Neither the $5\text{-HT}_{1\text{A}}$- nor the $5\text{-HT}_{2}$-Receptor Subtype Mediates the Effect of Fluvoxamine, a Selective Serotonin Reuptake Inhibitor, on Forced-Swimming-Induced Immobility in Mice

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ABSTRACT—The effect of fluvoxamine, a selective serotonin (5-HT) reuptake inhibitor, was studied in the forced-swimming test, a model of depression, in mice. Fluvoxamine at 60 mg/kg, p.o. significantly decreased the immobility time in the forced-swimming test. A similar effect was observed by the selective norepinephrine reuptake inhibitor desipramine at the same dose. Furthermore, the suppression of immobility time was slightly potentiated by repeated administration of fluvoxamine, and a significant effect was observed at 30 mg/kg, p.o. The effect of fluvoxamine on forced-swimming was unaffected by the 5-HT$_2$ antagonist ritanserin. On the other hand, the 5-HT$_{1\text{A}}$ antagonist NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine) potentiated the effect of fluvoxamine on forced-swimming. It is expected, however, that a 5-HT$_{1\text{A}}$ antagonist should antagonize the effect of fluvoxamine when 5-HT$_{1\text{A}}$ mediates the suppressive effect of fluvoxamine on the immobility time in forced-swimming. From these results, neither the 5-HT$_{1\text{A}}$- nor the 5-HT$_2$-receptor subtype is involved in the suppressive effect of fluvoxamine on the immobility associated with forced-swimming.

Keywords: Fluvoxamine, Selective serotonin reuptake inhibitor, Forced-swimming, Depression model

Selective serotonin (5-HT) reuptake inhibitors (SSRIs; fluvoxamine, fluoxetine, etc.) have established their status as effective antidepressants with effects comparable to the more traditionally used tricyclic and tetracyclic antidepressants (1). Furthermore, it is recognized that the binding sites for the antidepressant imipramine are the 5-HT-reuptake pumps (2) and that 5-HT$_2$ receptors are also down-regulated by the repeated administration of antidepressants (3). These lines of evidence support the 5-HT theory of depressive illness, and it is apparent, therefore, that malfunction of 5-HT transmission is involved in depressive illness.

The forced-swimming test has been used elsewhere to evaluate the efficacy of antidepressants (4). There is, however, few reports about the effect of fluvoxamine on the forced-swimming test (5). Furthermore, there is no report about the subchronic effect of fluvoxamine on this model or which 5-HT receptor subtype mediates the effect of fluvoxamine on immobility.

In the present experiments, therefore, we investigated the acute and subchronic effects of fluvoxamine and other tricyclic antidepressants on forced-swimming in mice and whether the effect of fluvoxamine is mediated by the 5-HT$_{1\text{A}}$- or the 5-HT$_2$-receptor subtype.

MATERIALS AND METHODS

Drugs and animals

Drugs used in the present experiments were fluvoxamine maleate (Solvay-Duphar, Weesp, The Netherlands); desipramine hydrochloride (Sigma, St. Louis, MO, USA); ritanserin and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190) (Research Biochemicals, Inc., Natick, MA, USA). Fluvoxamine and desipramine were dissolved in purified water and administered orally in a volume of 0.1 ml/10 g of body weight. Ritanserin and NAN-190 were dissolved in 0.1% Tween 80 and injected subcutaneously.

Male ICR strain mice weighing 20–30 g (Nihon SLC, Atsugi) were used in the experiment. All animals were housed in the animal rooms (temperature, 20–25°C; humidity, 60±5%; light on at 7 a.m.; light off at 7 p.m.) for

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at least 7 days before the behavioral tests. All experiments were carried out between 10 a.m. and 5 p.m. in a sound-proof behavioral testing room under the same environmental conditions as the animal rooms.

**Behavioral test**

All the following procedures were conducted in accordance with the guiding principles for the care and use of laboratory animals by the Japanese Pharmacological Society and with the guidelines of animal care in our laboratories, as approved by the Meiji Seika Pharmaceutical Research Center committee on animal care and use.

For the forced-swimming test, a cylinder (25 cm height 10 cm diameter) was used, and this was filled with water (24°C) to a depth of 15 cm. The immobility time of each mouse in the cylinder was recorded by an AXIS30 Video Image Motion Analyzer System (Neuroscience, Tokyo). This system is composed of a CCD camera (NEC, Tokyo), AXIS30 Video Image Motion Analyzer (Neuroscience), video tape recorder (JVC, Tokyo), video monitor (Panasonic, Tokyo) and a personal computer (NEC). In this system, the movement of an animal in the cylinder was monitored by the camera over the cylinder, and it was transformed by software (TARGET/7) to the swimming speed; i.e., the distance swum (mm) in a 0.1-sec interval. The criterion for immobilization was defined as a swimming speed of less than 0.2 mm/0.1 sec for at least 10 successive segments (i.e., 1 sec). The total immobility time of each animal was expressed in seconds as the sum of each immobility period during a 5-min forced-swimming test.

Each animal was exposed to 5 min of forced-swimming each day for 4 days (pretests) to stabilize the immobility time before the drug challenge. On the test day (5th day), fluvoxamine, desipramine or vehicle was orally administered to each animal 60 min before the 5-min forced-swimming test. In the experiments with concurrent administration of antagonists, ritanserin, NAN-190 or vehicle was injected subcutaneously at 30 min before the test. The immobility time on the test day was expressed as the percentage change from that of pretest day 4. To examine the subchronic effect of fluvoxamine, the animals were treated in a similar manner to the acute experiments, except that these animals received repeated administrations of fluvoxamine or vehicle twice a day (9–10 a.m. and 5–6 p.m.) for 14 days. The immobility time of each animal was measured 1 hr after the first administration on the 1st, 3rd, 7th and 14th day of the repeated administration period.

**Statistical analyses**

The Steel multiple range test was used for the statistical analyses of the data for acute and subchronic fluvoxamine. Analysis of variance and post-hoc Bonferroni analysis were used to test statistical significance in the combination experiments with antagonists.

**RESULTS**

The effects of fluvoxamine and desipramine on the forced-swimming test in mice are shown in Fig. 1. Doses of 15 and 30 mg/kg, p.o. fluvoxamine had no significant effect on the immobility time of the forced-swimming test. However, a dose of 60 mg/kg, p.o. fluvoxamine significantly reduced the immobility time (P < 0.05). Similar-
ly, desipramine also significantly reduced the immobility time (P < 0.01) at the dose of 60 mg/kg, p.o. Both antidepressants showed similar significant effects; however, the potency of 60 mg/kg fluvoxamine on this model was weaker than that of 60 mg/kg desipramine (P < 0.01, by a direct comparison of both groups).

The effects of NAN-190 and ritanserin on the reduction of immobility time by fluvoxamine are shown in Fig. 2.

![Fig. 2. Effect of NAN-190 and ritanserin on fluvoxamine induced suppression of the immobility time in the forced-swimming test. NAN-190 and ritanserin (0.6 mg/kg, respectively) were subcutaneously injected at 30 min prior the test. Each column indicates median and interquarter values of 15 or 16 animals. An asterisk indicates a significant difference (*P < 0.05 or **P < 0.01) from the vehicle + vehicle group. Crosses (††) indicate a significant difference (P < 0.01) from the fluvoxamine + vehicle group. See the legend of Fig. 1 for the other details.](image1)

![Fig. 3. Effect of acute and repeated fluvoxamine on the immobility time in the forced-swimming test in mice. The 3 columns on the left indicate the effects of vehicle and fluvoxamine on day 1 (acute effect). The open column in the middle indicates the effect of repeated vehicle administration for 14 days. The next 2 shaded columns indicate the acute effect of fluvoxamine after the repeated vehicle administration. The last 2 columns on the right indicate the subchronic effect of fluvoxamine. Each column indicates median and interquarter values of 15 or 16 animals. “fluv.” is the abbreviation for fluvoxamine. See the legend of Fig. 1 for the other details.](image2)
The significant suppressive effect of fluvoxamine was significantly potentiated (P<0.01) by NAN-190 at 0.6 mg/kg, s.c., while it was not affected by ritanserin at 0.6 mg/kg, s.c. Two antagonists at 0.6 mg/kg showed no significant effect on locomotor activity in mice (data not shown).

The acute and subchronic effects of fluvoxamine on the forced-swimming are shown in Fig. 3. Fluvoxamine at 60 but not 30 mg/kg, p.o. significantly reduced the immobility time (P<0.05) on the 1st test day. On the other hand, both 30 and 60 mg/kg, p.o. fluvoxamine significantly reduced the immobility time by day 14 (P<0.05, respectively).

DISCUSSION

Both fluvoxamine and desipramine significantly suppressed the immobility time of mice on the forced-swimming test. These results are consistent with other previous reports (6, 7). The suppression of the immobility time has been proposed as an index of clinical antidepressive effects (4). The serotonergic drugs employed in the present paradigm suppressed the immobility time in forced-swimming. These results are consistent with findings that serotonergic drugs are clinically active in the treatment of depression (1). It has been suggested that SSRIs are not effective in the forced-swimming test (8), and another report indicated that norepinephrine reuptake inhibitors, but not SSRIs are effective in this model (9). However, we found the significant effect of fluvoxamine in this model. The discrepancy of the results may mainly depend on the species used (rat and mouse). Certainly, in the present experiment, fluvoxamine showed a weaker suppression of immobility time during forced-swimming than desipramine. Alternatively, a new tail-suspension test has been reported as a good screening method to evaluate the antidepressant effects of SSRIs (10, 11), and we found that fluvoxamine suppressed the immobility time in the tail-suspension test (12) in mice. Thus, there may be some differences between models in terms of the precise neurotransmitter systems most active in performing the behavioral task, and this may be reflected in the different actions of different classes of antidepressants in each of these models. In the present study, both fluvoxamine and desipramine significantly suppressed the immobility time. Therefore, we found that the forced-swimming test in mice is also useful for evaluating the efficacy of SSRIs.

The effect of fluvoxamine was not significantly increased by repeated administration in this model, although the effect of fluvoxamine on marble-burying behavior was slightly attenuated by repeated administration (13). The difference of the effects of repeated fluvoxamine in two different models indicates that these changes by repeated administration do not reflect pharmacokinetic changes.

Fluvoxamine inhibits 5-HT reuptake and thus increases 5-HT availability, so it is reasonable to assume that increased 5-HT by fluvoxamine acts at all 5-HT-receptor subtypes. Thus, we examined whether the 5-HT1A- or the 5-HT2-receptor subtype is involved in its effect on forced-swimming. Ritanserin, a 5-HT2 antagonist, did not alter the effect of fluvoxamine. It is suggested, therefore, that there is no involvement of 5-HT2 receptors in the effect of fluvoxamine. There are some reports that a 5-HT2 antagonist was effective in another depression model (namely, the response rate of differential-reinforcement-of-low-rate schedule (14), and the antagonist has therapeutic benefits in patients with dysthymia (15). However, the present results suggest that the 5-HT1A receptors were not involved in either production of the immobility itself or the effect of fluvoxamine on immobility.

If the 5-HT1A receptor is involved in the effect of fluvoxamine, then a 5-HT1A antagonist should suppress it. In fact, we have already found that the 5-HT1A antagonist NAN-190 completely suppressed the effect of fluvoxamine on the marble-burying behavior test in mice (15). However, NAN-190 potentiated the effect of fluvoxamine on forced-swimming-induced immobility in the present study. It is difficult to explain the potentiation, but there are some possible interpretations: 1) NAN-190 non-selectively stimulates the motor activity of animals. 2) NAN-190 shows the agonistic action on 5-HT1A because of its partial agonistic effect. 3) Excitation of postsynaptic 5-HT1A receptors in the terminal field induces immobility itself. However, the first possibility can be excluded by the fact that NAN-190 showed no effect on locomotor activity in mice. The second possibility is unlikely because it is assumed that the partial antagonist may behave as an antagonist rather than an agonist when the 5-HT level is increased in the synaptic cleft by the reuptake inhibition. The third possibility is also controversial in view of the fact that fluvoxamine increased the 5-HT level in the synaptic cleft, which acts on various 5-HT-receptor subtypes, including 5-HT1A, and reduced immobility. Therefore, we concluded that the 5-HT1A subtype in the terminal field does not mediate fluvoxamine's anti-immobility, and that other 5-HT-receptor subtypes probably mediate its anti-immobility. Based on this hypothesis, NAN-190 antagonizes the suppression of firing of the raphe nuclei by fluvoxamine, and fluvoxamine increases the 5-HT level in the terminal field by reuptake inhibition; then both drugs act synergistically to increase the 5-HT level in the terminal field. This hypothesis is supported by a report that 5-HT1B probably mediates the anti-immobility effect of desipramine (16). However, this does not agree with...
another report that a 5-HT1A agonist suppresses forced-swimming-induced immobility (17). Therefore, it is not clear which receptor subtype is mainly involved in the anti-immobility effect of fluvoxamine and immobility itself. Further investigations are necessary to elucidate which receptor subtype mediates these effects.

In conclusion, it is suggested that neither the 5-HT1- nor the 5-HT2-receptor subtype mediates the effect of fluvoxamine on forced-swimming-induced immobility in mice.

Acknowledgments

The authors wish to thank Miss Kyoko Masuda for her technical assistance. We thank Dr. Fiona M. Inglis (Dept. Psychiatry, Univ. British Columbia, Canada) for her review of the manuscript and her helpful suggestions during its development. We also thank Solvay Duphar B.V. for the kind donation of fluvoxamine.

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