Ki value) are 0.81 and 69 nM, respectively (25).

The present data are also in line with the previous behavioural studies in rats which indicated that ESC but not citalopram might enhance the effect of a low dose of risperidone in animal tests used to evaluate some positive, negative, cognitive, and depressive symptoms of schizophrenia without an increase extrapyramidal side-effects (49, 50).

In addition, the clinical study suggested that addition of MIR to risperidone therapy can efficiently improve treatment of both negative and some cognitive symptoms of schizophrenia (32, 33). The following study also indicated that MIR can be used safely with risperidone in schizophrenic patients, since the lack of a pharmacokinetic interaction between MIR and risperidone was observed in those patients (plasma concentration of risperidone and its metabolite 9-hydroxyrisperidone did not change for six weeks of therapy) (51).

However, a preliminary clinical study indicated that co-treatment with risperidone and ESC did not efficiently alleviate the negative symptoms of schizophrenic patients. ESC was well tolerated but was not more effective than placebo in the treatment of the negative symptoms of schizophrenia (52). A further study in subgroups of patients with negative symptoms is needed to evaluate whether those patients may still respond to co-treatment with atypical antipsychotic risperidone with ESC.

All the earlier and present behavioral data are in line with the previous clinical observations which suggested that adjunct treatment with antidepressants might enhance the effect of a sub-therapeutic dose of risperidone on the positive, negative, cognitive, and depressive symptoms of schizophrenia. Anxiolytic-like effects of risperidone and ESC or MIR were evaluated in the elevated plus-maze test. Our data showed that risperidone at a higher dose and ESC or MIR induced an anxiolytic-like activity in this test. Moreover, co-treatment with an ineffective dose of risperidone enhanced the anxiolytic-like actions of ESC or MIR. Furthermore, ESC co-treatment with the higher (effective) dose of risperidone reduced the anxiolytic-like effect of risperidone in this test. The lack of anxiolytic-like activity following co-treatment with the above drugs can result from the decreased exploratory activity of those rats (by ca. 30 – 40% as compared to control group), evaluated in the open field test.

In our earlier studies, we demonstrated that diazepam, i.e. the positive standard, when given at higher doses (2.5 and 5 mg/kg), evoked anxiolytic-like activity by significantly increasing the percentage of the time spent in the open arms (by up to 47.2 and 70.4%, respectively) and the percentage of entries into the open arms (by up to 73.8 and 76.2%, respectively) (53). Moreover, the action of atypical antipsychotic olanzapine (1 mg/kg) or risperidone (0.1 and 0.3 mg/kg) in this test was similar to that produced by diazepam at a lower dose (2.5 mg/kg), while the effect of fluoxetine (10 mg/kg) was similar to the action induced by the higher dose (5 mg/kg) of diazepam (40). The present data also showed that the action of atypical antipsychotic risperidone (0.1 mg/kg) and ESC or MIR (5 mg/kg) in this test was similar to that produced by diazepam at a lower dose (2.5 mg/kg), while the effect of MIR (10 mg/kg) was similar to the action evoked by the higher dose (5 mg/kg) of diazepam. The efficacy of atypical antipsychotic drugs in anxiety disorders has also been suggested by other authors. Anxiolytic-like effects of risperidone or olanzapine were observed in the multiple measures of fear in rats. In this test, risperidone (1 mg/kg) significantly decreased the number of avoidance responses, 22 kHz ultrasonic vocalization, 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 evoked some anxiolytic-like activity in these tests (23). Data from the conditioned fear stress paradigm in mice (a model of anxiety), showed that risperidone at a low dose (0.01 mg/kg), fluvoxamine (1.25 – 10 mg/kg) and milnacipran (0.5 – 4 mg/kg) induced 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) (20). Similarly, our early study in rats indicated 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 was significantly reduced by co-treatment with fluoxetine (5 mg/kg). The above drugs given alone did not disturb motor coordination measured in the rota-rod test, while co-treatment with fluoxetine and olanzapine or risperidone disturbed motor coordination of those rats. Thus, an important role of the motor coordination disturbance in reduction of the anxiolytic-like effect in this test has been suggested (40).

Although the effectiveness of combined therapy with antipsychotics and some SSRI in anxiety disorders has been recently demonstrated in clinical investigations (14, 15, 18), the mechanism of interaction of these drugs is not completely understood, yet. Since risperidone evoked antagonist action at 5-HT2A receptors (Ki value is 0.81 nM) (25), it was suggested to play an important role in the improved therapeutic effects not only of negative symptoms of schizophrenia, but also it is possible that risperidone applied alone or in combination with SSRI may exhibit an anxiolytic-like effect by antagonizing 5-HT2A receptors, especially the ones located on glutamatergic pyramidal neurons and GABAergic interneurons in the cortex and hippocampus (54, 55). On the other hand, it is known that 5-HT2A receptors are localized postsynaptically (56, 57), ADs (especially SSRIs) by increasing of endogenous 5-HT at nerve terminals might thought to indirectly activate the 5-HT2A receptors. Thus, it is suggested that the opposite effects of risperidone at a higher dose and ADs (especially SSRIs) on the 5-HT2A receptors might be involved in the reduction of their anxiolytic-like efficacy when they are co-treatment. All the earlier and present data indicate that atypical antipsychotic drugs (olanzapine or risperidone) and the ADs (ESC, fluvoxamine, fluoxetine, milnacipram and MIR) may be useful for the treatment of patients suffering from anxiety disorders, while a combined therapy with these drugs not always produced the anticipated therapeutic effects. Further studies are necessary to explain its mechanism of action.

In summary, the results obtained in this study indicate that risperidone applied at a low dose enhances the antidepressant-
like activity of ESC in the FST in rats, and among other mechanisms [5-HT$_{2A}$, α$_2$-adrenergic and histamine H$_1$ receptors (58)], the serotonin 5-HT$_{2A}$ receptors may be involved in this effect. Moreover, risperidone, ESC and MIR evoked anxiolytic-like effect in the elevated plus-maze test in rats, and risperidone used at a low dose enhanced the anxiolytic-like effect ESC or MIR in this test. The obtained results indicate that risperidone applied at a low dose may enhance the antidepressant- and anxiolytic-like effects of ESC in animal tests, and suggest that the combined therapy may be useful for the treatment of patients suffering from drug resistant depression and also for some patients with treatment-resistant anxiety disorders.

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