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Short Communication

Influence of Imipramine on the Duration of Immobility in Chronic Forced-Swim-Stressed Rats

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We studied the influence of imipramine on the duration of immobility in chronic forced-swim-stressed rats. Both single and chronic administration of imipramine potently shortened immobility in naïve rats during forced-swim testing. However, chronic, 14-day forced-swim stress testing blocked the immobility-decreasing effect induced by a single administration of imipramine. When imipramine was administered for 14 days concurrently with forced-swim stress testing, immobility was shortened significantly. From the viewpoint of imipramine's effect, these findings suggest that chronic forced-swim stress testing in rats may be an effective animal model for depression.

Key words: stress, depression, imipramine, forced-swim test, animal model

Stress has been suggested to be an important etiological factor in many psychiatric diseases, including affective disorders. The main target of stress research in the central nervous system is the monoaminergic system, and stress is reported to affect the function of brain monoaminergic receptors. In particular, serotonin (5-HT) receptors have been postulated to play an important role in the pathogenesis of affective disorders. Several studies have found elevated numbers of 5-HT_{2A} receptors in the post-mortem brains of suicide victims and depressed subjects [1–3]. In animal studies, the number of 5-HT_{2A} receptors is decreased in the rat frontal cortex following the chronic administration of antidepressants [4–5]. These findings suggest that the down-regulation of 5-HT_{2A} receptors may be related to the activity of antidepressants.

Previously, we developed an animal model for depression that exposed rats to chronic forced-swim stress testing for 14 days [6]. In that study, rats were individually forced to swim for 6 min in plastic cylinders. For the chronic tests, they were forced to swim once daily for 14 days. The chronic forced-swim stress test increased the number of frontal cortical 5-HT_{2A} receptors without altering the concentrations of 5-HT and 5-hydroxyindole acetic acids or the number of wet-dog shakes mediated by 5-HT_{2A} receptors. These results suggested that 5-HT_{2A} receptors are closely related to the rat's response to chronic forced-swim stress testing, which may therefore act as a model of depressive conditions in animals. However, previously we did not study the effect of antidepressants on chronic forced-swim stress in rats. As antidepressants possess broad therapeutic properties, in the present study we examined their efficacy in chronic forced-swim stress testing, an animal model of depres-

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sion.

In rodents, the forced-swim test is widely used to predict the activity of antidepressants in naïve rats [7]. Imipramine is a well-established tricyclic antidepressant that has been used clinically for many years. In animal studies, it is well known that both the single and chronic administration of imipramine reduces the duration of immobility in rats during forced-swim tests [7, 8]. However, these studies used naïve rats; there have been few attempts to examine imipramine's chronic effects in rats after chronic forced-swim stress testing. Therefore, we examined the influence of chronic forced-swim stress testing, which may be a model of depressive conditions in animals, on imipramine's effect on the duration of immobility in those tests.

Materials and Methods

Animals. Male Wistar rats (Charles River, Yokohama, Japan) weighing 180–230 g, and kept on a constant light-dark cycle (light 07:00–19:00 h), were sustained with standard laboratory food and tap water in an air-conditioned room (23 ± 1 °C with approximately 60 % humidity).

Drugs. The drug used in this study was imipramine hydrochloride (Wako Pure Chemicals, Osaka, Japan). On each day of testing, imipramine was dissolved in saline and injected into the rats at 2 ml/kg body weight. Control rats received an equivalent vehicle volume for the same treatment duration.

Forced-swim stress testing and measurement of immobility. The rats were individually forced to swim for 6 min in plastic cylinders (height 37 cm, diameter 15.5 cm), which contained water to a height of 20 cm, at 25 °C. The total period of immobility during the 6-min testing period was recorded using the TARGET series/7 M analysis program (Neuroscience, Tokyo, Japan).

Experiment 1: Effects of a single or 14-day chronic administration of imipramine on the duration of immobility in naïve rats. Single administration of imipramine: Two swim sessions were conducted in an initial 13-min pretest; a 6-min test followed 24 h later. The duration of immobility was observed 30 min after the administration of saline (2 ml/kg, i.p.) or imipramine (1–10 mg/kg, i.p.).

Chronic administration of imipramine: Saline (2 ml/kg, i.p.) or imipramine (1–10 mg/kg, i.p.) was given once daily to the rats for 14 days. On the 13th day, an initial 13-min pretest was performed before the administration of saline or imipramine. Following the final treatment with saline or imipramine on Day 14, 30 min elapsed before immobility was observed.

Experiment 2: Effects of a single or 14-day chronic administration of imipramine on the duration of immobility in chronic forced-swim stress in rats for 14 days. Single administration of imipramine: Rats were forced to swim 6 min daily for 14 days. We chronically administered saline (2 ml/kg, i.p.) for 13 days. On Day 14, a single dose of saline or imipramine (10 mg/kg, i.p.) was administered. Immobility was observed 30 min after the administration.

Chronic administration of imipramine: Rats were forced to swim for 6 min beginning 30 min after treatment with saline (2 ml/kg, i.p.) or imipramine (10 mg/kg, i.p.) once daily for 14 days. Immobility was observed 30 min after the final administration of saline or imipramine on Day 14.

Statistics. Values are expressed as the means (± S.E.M.) of each group. The data were assessed using one-way analysis of variance (ANOVA), and the group means were compared using Dunnett's test for multiple comparisons.

Results. Both the single and chronic administration of imipramine (1–10 mg/kg, i.p.) potently decreased the duration of immobility in naïve rats (single: $[F(3,39) = 7.81, P < 0.01]$ (Fig. 1A); chronic: $[F(3,28) = 7.72, P < 0.01]$ (Fig. 1B). We tested the effects of imipramine on the duration of immobility in a chronic forced-swim stress test (Fig. 1 C). The chronic forced-swim stress for 14 days blocked the ability of a single administration of imipramine (10 mg/kg, i.p.) to decrease immobility. However, the chronic administration of imipramine (10 mg/kg, i.p.) for 14 days significantly shortened immobility following the 14-day repetition of chronic forced-swim stress $[F(2,15) = 7.68, P < 0.01]$ (Fig. 1 C).

Discussion

In this study, we examined the influence of imipramine on the duration of immobility after chronic forced-swim stress testing. The chronic 14-day testing inhibited the ability of a single administration of imipramine (10 mg/kg, i.p.) to shorten immobility. However, the chronic administration of imipramine (10 mg/kg, i.p.) for 14 days

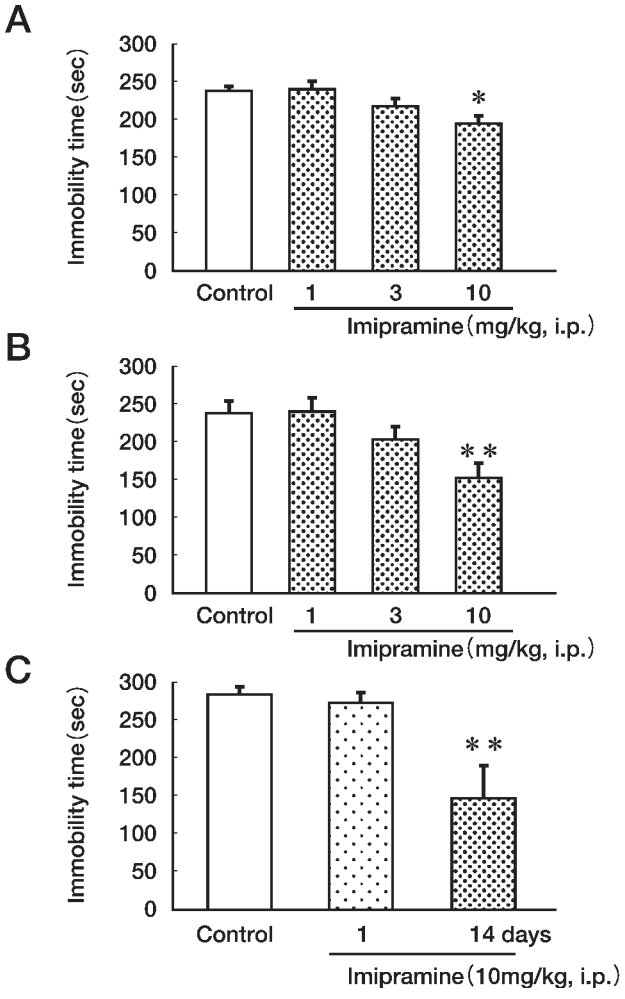

Fig. 1 Effects of single or 14-day chronic administration of imipramine on the duration of immobility during the forced-swim test in naïve rats and chronic forced-swim stress for 14 days in rats. (**A**) A single administration of imipramine (1–10 mg/kg, i.p.) was administered 30 min prior to testing. (**B**) Rats were administered imipramine (1–10 mg/kg, i.p.) once daily for 14 days. The final administration of imipramine was given 30 min prior to testing. (**C**) Rats were forced to swim 6 min daily for 14 days. A single administration of imipramine (10 mg/kg, i.p.) was administered 30 min prior to the forced swim on Day 14 only. In the chronic administration of imipramine, rats were treated with imipramine (10 mg/kg, i.p.) once daily for 14 days. The final administration of imipramine was given 30 min prior to testing. Values are expressed as the means ± S.E.M. of 6–11 animals per group. Data were analyzed by one-way ANOVA, followed by Dunnett's test. $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control.

shortened immobility in chronic forced-swim stress for 14 days. The chronic stress testing clearly inhibited imipramine's immobility-shortening effect, suggesting a link between the mechanism of drug action and the resulting behavioral changes.

We previously reported that chronic, but not acute, forced-swim stress in rats increased frontal cortical 5-HT_{2A} receptor levels and the severity of the wet-dog shakes that they mediated [6]. Clinically, the up-regulation of 5-HT_{2A} receptors was reported in the platelets of depressed patients and in the frontal cortex of suicide victims [1–3]. Furthermore, it was reported that the number of 5-HT_{2A} receptors increases as a result of some types of chronic stress, such as forced running [9] and learned helplessness [10]. Therefore, this animal model of chronic forced-swim stress may shed light on the mechanism governing 5-HT_{2A} receptor up-regulation, which may be associated with the pathophysiology of depression.

In rodents, the forced-swim test is widely used as a predictor of activity of antidepressant [7–8]. A single administration of antidepressants, including imipramine, screened in the forced-swim test, reduced the immobility of rodents in the forced-swim test. Although previous studies evaluated the effects of antidepressants on immobility in the forced-swim test in naïve rats, few attempts have been made to examine their effects on a model of chronic forced-swim stress testing. In this study, we demonstrated for the first time that chronic forced-swim stress testing blocked the ability of a single administration of imipramine to shorten immobility. The precise mechanism underlying this inhibition remains unclear.

Previously, we reported the effects of adrenocorticotropic hormone (ACTH), which up-regulates the hypothalamic-pituitary-adrenal axis, on the immobilization of rats in the forced-swim test after the administration of imipramine [8]. Treatment with ACTH for 14 days blocked the shortening of immobility induced by either single or chronic administration of imipramine (10 mg/kg, i.p.) for 15 days. On the other hand, chronic ACTH treatment increased the wet-dog shakes induced by a 5-HT_{2A} receptor agonist, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). The 14-day administration of imipramine did not inhibit the 14-day ACTH treatment from increasing DOI-induced wet-dog shakes. However, the chronic co-administration of imipramine and lithium decreased both the DOI-induced wet-dog

shakes and immobilization in forced-swim test rats treated with ACTH for 14 days [8, 11]. These findings suggest that inhibition of the 5-HT_{2A} receptor function may contribute to shortening the effect of immobility in forced swim testing. In fact, we determined that the chronic, 14-day administration of imipramine decreased the DOI-induced wet-dog shake response after chronic, 14-day forced-swim stress testing (data not shown). On the other hand, imipramine metabolizes to desipramine [12]. Desipramine is well known as a potent and selective inhibitor of noradrenaline reuptake. Porsolt *et al*. [9] suggested that desipramine shortened immobility more than did 2 selective serotonin reuptake inhibitors, citalopram and fluoxetine. Therefore, the possibility that the noradrenergic system is involved in the effects observed in the present study should not be ignored. Further studies are in progress to clarify the functions of the 5-HT_{2A} receptor and of the noradrenergic function in chronic forced-swim stress testing in rats.

Clinically, it is well established that 2 to 3 weeks of treatment with antidepressants is necessary before the first signs of therapeutic efficacy appear. Likewise, the chronic administration of imipramine significantly shortened immobility in the forced-swim test, even when administered concurrently with chronic forced-swim stress in this study. Furthermore, the immobility-shortening effect of a single administration of imipramine was inhibited in chronic forced-swim-stressed rats. Therefore, the results suggested that chronic forced-swim-stressed rats may serve as an animal model for depressive conditions.

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