

## Comparison of Noradrenergic and Serotonergic Antidepressants in Reducing Immobility Time in the Tail Suspension Test

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**ABSTRACT**—We examined the effects of two noradrenergic tricyclic antidepressants and two selective serotonin re-uptake inhibitors in the tail suspension test, with a suspension period of 30 min instead of the usual 10 min. Within the first 10 min, desipramine, nortriptyline and fluvoxamine significantly reduced the duration of immobility. Whereas desipramine and nortriptyline were also efficacious in the rest of the test period, fluvoxamine was not. Fluoxetine showed no significant effect throughout the study period. These results suggest that a prolonged tail suspension test results in functional changes in the noradrenergic and serotonergic systems and alters the sensitivity to antidepressants.

**Keywords:** Tail suspension test, Antidepressant, Immobility time

Tricyclic antidepressants (TCAs) have long been recognized as effective pharmacotherapy for patients with mood disturbance. However, selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) have recently been developed, and these are preferentially used as daily drug therapy for depressive patients due to their weak side effects. All of these antidepressants modulate noradrenergic and/or serotonergic neurotransmission in the brain.

Several animal models have been developed to evaluate putative antidepressants. Among these, the tail suspension test (TST) proposed by Steru and co-workers (1, 2) is a convenient model in which many antidepressants reduce the immobility time, indicating that this is an index of antidepressant activity (3, 4). However, there are few reports of differences between noradrenergic and serotonergic antidepressants in this test. One of the reasons why the TST does not discriminate between noradrenergic and serotonergic antidepressants may be because of the short duration of suspension, usually less than 10 min, in most TST studies (1, 4–6). In the present study, therefore, we examined the effects of four antidepressants in clinical use, i.e., the noradrenergic TCAs desipramine and nortriptyline and the SSRIs fluvoxamine (7) and fluoxetine, in the TST with a prolonged suspension time of 30 min.

Experiments were carried out according to the guidelines of the Animal Care and Use Committee of Pharmaceutical

Research Center, Meiji Seika Kaisha, Ltd. Male ICR mice weighing 24–28 g were purchased from Nihon SLC (Hamamatsu). They were housed for at least 7 days before the experiment in a room adjusted to 22–25°C and 50–70% humidity with lights on from 7:00–19:00. All experiments were carried out between 9:00–16:00. Desipramine hydrochloride (Sigma, St. Louis, MO, USA), nortriptyline hydrochloride (Sigma), fluoxetine hydrochloride (Nihon Bulk, Tokyo) and fluvoxamine maleate (Meiji Seika) were dissolved in saline and administered intraperitoneally at a volume of 1 ml/100 g of body weight. Mice were divided into 13 groups, 12 of which were treated with 10, 30 and 90 mg/kg of each antidepressant, respectively. The remaining group was injected with saline as a control. Thirty minutes after drug administration, each mouse was suspended individually by its tail from a hook connected to a strain gauge that was adjusted to detect all movements of the animal (Neuroscience, Tokyo). The mice were suspended in separate chambers so that they could not see each other. The movements of mice were measured for 30 min and digitized and processed at 1-min intervals using multipurpose analysis software, Super Scope II (GWI; Somerville, MA, USA). The threshold level was set so as to exclude the respiration movement. The immobility time was defined as the total duration that the animal showed no movement.

The results are shown in Figs. 1 and 2. The measurement period was divided into six 5-min blocks for analysis. In the control group, immobility time increased rapidly in the first two blocks and gradually thereafter. Figure 1

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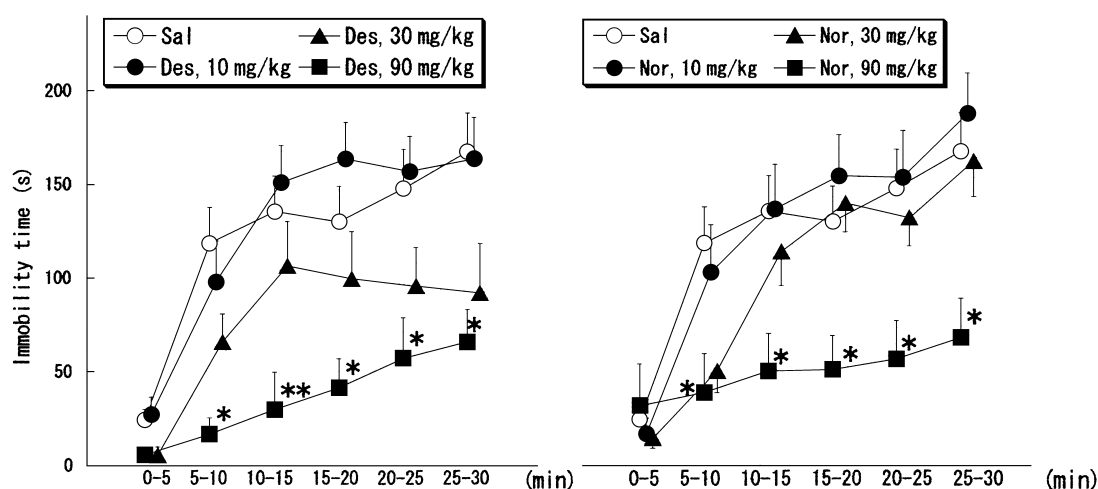


Fig. 1. The effects of desipramine (Des) and nortriptyline (Nor) in the mouse tail suspension test. Asterisks indicate a statistically significant difference compared to the control group (\* $P < 0.05$ , \*\* $P < 0.01$ , Dunnett's multiple comparison). Each point represents the mean  $\pm$  S.E.M. of 10 to 12 animals.

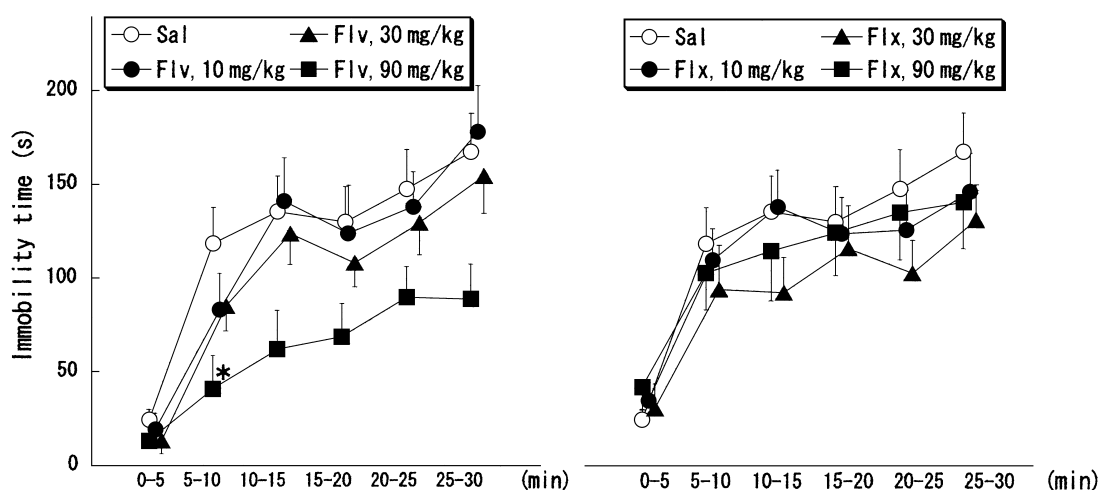


Fig. 2. The effects of fluvoxamine (Flv) and fluoxetine (Flx) in the mouse tail suspension test. Asterisk indicates a statistically significant difference compared to the control group (\* $P < 0.05$ , Dunnett's multiple comparison). Each point represents the mean  $\pm$  S.E.M. of 10 to 12 animals.

shows the immobility time with the noradrenergic antidepressants desipramine and nortriptyline. A two-way repeated measures analysis of variance (ANOVA, treatment  $\times$  block) showed that both treatment ( $P < 0.001$  for desipramine and  $P < 0.01$  for nortriptyline) and time course ( $P < 0.001$  for both drugs) had significant effects. Interaction between both factors was also detected ( $P < 0.01$  for desipramine and  $P < 0.001$  for nortriptyline). Dunnett's multiple comparison was performed for each block and showed that both drugs at a dose of 90 mg/kg significantly reduced the immobility time throughout the measurement period, except in the first block. In addition, the immobility time was substantially suppressed in blocks 4 through 6 in the

30 mg/kg desipramine group, although it was not significant.

Figure 2 shows the results following treatment with the serotonergic antidepressants fluvoxamine and fluoxetine. A two-way repeated measures ANOVA showed that both treatment ( $P < 0.05$ ) and time course ( $P < 0.001$ ) had significant effects for fluvoxamine, but there was no significant interaction between these factors. Fluvoxamine at 90 mg/kg produced substantial suppression throughout the measurement period. However, Dunnett's multiple comparison for each block showed that the immobility time was significantly reduced only in the second block. On the other hand, fluoxetine had no significant effect at the doses tested.

In the conventional TST, immobility is measured for 10 min or less of suspension, which corresponds to the first two blocks in this study. Indeed, the present data indicated that three of the four antidepressants tested exhibited significant efficacy in the second block, which is in agreement with previous reports.

Fluoxetine had no significant effect in this study; however, several reports have indicated the lack of efficacy of SSRIs compared to TCAs in the TST (8).

None of the four antidepressants tested had a significant effect in the first block, which might reflect the notion that the hyperactivity of mice in response to suspension offsets the rapid increase in immobility in the initial part of measurement. In fact, Ukai et al. (9) only recorded last 6 min of a total 7-min observation period because vigorous activity was seen in the very early part of the measurement. In addition, the duration of immobility in the first block might be too short to be appreciably reduced by antidepressants.

The most important findings in this study were observed in the later blocks (blocks 3 through 6), in which the two noradrenergic TCAs, desipramine and nortriptyline, at higher doses significantly reduced the duration of immobility, whereas the two SSRIs, fluvoxamine and fluoxetine, had no significant effects. The effects at these doses are quite unique. By a two-way repeated measures ANOVA, significant interaction was observed between treatment and block with the TCAs desipramine and nortriptyline. On the other hand, the effect of the SSRI fluvoxamine tended to decrease with time.

It is not likely that the pharmacokinetic profiles of SSRIs caused their lack of efficacy in the latter half of this study. In fact, fluvoxamine has been reported to elevate the extracellular serotonin concentration in the rat brain for at least 180 min after intraperitoneal injection, even though its half-life has been reported to be the shortest among the SSRIs (10, 11).

The results also suggest that central noradrenergic and serotonergic systems might be affected differently during 30 min of suspension. Fagette et al. (12) reported that tail suspension markedly reduced the turnover of noradrenaline in a specific part of the brain in rats. In addition, Izumi et al. (13) reported that stress induced by either handling alone or saline injection increased the tissue content of noradrenaline as well as immobility in the TST in rats, whereas the tissue content of serotonin was not affected. These facts suggest that prolonged suspension might decrease noradrenergic function in the brain. Our present finding that noradrenergic drugs remained efficacious throughout the entire measurement period might support this supposition. On the other hand, the function of the serotonergic system in the TST is almost unknown and further study is needed.

Electroconvulsive therapy is used to treat depressive disorders, especially in drug-resistant patients, and it has

also been reported to be effective in the TST. Teste et al. (14) reported that electroconvulsive therapy leads to an increase in noradrenergic and dopaminergic activities as well as reduces immobility in the TST. In addition, treatment with reserpine induces a depressive state in animals and is used as a model of depression. Teste et al. (4) investigated the efficacy of various antidepressants in normal and reserpinized mice in the TST. They reported that reserpine increased the duration of immobility in the TST, and three SSRIs, including fluvoxamine, had weak effects in normal mice and no effect in reserpinized mice. On the other hand, noradrenergic drugs, including desipramine, were more effective in reserpinized mice than in normal mice. These results suggest that a prolonged TST may put mice in a state of severe depression.

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