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**Short communication**

# Effects of co-treatment with mirtazapine and low doses of risperidone on immobility time in the forced swimming test in mice

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**Abstract:**
The aim of the present study was to examine the effect of mirtazapine (MIR) and risperidone (an atypical antipsychotic drug), given separately or jointly, on immobility time in the forced swimming test in male C57BL/6J mice. Fluoxetine (FLU) was used as a reference drug. MIR (2.5, 5 and 10 mg/kg) and FLU (5 and 10 mg/kg), or risperidone in low doses (0.05 and 0.1 mg/kg) given alone did not change the immobility time of mice in the forced swimming test. Joint administration of MIR (5 and 10 mg/kg) or FLU (10 mg/kg) and risperidone (0.1 mg/kg) produced antidepressant-like activity in the forced swimming test. WAY 100636 (a 5-$HT_{1A}$ receptor antagonist) inhibited, while yohimbine (an $\alpha_2$-adrenergic receptor antagonist) potentiated the antidepressant-like effect induced by co-administration of MIR and risperidone. Active behavior in that test did not reflect an increase in general activity, since combined administration of antidepressants and risperidone failed to enhance the locomotor activity of mice. The obtained results indicate that risperidone applied in a low dose enhances the antidepressant-like activity of MIR and that, among other mechanisms, 5-$HT_{1A}$-, and $\alpha_2$-adrenergic receptors may play a role in this effect.

**Key words:**
mirtazapine, fluoxetine, risperidone, forced swimming test, mice

## Introduction

Major depression occurs in up to 10% of the human population. Moreover, the currently used antidepressant drugs (ADs) show therapeutic efficacy as a monotherapy in about 60–70% of depressive patients [e.g., 1, 11, 14]. Therefore, in order to improve therapy, a combination of ADs belonging to various pharmacological groups, or a combination of an AD and a substance enhancing its effect is used in the clinic [e.g., 2, 6, 13]. Among the agents that are expected to potentiate the efficacy of ADs are atypical antypsychotics (e.g., olanzapine, risperidone, quetiapine, ziprasidone or aripiprazole) which produce minimal extrapyramidal side-effects and which have also been found to be effective and tolerable in some patients with treatment-resistant depression [23, 24]. Several clinical reports have postulated a beneficial effect of additional risperidone to ongoing treatment with ADs (especially with selective serotonin reuptake inhibitors [SSRI] such as, e.g., fluoxetine, fluvoxamine or paroxetine) [9, 10, 15, 16, 18]. Like other atypical antipsychotic drugs, risperidone is known to produce minimal extrapyramidal side-effects compared to classic antipsychotics (e.g., chlorpromazine) [12]. This

drug is ca. 20–50 times more potent in its binding to 5-$HT_{2A}$ serotonin receptors than to $\alpha_1$-adrenergic, dopamine $D_2$, histamine $H_1$ and $\alpha_2$-adrenergic ones [19, 22].

To understand the mechanism of the clinical efficacy of an AD and an atypical antypsychotic (a combination therapy) in treatment-resistant depression, the present study was aimed at examining the effect of another AD, mirtazapine (MIR), which enhances noradrenaline and serotonin neurotransmission *via* its antagonistic action on central $\alpha_2$-adrenergic receptors [e.g., 3], and that of risperidone, given separately or jointly, on immobility time in the forced swimming test (FST – an animal model of depression) in male C57BL/6J mice. The effect of co-treatment with MIR and risperidone on the immobility time of mice subjected to the FST had not been studied before. We also used 5-$HT_{1A}$ and $\alpha_2$-adrenergic receptor antagonists to determine the role of those receptors in the antidepressant-like effect induced by joint treatment with MIR and risperidone in the FST. Fluoxetine (FLU) was used as a reference drug.

## Materials and Methods

### Animals

The experiments were carried out on male C57BL/6J mice (23 ± 2 g) (Charles River Laboratories, Sulzfeld, Germany). The animals were housed 8 per cage (57 × 35 × 20 cm) in a colony room kept at 21 ± 1°C with a 40–50% humidity, on a 12-h light-dark cycle (the light on at 7 a.m.). The mice 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 experimental protocols were approved by the Local Bioethics Commission for Animal Experiments at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

### Drugs administration

Fluoxetine hydrochloride (FLU; Pliva, Kraków, Poland), WAY 100635 (synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) and yohimbine hydrochloride (Research Biochemicals Incorporated, USA) were dissolved in distilled water; mirtazapine (MIR, Organon, The Netherlands) and risperidone (Tocris, UK) were suspended in a 1% aqueous solution of Tween 80. WAY 100635 (0.1 mg/kg, *sc*) and yohimbine (2 mg/kg, *ip*) were given 10 min before MIR or FLU. MIR (2.5, 5 or 10 mg/kg, *ip*) and FLU (5 or 10 mg/kg, *ip*) were given at 60 min, and risperidone (0.05 or 0.1 mg/kg, *ip*) at 30 min before the FST and locomotor activity test.

### FST in mice

FST was evaluated in mice according to the slightly modified method of Porsolt et al. [17]. Briefly, each mouse was individually placed in a glass cylinder, filled with water up to 9 cm, at 22–23°C. Immobility time was recorded for the last 4 min of the 6-min FST. The animals were used only once for each experiment. Groups consisted of 8 mice each.

### Locomotor activity test

The locomotor activity of mice was recorded using the Opto-M3 System (Columbus Instruments, Columbus, OH, USA), which is a multi-channel activity monitor supporting sensors (0.5" beam spacing) attached to the computer and which measures both ambulatory activity and total counts every 10 min for 30 min [25]. Each group consisted of 8 mice.

### 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

The obtained results showed that neither the antidepressant drug MIR (2.5, 5 and 10 mg/kg) nor FLU (5 and 10 mg/kg) given alone modified the immobility time of mice in the FST ($F(3,28) = 2.23$ ns, and $F(2,21) = 0.62$ ns, respectively; data not shown). The atypical antypsychotic drug risperidone in doses of 0.05 and 0.1 mg/kg did not change the immobility

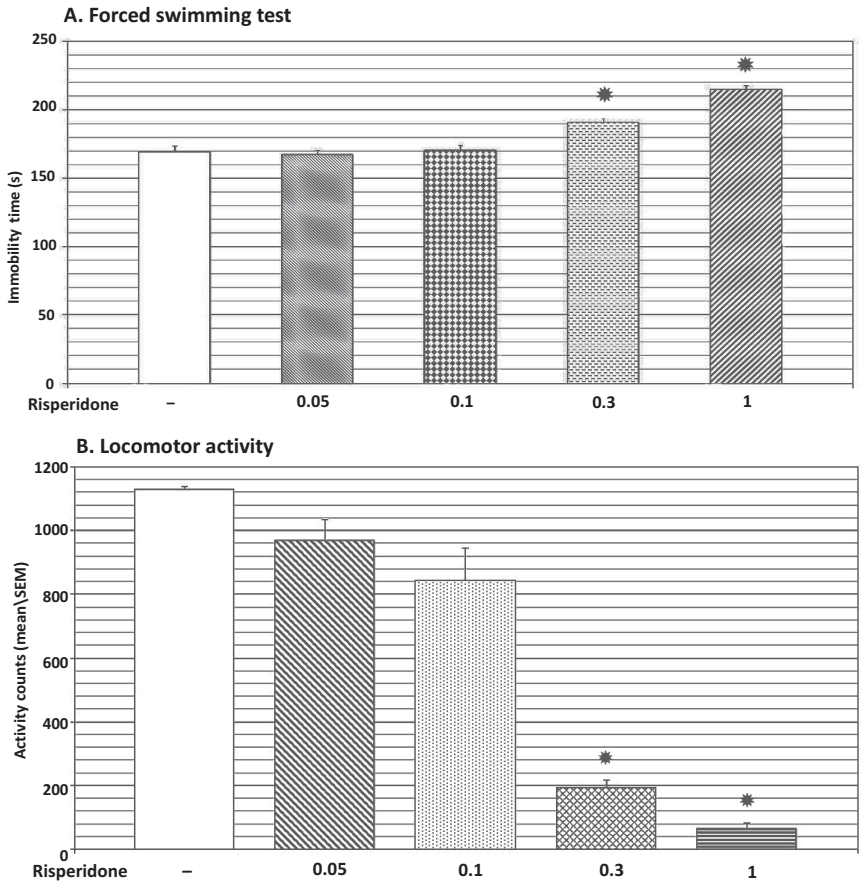


**Fig. 1.** The effect of risperidone (RIS, 0.05, 0.1, 0.3 and 1 mg/kg, *ip*) on immobility time in the forced swimming test (**A**) and on the locomotor activity (**B**) of mice. RIS was given 30 min before the test. The results are shown as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * $p < 0.001$ *vs.* vehicle-treated group

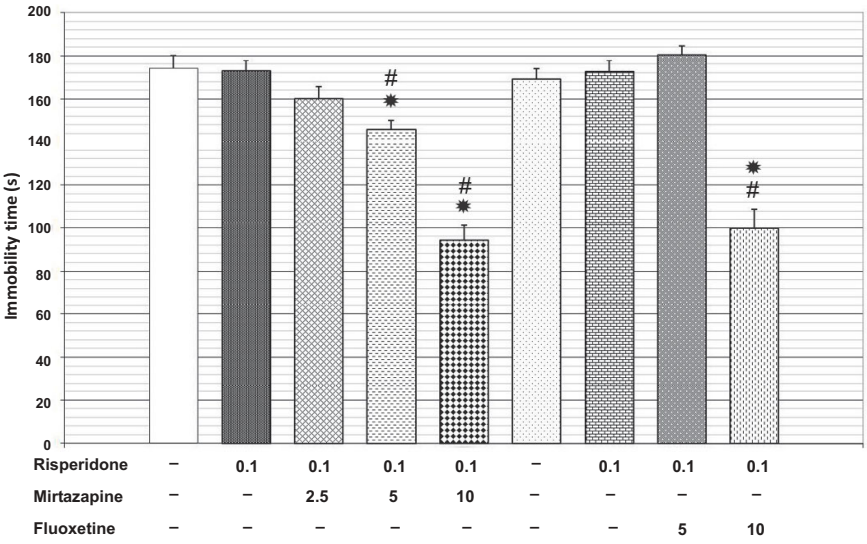


**Fig. 2.** The effect of mirtazapine (MIR, 2.5, 5 and 10 mg/kg, *ip*) or fluoxetine (FLU, 5 and 10 mg/kg, *ip*), given alone or in combination with risperidone (RIS, 0.1 mg/kg, *ip*) on immobility time in the forced swimming test in mice. MIR and FLU were given 60 min and RIS 30 min before the test. The results are shown as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * $p < 0.001$ *vs.* vehicle-treated group, # $p < 0.001$ *vs.* RIS-treated group

time of mice, while its higher doses (0.3 and 1 mg/kg) exhibited pro-depressive activity by increasing the immobility time of mice in that test ($F(4,35) = 34.50$, $p < 0.001$; Fig. 1A). Co-treatment with MIR (5 and 10 mg/kg) or FLU (5 and 10 mg/kg) and a lower dose of risperidone (0.05 mg/kg) did not change the immobility time of mice ($F(5,42) = 1.15$, ns; data not shown). On comparison, joint administration with MIR (5 or 10, but not 2.5 mg/kg) and a higher dose of risperidone (0.1 mg/kg) revealed antidepressant-like activity by shortening the immobility time of mice in that test ($F(4,35) = 34.89$, $p < 0.001$; Fig. 2). Like in the case

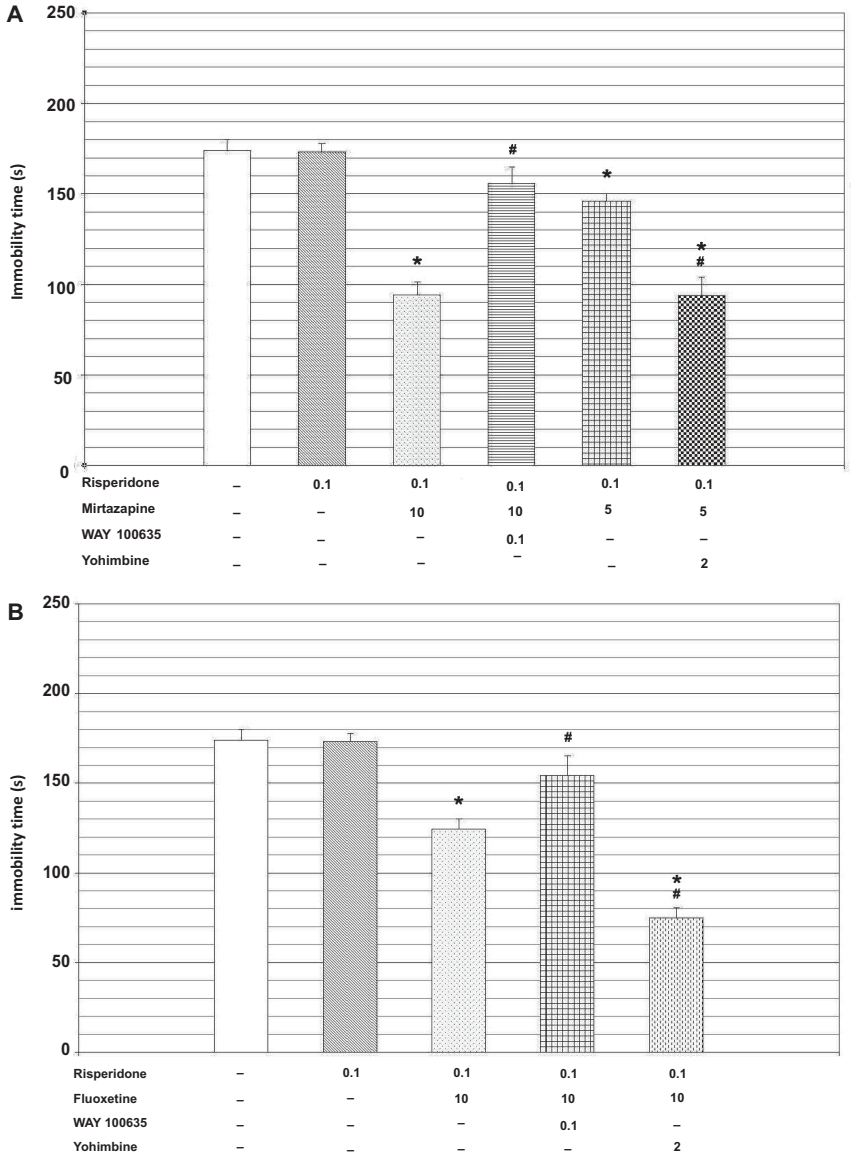


**Fig. 3.** The influence of WAY 100635 (0.1 mg/kg, *sc*) or yohimbine (2 mg/kg, *ip*) on the effect of combined treatment with mirtazapine (MIR) (**A**) or fluoxetine (FLU) (**B**) with risperidone (RIS) in the forced swimming test in mice. MIR and FLU were given 60 min and RIS 30 min before the test. WAY 100635 or yohimbine was given 10 min before MIR or FLU. The results are shown as the mean ± SEM of 8 animals/group. The data were statistically evaluated by ANOVA, followed by individual comparisons using Dunnett's test. * $p < 0.001$ *vs.* vehicle- or RIS-treated group, # $p < 0.001$ *vs.* RIS + MIR- or RIS + FLU-treated group

of MIR, co-treatment with FLU (10 but not 5 mg/kg) and risperidone (0.1 mg/kg) also exhibited antidepressant-like activity in that test ($F(3,28) = 39.49$, $p < 0.001$; Fig. 2). The present data are in line with some earlier observations that risperidone in a dose of 0.1 mg/kg enhanced the antidepressant-like effect of FLU (10 and 20 mg/kg) or venlafaxine (4 and 8 mg/kg) in the FST without altering the locomotor activity of mice [5].

Moreover, also our earlier [20] and present results showed that WAY 100635 (a 5-$HT_{1A}$ receptor antagonist) in a dose of 0.1 mg/kg was ineffective in the FST (data not shown), but inhibited the antidepressant-like effect induced by co-administration of MIR or FLU (10 mg/kg) and risperidone (0.1 mg/kg). On comparison, yohimbine (an $\alpha_2$-adrenergic receptor antagonist) in a dose of 2 mg/kg did not change immobility time (data not shown), but potentiated the antidepressant-like effect induced by co-administration of MIR (2.5 mg/kg) or FLU (10 mg/kg) and risperidone (0.1 mg/kg) (Fig. 3). Similarly, in an earlier study the antidepressant-like effect of a combination of risperidone (0.1 mg/kg) and FLU (10 mg/kg) or venlafaxine (4 mg/kg) was enhanced by the addition of yohimbine (2 mg/kg) [5].

It is widely accepted that false positive effects in the FST can be induced by various dopamine stimulants used in doses that increase locomotor activity. The present experiment showed that none of the tested

drugs, i.e., neither MIR (2.5, 5 or 10 mg/kg) nor FLU (5 or 10 mg/kg) nor risperidone (0.05 or 0.1 mg/kg) – alone or in combination with MIR – nor FLU given jointly with risperidone enhanced locomotor activity (data not shown). Risperidone in a dose of 0.1 mg/kg slightly reduced the locomotor activity of mice by ca. 25% (but did not change the immobility time of mice in that test), while its higher doses (0.3 and 1 mg/kg) increased immobility time and significantly decreased locomotor activity by ca. 83 and 95%, respectively ($F(4,25 = 48.53)$, $p < 0.001$; Fig. 1). Some earlier data suggested that the major effect of risperidone given in higher doses was *via* blockade of dopamine $D_2$ receptors, while its lower doses used in the present study (0.05 and 0.1 mg/kg) acted *via* involvement of 5-$HT_{2A}$ serotonin receptors (see Discussion in [5]). Furthermore, the present results demonstrated that WAY 100635 (0.1 mg/kg) and the $\alpha_2$-adrenergic receptor antagonist yohimbine (2 mg/kg) in the doses used in the FST neither changed locomotor activity in a statistically significant manner nor decreased the activity of mice after joint administration of MIR or FLU and risperidone (0.1 mg/kg) (data not shown). All the above data indicated that potentiation of the antidepressant-like effect of MIR or FLU by risperidone in the FST did not reflect the increase in general activity, since combined administration of the drugs studied failed to alter the locomotor activity of mice, measured in the locomotor activity test. All the same, they suggested that 5-$HT_{1A}$ and $\alpha_2$-adrenergic receptors may be involved in that effect. Furthermore, there was postulated an important role of 5-$HT_{2A}$ receptors in mediating that action in the FST. It is well known that MIR, a new antidepressant, enhances noradrenergic and 5-$HT_{1A}$-mediated serotonergic neurotransmission by antagonizing central $\alpha_2$-auto- and hetero-adrenoreceptors, but does not inhibit the uptake of noradrenaline and 5-HT. Moreover, it blocks 5-$HT_2$ and 5-$HT_3$ receptors and displays very low affinity for dopaminergic receptors and high affinity for histamine $H_1$ receptors (e.g., [3]). By comparison, risperidone is about 20–50 times more potent in binding to 5-$HT_{2A}$ receptors than to $\alpha_1$-adrenergic, dopamine $D_2$-, and $\alpha_2$-adrenergic ones, and also show sligh affinity for histamine $H_1$ receptors [19, 22]. It is suggested that the selectivity of risperidone for 5-$HT_{2A}$- *vs*. 5-$HT_{2C}$ receptors offers a more favorable therapeutic option in various mood disorders including depression. However, the addition of risperdone to serotonergic antidepressants may trigger complex interactions between the serotonergic, dopaminergic and/or noradrenergic systems. It is postulated that administration of SSRIs leads to a decrease in norepinephrine neuronal firing [21] and, subsequently, builds up resistance to its antidepressant action, which can be overcome by administration of risperidone, a 5-$HT_{2A}$ receptors antagonist. Risperidone is known to reverse the SSRI-induced inhibition of the activity of norepinephrine neurons by a mechanism involving 5-$HT_{2A}$ receptors [7]. Hence, the drugs that exert both those effects (serotonin reuptake inhibition and 5-$HT_{2A}$ receptor antagonism) may have a more beneficial therapeutic action compared to SSRIs.

Some earlier studies suggested that $\alpha_2$-adrenoreceptors profoundly affected monoaminergic neurotransmission by enhancing both tone and serotonergic firing rate [4]. Moreover, those studies and the present findings indicate that yohimbine, an $\alpha_2$-adrenergic receptor antagonist, enhance the antidepressant-like action of venlafaxine and FLU and potentiate the effect of joint treatment with those ADs and risperidone [5], and the present data in the mouse FST suggest a role of $\alpha_2$-adrenoreceptors in those effects.

Moreover, some biochemical data indicate that a combination of 5-$HT_{2A}$ antagonism and 5-$HT_{1A}$ agonism may potentiate the antidepressant-like effect [8]. The above observations are in line with the present results, which show that WAY 100635 (a 5-$HT_{1A}$ receptor antagonist) inhibits the antidepressant-like effect induced by co-administration of MIR or FLU and risperidone in the FST in mice. In conclusion, the obtained results reveal that risperidone (a 5-$HT_{2A}$ receptor antagonist) applied in a low dose enhances the antidepressant-like activity of MIR or FLU in the FST in mice. Since a combination of antidepressant drugs and risperidone is enhanced by the addition of yohimbine (an $\alpha_2$-adrenergic receptor antagonist), or inhibited by WAY 100635 (a 5-$HT_{1A}$ receptor antagonist), an important role of $\alpha_2$-adrenergic, 5-$HT_{1A}$ and also 5-$HT_{2A}$ receptors in mediating their action has been suggested.

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