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Short communication

Enhancement of the anti-immobility action of antidepressants by risperidone in the forced swimming test in mice

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Abstract:
The aim of the present study was to examine the effect of antidepressants (ADs) belonging to different pharmacological groups and risperidone (an atypical antipsychotic drug), given separately or jointly, on immobility time in the forced swimming test in male C57BL/6J mice. The antidepressants: citalopram, fluvoxamine, sertraline, reboxetine, milnacipran (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. Co-treatment with reboxetine or milnacipran (10 mg/kg) and risperidone in a lower dose of 0.05 mg/kg or with sertraline, reboxetine (5 and 10 mg/kg), citalopram, fluvoxamine, milnacipran (10 mg/kg) and risperidone in a higher dose of 0.1 mg/kg produced antidepressant-like effect in the forced swimming test. WAY 100635 (a 5-HT_{1A} receptor antagonist) inhibited the effects induced by co-administration of ADs and risperidone. Active behavior in the forced swimming test was not a consequence of an increased general activity, since the combined treatment with ADs and risperidone failed to enhance the locomotor activity of mice. The obtained results indicate that a low dose of risperidone enhances the activity of ADs in an animal model of depression, and that, among other mechanisms, 5-HT_{1A} receptors may play a role in these effects.

Key words:
antidepressant drugs, risperidone, forced swimming test, mice

Introduction

All currently used antidepressant drugs (ADs), including tricyclic and newer agents, such as venlafaxine or milnacipran, have shown therapeutic efficacy in monotherapy in ca. 60–70% of patients [e.g., 1, 8, 10, 22]. The problem of AD-resistant depression has been the subject of a number of comprehensive studies, with no apparent therapeutic success, though. Hence, there is a strong need for alternative antidepressant treatment. Agents that are expected to potentiate the efficacy of ADs comprise atypical antipsychotics (e.g., olanzapine, risperidone, quetiapine, ziprasidone, aripiprazole), which produce minimal extrapyramidal side-effects and which have also been found to be efficient and tolerable in some patients with treatment-resistant depression [11, 22, 23]. Several clinical reports have postulated a beneficial effect of the addition of a low dose risperidone to the ongoing treatment with ADs (in particular, selective serotonin reuptake inhibitors [SSRI], such as fluoxetine, fluvoxamine or paroxetine) [5, 7, 12, 13, 16]. Like other atypical antipsychotic drugs, risperidone is known to produce mini-

mal extrapyramidal side-effects compared to classic antipsychotics (e.g., chlorpromazine) [9]. This drug is ca. 20–50 times more potent in the binding to 5-HT_{2A} serotonin receptors than to α_1-adrenergic, dopamine D_2, histamine H_1 and $_2$-adrenergic ones [17, 21]. It is proposed that in lower doses, risperidone mainly acts through blocking the 5-HT_{2A} serotonin receptors and in higher doses it blocks D_2 dopamine receptors *in vivo*. Our previous studies indicated that risperidone applied in a low dose enhanced the antidepressant-like activity of mirtazapine and fluoxetine in the forced swimming test (FST), and that 5-HT_{1A}- and α_2-adrenergic receptors might play some role in that effect [18].

In order to understand the mechanism of clinical efficacy of the combination therapy with an AD and an atypical antipsychotic in treatment-resistant depression, in the present study we examined the effect of other ADs belonging to different pharmacological groups [citalopram (CIT), fluvoxamine (FLUV), sertraline (SER), SSRI], reboxetine (REB, selective noradrenaline reuptake inhibitor), milnacipran (MIL, selective serotonin and noradrenaline reuptake inhibitor)] and a low dose of risperidone, given separately or jointly, on the immobility time in the FST (an animal model of depression) in male C57BL/6J mice. The effect of co-treatment with the above ADs and risperidone on the immobility time of mice subjected to the FST had not been studied before. We also used 5-HT_{1A} receptor antagonists to determine the role of those receptors in the antidepressant-like effect induced by joint treatment with ADs and risperidone in the FST.

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 the 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

CIT (hydrobromide, H. Lundbeck A/S, Denmark), FLUV (maleate, Tocris, UK), MIL (hydrochloride, Centre de Recherche Pierre Fabre, France), SER (hydrochloride, Sigma, USA), REB (hydrochloride, PNU-0155950E, Pharmacia & Upjohn, USA), WAY 100635 (synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) were dissolved in distilled water and risperidone (Tocris, UK) was suspended in a 1% aqueous solution of Tween 80. All drugs were injected in a volume of 10 ml/kg. WAY 100635 (0.1 mg/kg, *sc*) was given 10 min before the ADs studied. CIT, FLUV, SER, REB, MIL (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

The FST was evaluated in mice according to the method of Porsolt et al. [14, 15]. 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 which measures both ambulatory activity and total counts every 10 min for 30 min [18]. 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 the studied ADs: CIT, FLUV, SER, REB, MIL (5 and 10 mg/kg) given alone did not change the immobility time of mice in the FST (data not shown). The present (Figs. 1–3) and earlier data showed that an atypical antipsychotic drug risperidone in doses of 0.05 and 0.1 mg/kg did not change the immobility time of mice, while its higher doses (0.3 and 1 mg/kg) exhibited pro-depressive activity by increasing the immobility time in that test [18]. Co-treatment with SSRI: CIT, FLUV, SER (5 and 10 mg/kg) and a lower dose of risperidone (0.05 mg/kg) did not change the immobility time of mice (data not shown), while joint administration with REB or MIL (10, but not 5 mg/kg) and risperidone (0.05 mg/kg) revealed antidepressant-like activity by shortening the immobility time of mice in that test [$F(3,28) = 5.69$, * $p < 0.001$ or $F(3,28) = 20.93$, $p < 0.001$, respectively; Figs. 1A and 1B]. Moreover, co-treatment with REB, SER (5 and 10 mg/kg), MIL, CIT, FLUV (10 but not 5 mg/kg) and risperidone in a higher dose (0.1 mg/kg) exhibited antidepressant-like activity in that test (Figs. 2 and 3). 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 fluoxetine (10 and 20 mg/kg) or venlafaxine (4 and 8 mg/kg) and mirtazapine (5 and 10 mg/kg) in the FST without altering the locomotor activity of mice [2, 18]. Moreover, an earlier [18, 19] and present results showed that WAY 100635 (a 5-HT_{1A} receptor antagonist) in a dose of 0.1 mg/kg

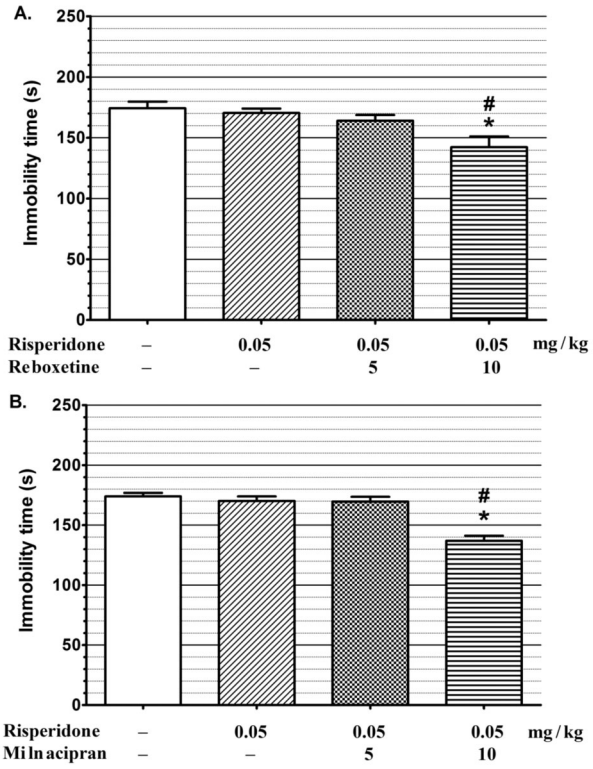

Fig. 1. The effect of reboxetine (REB, 5 and 10 mg/kg, *ip*) (**A**) or milnacipran (MIL, 5 and 10 mg/kg, *ip*) (**B**), given alone or in combination with risperidone (RIS, 0.05 mg/kg, *ip*) on immobility time in the forced swimming test in mice. REB and MIL were given 60 min and RIS 30 min before the 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

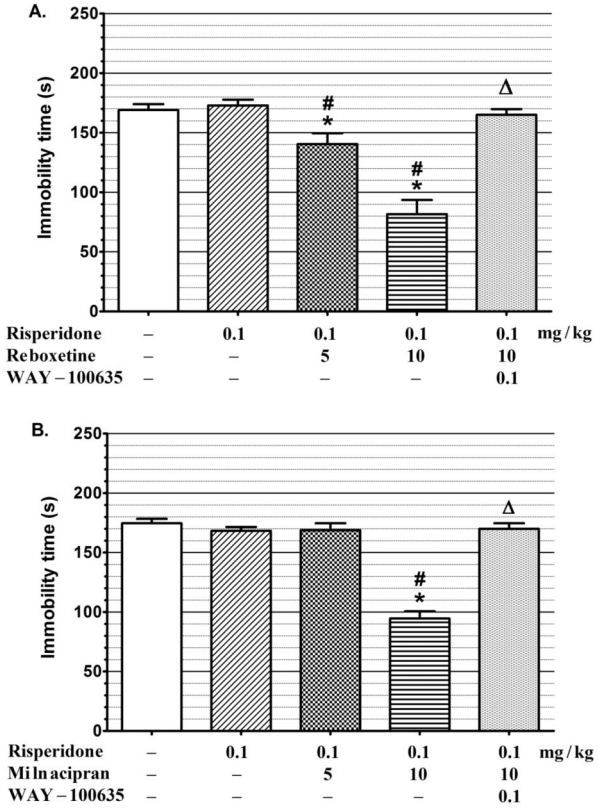

Fig. 2. The influence of WAY 100635 (0.1 mg/kg *sc*) on the effect of combined treatment with reboxetine (REB) (**A**) or milnacipran (MIL) (**B**) with risperidone (RIS, 0.1 mg/kg, *ip*) in the forced swimming test in mice. REB and MIL (5 or 10 mg/kg) were given 60 min and RIS 30 min before the test. WAY 100635 was given 10 min before ADs studied. 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, Δ $p < 0.001$ *vs.* AD + RIS-treated group

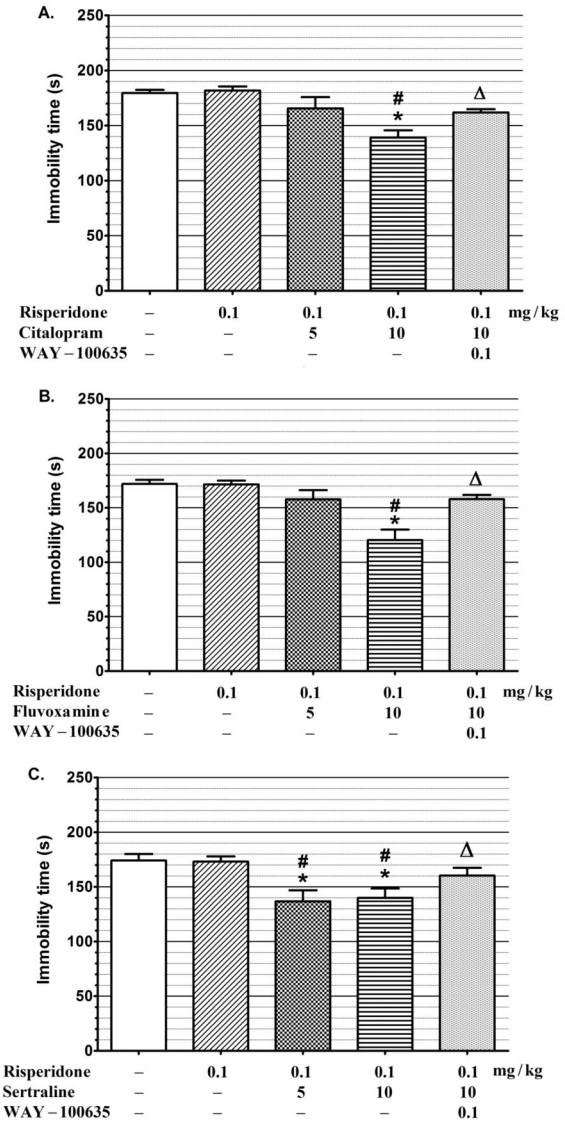

Fig. 3. The influence of WAY 100635 (0.1 mg/kg, *sc*)) on the effect of combined treatment with citalopram (CIT) (**A**), fluvoxamine (FLUV) (**B**) or sertraline (SER) (**C**) with risperidone (RIS, 0.1 mg/kg, *ip*) in the forced swimming test in mice. CIT, FLUV and SER (5 or 10 mg/kg) were given 60 min and RIS 30 min before the test. WAY 100635 was given 10 min before ADs studied. 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, Δ $p < 0.001$ *vs.* AD + RIS-treated group

was ineffective in the FST (data not shown), but partially inhibited the antidepressant-like effect induced by co-administration of the studied ADs (10 mg/kg) and risperidone (0.1 mg/kg): REB, $F(4,35) = 24.41$, $p < 0.001$; MIL, $F(4,35) = 50.42$, $p < 0.001$; CIT, $F(4,35) = 8.08$, $p < 0.001$; FLUV, $F(4,35) = 10.80$, $p < 0.001$; and SER, $F(4,35) = 6.33$, $p < 0.001$ (Figs. 2 and 3). Similarly, in our previous study the antidepressant-like effect of a combination of fluoxetine or mirtazapine (10 mg/kg) and risperidone (0.1 mg/kg) was inhibited by the addition of WAY 100635 (0.1 mg/kg) [18].

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 ADs under study (5 or 10 mg/kg) nor risperidone (0.05 or 0.1 mg/kg), alone or in combination 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 [18]. Some earlier data suggested that the major effect of risperidone given in higher doses was elicited *via* dopamine D_2 receptor blockade, while its lower doses used in the present study (0.05 and 0.1 mg/kg) acted *via* 5-HT_{2A} serotonin receptors (see Discussion, [2]). Furthermore, the present results demonstrated that WAY 100635 (0.1 mg/kg) in the dose used in the FST neither changed locomotor activity in a statistically significant manner nor did it decrease the activity of mice after joint administration of ADs and risperidone (0.1 mg/kg) (data not shown). All the above data indicated that potentiation of the antidepressant-like effect of ADs studied by risperidone was not a consequence of an increased general activity, since the combined treatment with ADs and risperidone failed to enhance the locomotor activity of mice, measured in the locomotor activity test, and they suggested that 5-HT_{1A} receptors may be involved in that effect.

Furthermore, it was postulated that 5-HT_{2A} receptors play an important role in mediating that action in the FST. By comparison, risperidone is about 20–50 times more potent in binding to 5-HT_{2A} receptors than to α_1-adrenergic, dopamine D_2-, and α_2-adrenergic ones, and also shows a slight affinity for histamine H_1 receptors [17, 21]. 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 ADs may trigger complex interactions between the serotonergic, dopaminergic and/or noradrenergic systems. It has been postulated that administration of SSRIs leads to a decrease in norepinephrine neuronal firing [20] and, subse-

quently, 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 [3]. 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.

Moreover, some biochemical data have indicated that a combination of 5-HT_{2A} antagonism and 5-HT_{1A} agonism may potentiate the antidepressant-like effect [4]. It was shown that the combination of risperidone and CIT produced significantly greater increases in the efflux of both dopamine and noradrenaline than risperidone alone. However, the effect of this combination on extracellular 5-HT concentration was not significantly different than that of CIT alone. The augmentation of dopamine and norepinephrine efflux induced by risperidone plus CIT was partially blocked by the 5-HT_{1A} receptor antagonist, WAY 100635 [6]. The above observations are in line with the present results which show that WAY 100635 inhibits the antidepressant-like effect induced by co-administration of an ADs 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 ADs in the FST in mice. Since the effect exerted by the combination of ADs belonging to different pharmacological groups and risperidone is inhibited by WAY 100635, among other mechanisms, an important role of 5-HT_{1A}- and also 5-HT_{2A} receptors in mediating their action has been suggested.

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