The 5-HT₂ Receptor Antagonist Reduces Immobility of Mice Treated with the Atypical Antidepressant Mianserin in the Forced Swimming Test

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Effects of co-administration with the 5-HT₂ receptor antagonist LY 53857 and the antidepressant mianserin on immobility time in the forced swimming test were investigated in mice. Mianserin did not affect the immobility time at ranges from 1–10 mg/kg, although it elicited anti-immobility effects at 20 mg/kg. Co-administration of the 5-HT₂ receptor antagonist LY 53857 and a subactive dose of mianserin induced apparent anti-immobility effects. Since mianserin facilitates noradrenaline (NA) release from nerve terminals *via* inhibition of α_2 receptors, the blockade of the 5-HT₂ receptor may increase the antidepressant effect of drugs modifying NA levels in the synaptic cleft.

Key words mianserin; 5-HT₂ receptor; forced swimming test; immobility; antidepressant

Forced swimming test is widely used as the screening model for assessments of antidepressants.^{1,2)} Most antidepressants reduce immobility time in both mice and rats and the mechanisms of antidepressants have been investigated using this model.^{1–3)} It is well recognized that depression is associated with the noradrenergic and serotonergic systems. Drugs inhibiting noradrenaline (NA) and/or 5-HT reuptake at nerve terminals, or inhibiting metabolism of these amines (monoamine oxidase inhibitors) improve depression.^{4–6)}

We previously demonstrated that the tricyclic antidepressant imipramine which inhibits reuptake of both 5-HT and NA, induces anti-immobility effects and that blockade of the 5-HT₂ receptor leads to potentiation of the antidepressant effects of imipramine.⁷⁾ Furthermore, it was found that the 5-HT₂ receptor antagonist reduces immobility time in mice treated with NA reuptake inhibitors, but not with selective serotonin reuptake inhibitors (SSRIs).⁸⁾ These results indicate that the blockade of the 5-HT₂ receptor selectively potentiate antidepressant effects of drugs that inhibit NA reuptake and that this receptor may participate in antidepressant effects by modifying NA transmission.

Mianserin is an atypical antidepressant, which inhibits α_2 adrenoceptor located in nerve terminals of noradrenergic neurons, resulting in facilitating NA release and increasing NA levels in the synaptic cleft.⁹⁾ As described above, the blockade of the 5-HT₂ receptor enhances the anti-immobility effects of NA reuptake inhibitors. Since mianserin increases NA release, the 5-HT₂ receptor antagonist may affect the immobility time of mice treated with mianserin. In the present paper, we examined the immobility time following co-administration of the 5-HT₂ receptor antagonist LY 53857 and mianserin.

MATERIALS AND METHODS

Male ddY mice weighing 25–30 g were purchased from SLC Japan Inc. (Japan). The mice were given free access to food and water and were housed under a controlled 12-h/12-h light–dark cycle (light from 7:00 a.m. to 7:00 p.m.), with room temperature 23 ± 1 °C and humidity 55 ± 5%.

Mianserin HCl and 4-isopropyl-7-methyl-9-(2-hydroxy-1-

methyl-propoxycarbonyl)-4,6A,7,8,9,10,10A-octahydro-indolo[4,3-FG]quinolone maleate (LY 53857) were obtained from Research Biochemicals Inc. (U.S.A.) and dissolved in saline. Mianserin was given i.p. LY 53857 was administered i.p. 30 min before the injection of mianserin.

The forced swimming test was performed according to the methods described by Porsolt *et al.*¹⁾ Each mouse was placed in a 25 cm glass cylinder (10 cm diameter) containing 10 cm of water maintained at 23 ± 1 °C. Immobility was recorded during a 6-min swimming test.

Locomotor activity for 10 min was measured by a digital counter with an infrared sensor (Neuroscience Inc., Japan) 30 min after the injection of mianserin.

Dose-related effects of mianserin on immobility were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. Other results were analyzed by two-way ANOVA followed by Tukey's test.

RESULTS

Effects of Mianserin on Immobility in Forced Swimming Test Figure 1 shows effects of mianserin on immobility in mice. Mianserin at doses ranging from 1–10 mg/kg did not affect immobility time. At 20 mg/kg, mianserin reduced immobility time significantly.

Fig. 1. Effects of Mianserin on Immobility in Forced Swimming Test
Results are shown as mean ± S.E. ($n=6-9$). Mianserin was given i.p. *** $p<0.001$.

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Fig. 2. Effects of Co-administration of LY 53857 and Mianserin on Immobility in the Forced Swimming Test

Results are shown as mean $\pm$ S.E. ($n=5-8$). Mianserin at 5 mg/kg was given i.p. LY 53857 was injected i.p. 30 min before mianserin. *** $p<0.001$ vs. saline of respective group. # $p<0.05$, ### $p<0.001$ vs. saline+mianserin-treated group.

Fig. 3. Effects of Co-administration of LY 53857 and Mianserin on Locomotor Activity in Mice

Results are shown as mean $\pm$ S.E. ($n=5-8$). Mianserin at 5 mg/kg was given i.p. LY 53857 was injected i.p. 30 min before mianserin.

Immobility Time of Mice Treated with LY 53857 and Mianserin Figure 2 shows the immobility after co-administration of the 5-HT₂ receptor antagonist LY 53857 and mianserin at 5 mg/kg. Mianserin at 5 mg/kg did not affect immobility time. Apparent anti-immobility appeared following the administration of LY 53857 and mianserin. LY 53857 alone did not affect immobility.

Locomotor Activity with Treated with LY 53857 and Mianserin As shown in Fig. 3, treatment with LY 53857 (5 mg/kg) and mianserin (5 mg/kg) did not affect locomotor activity.

DISCUSSION

Mianserin, an atypical antidepressant, does not affect uptake of monoamines, but antagonizes noradrenergic α_2 receptors.⁹⁾ Mianserin is also recognized as a 5-HT_{2A/2C} receptor antagonist.¹⁰⁻¹²⁾ It is well known that several antidepressants reduce immobility in the forced swimming test.^{1,2)} It has been reported that mianserin also reduces immobility time in mice.^{1,3,13)} In the present study, we also found that administration of mianserin decreases immobility, although this effect appeared only at a dose of 20 mg/kg. Our results are consistent with previous reports.^{1,3,13)}

The present results demonstrated that co-administration of a subactive dose of mianserin with the 5-HT₂ receptor antag-

onist LY 53857 induces an apparent anti-immobility effect. Thus, it is suggested that blockade of the 5-HT₂ receptor increases the sensitivity to mianserin in the forced swimming test. Drugs such as amphetamines increasing locomotor activity reduces immobility in forced swimming test.¹⁾ However, as shown in results, LY 53857 itself did not affect locomotor activity. Furthermore, co-administration of LY 53857 and mianserin did not alter locomotor activity, either. These results indicate that the enhancement of anti-immobility effects elicited by LY 53857 is not related to changes in locomotor activity.

The mechanisms for the antidepressant effects of mianserin have been suggested to be involved in enhancing noradrenergic neurotransmission based on its blockade of α_2 receptors.^{3,9)} Our previous reports indicated that the 5-HT₂ receptor antagonist LY 53857 reduced immobility in mice treated with antidepressants inhibiting reuptake of NA but not those of SSRIs.^{7,8)} For example, treatment with LY 53857 and a subactive dose of the selective NA reuptake inhibitor maprotiline induces apparent anti-immobility effects, although LY 53857 did not alter the duration of immobility of mice treated with the SSRI fluoxetine.⁸⁾ It indicates that the 5-HT₂ receptor may participate in antidepressant effects by modifying NA transmission.

Our results demonstrated that treatment with LY 53857 and subactive dose of mianserin reduced immobility similar to that by maprotiline. Since mianserin increases NA release through α_2 receptor inhibition, augmentation of anti-immobility effects elicited by LY 53857 may be associated with the noradrenergic system. This result confirms that LY 53857 enhances anti-immobility effects by modulating NA transmission.

It has been reported that LY 53857 has a high affinity with the 5-HT_{2A/2B/2C} receptor subtypes.¹⁴⁾ The dose of LY 53857 in the present study was sufficient to inhibit 5-HT_{2A} and 5-HT_{2C} receptor-mediated head-shake responses and hypophagia in rats.¹⁵⁾ The presence of an interaction between 5-HT₂ receptors and catecholaminergic neurons has been suggested. It was reported that the 5-HT_{2A} receptor activation may enhance NA release from nerve terminals of rat brain.¹⁶⁾ Recent findings suggested that the blockade of 5-HT_{2C/2B} receptor increases noradrenaline or dopamine release in the brain, as measured by microdialysis in rats.^{17,18)} Since mianserin increases NA release by blocking α_2 receptors, blockade of the 5-HT_{2B/2C} receptor by LY 53857 may lead to increased potentiation of noradrenergic transmission, which results in apparent anti-immobility effects.

It has been suggested that mianserin itself is a 5-HT_{2A/2C} receptor antagonist.¹⁰⁻¹²⁾ Hand *et al.* demonstrated that antidepressant effects of mianserin are associated with the 5-HT₂ receptor antagonism.¹⁹⁾ Therefore, because mianserin antagonizes 5-HT₂ receptor, probably the 5-HT_{2C} receptor, in addition to α_2 receptors, co-administration of LY 53857 with mianserin may appear apparent anti-immobility effects. Recent evidence has shown that the 5-HT_{2C} receptor inverse agonist may be effective in depression.²⁰⁾ Thus, inhibition of the 5-HT_{2C} receptor activity may lead to antidepressant effects.

In summary, our results demonstrated that inhibition of the 5-HT₂ receptor by LY 53857 enhances the anti-immobility effects of mianserin. Since LY 53857 potentiates anti-immobility effects of NA reuptake inhibitors, the results obtained

with mianserin further support the postulation that the 5-HT₂ receptor antagonism may increase the sensitivity to antidepressants by moderating the noradrenergic system.

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