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Effect of chronic administration of flesinoxan and fluvoxamine on freezing behavior induced by conditioned fear

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Abstract

The present study investigated the acute effects of flesinoxan (a selective 5-HT_{1A} receptor agonist), fluvoxamine (a selective serotonin reuptake inhibitor) and their co-administration on the expression of conditioned freezing, and index of anxiety in rats. This study also examined the acute effects of fluvoxamine and flesinoxan following chronic flesinoxan or chronic fluvoxamine on the expression of conditioned freezing. Acute administration of flesinoxan (s.c.; 0.1–3 mg/kg) reduced freezing dose dependently, and fluvoxamine (i.p.) at a high dose (60 mg/kg) reduced freezing significantly. Acute co-administration of fluvoxamine (30 mg/kg) and flesinoxan (0.3 mg/kg) showed an additive inhibitory effect on freezing. Chronic flesinoxan treatment (0.3 mg/kg, for 13 days) did not affect the inhibitory effect of acute flesinoxan treatment, but enhanced that of acute fluvoxamine (30 mg/kg) on conditioned freezing. Chronic fluvoxamine treatment (30 mg/kg, for 13 days) enhanced the inhibitory effect of acute fluvoxamine (30 mg/kg) and the inhibitory effect of acute flesinoxan (0.3 mg/kg) on conditioned freezing. These results suggest that co-administration of a selective serotonin reuptake inhibitor and a 5-HT_{1A} receptor agonist is useful for the treatment of anxiety disorders.

Keywords: Fluvoxamine; Flesinoxan; Anxiety; Conditioned fear stress; 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, selective; 5-HT_{1A} receptor agonist

1. Introduction

It has been shown that acute administration of 5-HT_{1A} receptor agonists reduces the neuronal firing of serotonergic neurons by activating presynaptic 5-HT_{1A} autoreceptors (Blier and de Montigny, 1987; Sprouse and Aghajanian, 1987), but long-term administration of these agents reverses the inhibition of neuronal firing, consequently leading to increased 5-HT neurotransmission (Dong et al., 1998; Haddjeri et al., 1999). Increased 5-HT neurotransmission, by gradual desensitization of somatodendritic 5-HT_{1A} autoreceptors after chronic treatment with 5-HT_{1A} receptor agonists, has been considered to be the possible reason for the slow anxiolytic and antidepressive responses to these agents, which are partial agonists, because it usually takes 2 to 4 weeks until a fully developed therapeutic effect is exerted clinically (Balon et al., 1990; Wilcox et al., 1996).

Selective serotonin reuptake inhibitors (selective 5-HT reuptake inhibitors) have been clinically demonstrated to be effective in the treatment of various anxiety disorders, in addition to depressive disorders (Erikkson and Humble, 1990; Lane et al., 1995). As with 5-HT_{1A} receptors agonists, chronic treatment with selective 5-HT reuptake inhibitors is necessary for a fully developed therapeutic effect (Åsberg et al., 1986; Schatzberg et al., 1987). It has been suggested that repeated selective 5-HT reuptake inhibitor administration desensitizes 5-HT_{1A} autoreceptors by decreasing the amount of Gi/o proteins (Li et al., 1996) and attenuates the inhibition of neuronal firing by acute selective 5-HT reuptake inhibitor administration, producing an enhancement of 5-HT release in the axon terminal areas (Chaput et al., 1986; Invernizzi et al., 1994).

In our previous study, we found acute anxiolytic effects of selective 5-HT reuptake inhibitors, 5-HR_{1A} receptor agonists and a 5-HT precursor on freezing behavior, an index of anxiety induced by conditioned fear stress (re-exposure to an environment previously paired with inescapable electric footshock) (Hashimoto et al., 1996; Inoue et al., 1996a; Muraki et al., 1999). However, the cooperative effect of a 5-HT_{1A} receptor agonist and of a

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selective serotonin reuptake inhibitor has not been reported for animal models of anxiety, though co-administration of a 5-HT_{1A} receptor agonist and a selective 5-HT reuptake inhibitor was reported to be more effective for the treatment of depressive disorders clinically than a selective 5-HT reuptake inhibitor alone. (Joffe and Schalla, 1993). Furthermore, chronic effects of a 5-HT_{1A} receptor agonist and a selective 5-HT reuptake inhibitor on conditioned freezing have not been examined. The present study was undertaken to investigate the effect of chronic treatment with the selective 5-HT_{1A} receptor agonist, flesinoxan, and the selective 5-HT reuptake inhibitor, fluvoxamine, on conditioned freezing and on the anxiolytic effect of acute flesinoxan or fluvoxamine treatment. The effect of co-administration of acute flesinoxan and fluvoxamine on conditioned freezing was also examined.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 230–250 g at the time of the test, were housed in groups of four per Plexiglass cage ($38 \times 33 \times 17$ cm), and maintained in a 12-h light–dark cycle (light phase: 0630–1830 h), temperature-controlled environment (22 ± 1 °C) with free access to food and water. Experiments began after 1-week period of acclimatization. All experiments were performed between 0800 and 1300 h.

2.2. Drugs

Flesinoxan (*R*(+)-*N*-[2[4-(2,3-dihydro-2-2-hydroxy-metnyl-1, 4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzoamide) (Solvay-Seiyaku, Japan) and fluvoxamine (Meiji-Seika, Japan) were dissolved in 0.9% saline. Fluvoxamine was administered intraperitoneally (i.p.) as a volume of 2 ml/kg, and flesinoxan was administered subcutaneously (s.c.) as a volume of 1 ml/kg.

2.3. Procedures

2.3.1. Conditioned fear stress-induced freezing

As described previously (Inoue et al., 1996b), the rats were individually subjected to inescapable electric footshocks for a total of 2.5 min [five footshocks (2.5-mA scrambled shock, 30-s duration) that were delivered at intershock intervals of 35–85 s (mean 60 s)] in a shock chamber with a grid floor ($19 \times 22 \times 20$ cm, Medical Agent, Japan). Electric shocks were administered by a Model SGS-02D Shock Generator (Medical Agent). This provides a high-voltage, high-resistance circuit with resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5-mA, this generator actually gave a 0.2-mA shock intensity to the rats. Twenty-four hours after footshock in the acute treatment experiments or 14 days after footshock in chronic experiments, the rats were individually placed in a shock chamber without shocks and were observed for 5 min. With these procedures, conditioned fear, as measured by freezing, develops from the contextual stimuli of the conditioned chamber (Fanselow, 1980). During the observation period, freezing behavior was recorded using a modification of a time-sampling procedure (Fanselow, 1980) previously described by Inoue et al., (1996b). Freezing was defined as the absence of any observable movement of the skeleton and the vibrissae, except those related to respiration. All other behavior was scored as activity. The animal was classified as showing either freezing or active behavior according to its behavior throughout the entire 10-s period. The percentage score (% freezing) represented the number of 10-s periods the animal froze for the entire 10-s. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee.

2.3.2. Effect of acute fluvoxamine on conditioned freezing

Twenty hours after footshock, the rats received a single intraperitoneal injection of fluvoxamine (10, 30, and 60 mg/kg). Four hours after the injection, the rats were individually placed in the shock chamber without shocks and were observed for 5 min.

2.3.3. Effect of acute flesinoxan on conditioned freezing

Twenty-three and a half hour after footshock, the rats received a single subcutaneous injection of flesinoxan (0.3, 1 and 3 mg/kg). Thirty minutes after the injection, the rats were individually placed in the shock chamber without shocks and were observed for 5 min.

2.3.4. Effect of acute co-administration of fluvoxamine and flesinoxan on conditioned freezing

Twenty hours after footshock, fluvoxamine (30 mg/kg) or saline was given intraperitoneally 4 h before testing. Flesinoxan (0.3 mg/kg) or saline was given subcutaneously 3.5 h after fluvoxamine treatment and 30 min before testing.

2.3.5. Effect of acute fluvoxamine following chronic fluvoxamine on conditioned freezing

From 24 h after footshock, fluvoxamine (30 mg/kg) or saline was administered intraperitoneally once a day for 13 days (day 2–14). On the 15th day, saline or fluvoxamine (30 and 60 mg/kg) was administered intraperitoneally at 20 h after the final injection and 4 h before testing. In this experiment, rats were randomly assigned to the following six groups (7–16 rats/group): chronic saline–acute saline, chronic saline–acute fluvoxamine 30 mg/kg, chronic saline–acute fluvoxamine 60 mg/kg, chronic fluvoxam-

ine-acute saline, chronic fluvoxamine-acute fluvoxamine 30 mg/kg, chronic fluvoxamine-acute fluvoxamine 60 mg/kg.

2.3.6. Effect of acute flesinoxan following chronic fluvoxamine on conditioned freezing

From 24 h after footshock, fluvoxamine (30 mg/kg) or saline was administered intraperitoneally once a day for 13 days (day 2–14). On the 15th day, saline or 0.3 mg/kg of flesinoxan was administered subcutaneously at 23.5 h after the final injection and 30 min before testing. In this experiment, rats were randomly assigned to the following four groups (7–8 rats/group): chronic saline–acute saline, chronic saline–acute flesinoxan 0.3 mg/kg, chronic fluvoxamine–acute saline, chronic fluvoxamine–acute flesinoxan 0.3 mg/kg.

2.3.7. Effect of acute fluvoxamine following chronic flesinoxan on conditioned freezing

From 24 h after footshock, flesinoxan (0.3 mg/kg) was administered subcutaneously once a day for 13 days (day 2–14). On the 15th day, saline or fluvoxamine (30 and 60 mg/kg) was administered intraperitoneally at 20 h after the final injection and 4 h before testing. In this experiment, rats were randomly assigned to the following six groups (8 rats/group): chronic saline–acute saline, chronic saline–acute fluvoxamine 30 mg/kg, chronic saline–acute fluvoxamine 60 mg/kg, chronic flesinoxan–acute saline, chronic flesinoxan–acute fluvoxamine 30 mg/kg, chronic flesinoxan–acute fluvoxamine 60 mg/kg.

2.3.8. Effect of acute flesinoxan following chronic flesinoxan on conditioned freezing

From 24 h after footshock, flesinoxan (0.3 mg/kg) or saline was administered subcutaneously once a day for 13 days (day 2–14). On the 15th day, saline or 0.3 mg/kg of flesinoxan was administered subcutaneously at 23.5 h after the final injection and 30 min before testing. In this experiment, rats were randomly assigned to the following four groups (8 rats/group): chronic saline–acute saline, chronic saline–acute flesinoxan 0.3 mg/kg, chronic flesinoxan–acute saline, chronic flesinoxan–acute flesinoxan 0.3 mg/kg.

2.3.9. Motor activity

Motor activity was measured for fluvoxamine (30 and 60 mg/kg) and flesinoxan (0.3 mg/kg), and their co-administration. Rats were housed individually for 3 days before testing, and their motor activity in the home cages was automatically recorded by an infrared sensor that detected thermal radiation from the animals, as described by Ohmori et al. (1994). Fluvoxamine (30 and 60 mg/kg) and flesinoxan (0.3 mg/kg) was administered at 4 h and at 30 min before testing for 10 min, respectively. In separate experiments, rats received the combined treatment with fluvoxamine (30 mg/kg, 4 h before testing) and flesinoxan (0.3 mg/kg, 30 min before testing). Horizontal movement was digitized and fed into a computer. Locomotion contributed predominantly to the count, but other body movements also contributed to the count when these movements contained substantial horizontal components. Rats were tested between 0800 and 1300 h.

2.4. Data analysis

All the data are presented as the means ± S.E.M. of the individual values for the rats from each group. Statistical analysis of the data was performed with a one-way analysis of variance (ANOVA) followed by Duncan's test for multiple comparisons or two-way ANOVA for the drug interaction.

3. Results

3.1. Effect of acute fluvoxamine on conditioned freezing

The selective 5-HT reuptake inhibitor, fluvoxamine, significantly reduced the expression of conditioned freezing in a dose-dependent manner [1-way ANOVA, $F(3,28) = 3.83$, $P < 0.05$]. The effect of 60 mg/kg fluvoxamine was statistically significant compared with that of the vehicle controls ($P < 0.05$, see Fig. 1a).

3.2. Effect of acute flesinoxan on conditioned freezing (Fig. 1b)

The selective 5-HT_{1A} receptor agonist, flesinoxan, significantly reduced the expression of conditioned freezing [1-way ANOVA, $F(3,28) = 38.90$, $P < 0.001$]. The effects of 0.3, 1, and 3 mg/kg flesinoxan were statistically significant compared with those of the vehicle controls ($P < 0.01$).

3.3. Effect of acute co-administration of fluvoxamine and flesinoxan on conditioned freezing. (Fig. 1c)

Two-way ANOVA revealed significant main effects of fluvoxamine [$F(1,28) = 5.11$, $P < 0.05$] and flesinoxan [$F(1,28) = 4.72$, $P < 0.05$], but no interaction between fluvoxamine and flesinoxan [$F(1,28) = 0.08$, NS]. Post hoc analysis revealed a significant inhibitory effect of co-administration of fluvoxamine (30 mg/kg) and flesinoxan (0.3 mg/kg) on conditioned freezing compared with the vehicle group.

3.4. Effect of chronic fluvoxamine on acute fluvoxamine-induced inhibition of conditioned freezing

Two-way ANOVA revealed a main effect of acute fluvoxamine treatment [$F(2, 57) = 9.27$, $P < 0.001$], and significant interaction between chronic fluvoxamine and

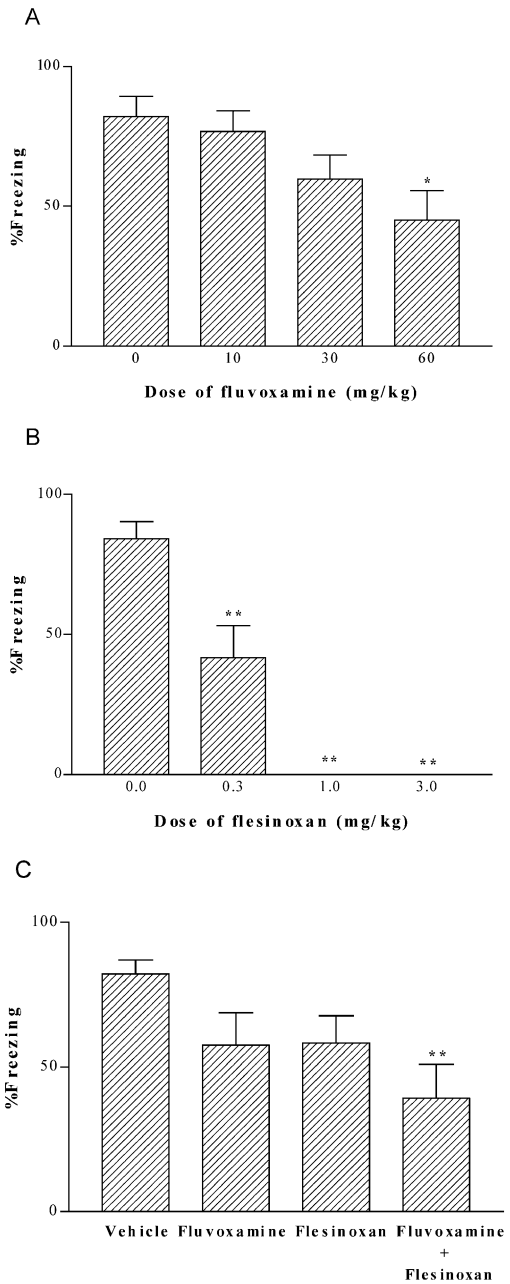

Fig. 1. Effect of acute fluvoxamine (A), flesinoxan treatment (B) and acute co-administration of fluvoxamine (30 mg/kg) and flesinoxan (0.3 mg/kg) (C) on conditioned freezing. Fluvoxamine was given intraperitoneally at 20 h after footshock and 4 h before testing, and flesinoxan was given subcutaneously at 23.5 h after footshock and 30 min before testing. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. Behavior was sampled at 10-s intervals. The number of rats per group for each experiment was 8; $^{*}P < 0.05$; $^{**}P < 0.01$ vs. vehicle controls.

acute fluvoxamine treatment [$F(2,57) = 3.67$, $P < 0.05$], but no significant effect of chronic fluvoxamine treatment [$F(1,57) = 2.05$, NS]. Post hoc analysis showed that there was a significant difference between chronic saline–acute fluvoxamine 30 mg/kg and chronic fluvoxamine–acute fluvoxamine 30 mg/kg ($P < 0.01$), but no significant difference between chronic saline–acute fluvoxamine 60 mg/kg and chronic fluvoxamine–acute fluvoxamine 60 mg/kg (see Fig. 2a).

3.5. Effect of chronic fluvoxamine on acute flesinoxan-induced inhibition of conditioned freezing (Fig. 2b)

Two-way ANOVA revealed significant main effects of chronic fluvoxamine treatment [$F(1,27) = 7.31$, $P < 0.05$] and acute flesinoxan [$F(1,27) = 16.76$, $P < 0.001$], but no significant interaction between chronic fluvoxamine and acute flesinoxan [$F(1,27) = 2.18$, NS]. Post hoc analysis revealed significantly inhibitory effects of acute flesinoxan (0.3 mg/kg) following chronic fluvoxamine on conditioned freezing compared with the acute vehicle groups. There was a significant difference between chronic saline–acute flesinoxan (0.3 mg/kg) and chronic fluvoxamine–acute flesinoxan (0.3 mg/kg) on conditioned freezing ($P < 0.01$).

3.6. Effect of chronic flesinoxan treatment on acute fluvoxamine-induced inhibition of conditioned freezing (Fig. 2c)

Two-way ANOVA revealed a significant main effect of acute fluvoxamine [$F(2,42) = 5.00$, $P < 0.05$] and significant interaction between chronic flesinoxan and acute fluvoxamine treatment [$F(2,42) = 6.04$, $P < 0.01$], but no significant main effect of chronic flesinoxan treatment [$F(1,42) = 3.23$, NS]. Post hoc analysis revealed significant inhibitory effects of chronic flesinoxan–acute fluvoxamine 30 mg/kg and chronic flesinoxan–acute fluvoxamine 60 mg/kg on conditioned freezing compared with the chronic flesinoxan–acute saline group. There was a significant difference between chronic saline–acute fluvoxamine 30 mg/kg and chronic flesinoxan–acute fluvoxamine 30 mg/kg on freezing ($P < 0.01$), but no significant diffirence between chronic saline–acute fluvoxamine 60 mg/kg and chronic flesinoxan–acute fluvoxamine 60 mg/kg.

3.7. Effect of chronic flesinoxan on acute flesinoxan-induced inhibition of conditioned freezing (Fig. 2d)

Two-way ANOVA revealed a significant main effect of acute flesinoxan [$F(1,27) = 19.56$, $P < 0.001$], but no significant main effect of chronic flesinoxan treatment [$F(1,27) = 0.08$, NS] and no significant interaction [$F(1,27) = 0.10$, NS]. Post hoc analysis showed that acute flesinoxan (0.3 mg/kg) following both chronic flesinoxan and chronic saline significantly decreased conditioned freezing compared with the respective acute vehicle groups.

3.8. Motor activity

Acute fluvoxamine (30 and 60 mg/kg) and flesinoxan (0.3 mg/kg) treatment failed to affect motor activity in the

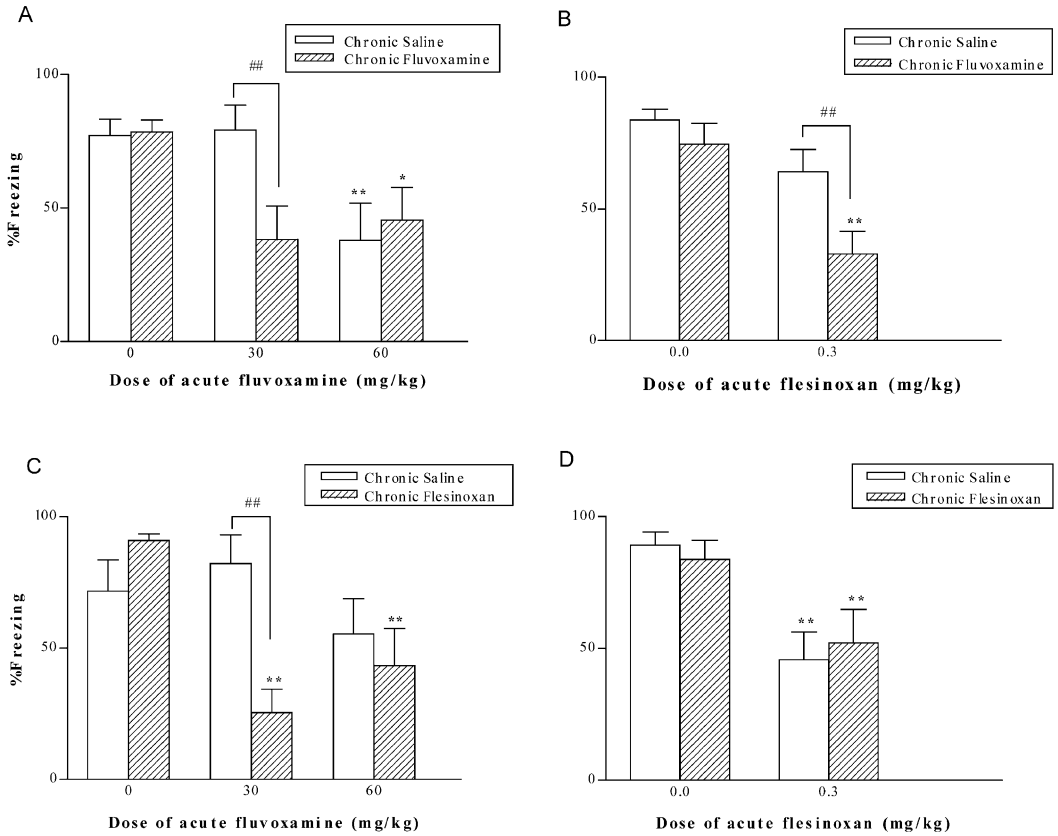

Fig. 2. Effect of chronic fluvoxamine treatment on acute fluvoxamine (A)- and acute flesinoxan (B)-induced inhibition of conditioned freezing, and effect of chronic flesinoxan treatment on acute fluvoxamine (C)- and acute flesinoxan (D)-induced inhibition of conditioned freezing. Chronic fluvoxamine was given intraperitoneally and flesinoxan was given subcutaneously for 13 days from 24 h after footshock. Acute fluvoxamine was given intraperitoneally 20 h after the final injection of chronic fluvoxamine and 4 h before conditioned fear stress, and acute flesinoxan was given subcutaneously 23.5 h after the final injection of chronic flesinoxan and 30 min before testing. The respective vehicle group was given saline. Represented are the mean percentages ± S.E.M. for freezing scored for a 5-min observation period. Behavior was sampled at 10-s intervals. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. vehicle controls; ## $P < 0.01$. In A, $N = 7$–16 rats; in B, $N = 7$–8 rats; in C and D, $N = 8$ rats.

home cages (data not shown). Co-administration of fluvoxamine (30 mg/kg) and flesinoxan (0.3 mg/kg) also failed to affect motor activity in the home cages (data not shown).

4. Discussion

In the present study, acute administration of fluvoxamine, a selective 5-HT reuptake inhibitor (Richelson and Nelson, 1984; Richelson and Pfenning, 1984), and flesinoxan, a selective 5-HT_{1A} receptor agonist (Bosker et al., 1996), decreased conditioned freezing in a dose-dependent manner. These results are consistent with those of our previous studies showing that acute administration of selective 5-HT reuptake inhibitors and selective 5-HT_{1A} receptor agonists decrease conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996a; Muraki et al., 1999).

Fluvoxamine (30–60 mg/kg) and flesinoxan (0.3 mg/kg) failed to affect motor activity in the home cages. Therefore, the reduction in freezing observed with these drugs appeared to be independent of any nonspecific effect on motor activity at doses required to significantly reduce freezing. The results of this study, thus, confirm the anxiolytic effects of a selective 5-HT reuptake inhibitor and a selective 5-HT_{1A} receptor agonist in an animal model of anxiety (Fanselow and Helmstetter, 1988; Rittenhouse et al., 1992; Hashimoto et al., 1996, 1997; Inoue et al., 1996a; Muraki et al., 1999).

In the experiment with acute co-administration of flesinoxan and fluvoxamine, the main effects of flesinoxan and fluvoxamine were significant, but the interaction between flesinoxan and fluvoxamine was not significant, suggesting that the combination of these drugs produced an additive effect on conditioned fear. In the mouse forced swimming test, a behavioral test to evaluate the efficacy of antidepressants, enhancement of the anti-immobility effect of selective 5-HT reuptake inhibitors by a 5-HT_{1A} receptor agonists was reported (Redrobe and Bourin, 1998). A previous in vivo microdialysis study showed that 5-HT_{1A} receptor agonists decreased the ability of a selective 5-HT reuptake inhibitor to increase extracellular 5-HT levels in terminal regions (Gobert et al., 1999). While 5-HT_{1A} receptor agonists alone decreased extracellular 5-HT levels

below baseline levels (Bosker et al., 1996; Gobert et al., 1999), extracellular 5-HT levels remained unchanged at baseline levels following the co-administration of 5-HT_{1A} receptor agonists and selective 5-HT reuptake inhibitors (Gobert et al., 1999). These results may account for the slight, but additive, effect of flesinoxan and fluvoxamine in the present study.

There is abundant evidence that several brain structures, including the amygdala, hippocampus and medial prefrontal cortex are closely involved in conditioned fear (Fendt and Fanselow, 1999; Fanselow, 2000). Conditioned fear stress induces the enhancement of serotonergic neuronal activity within the amygdala, medial prefrontal cortex and nucleus accumbens, suggesting that conditioned fear is related to the brain serotonergic systems (Inoue et al., 1993, 1994). The anxiolytic effects of 5-HT-related anxiolytics (e.g. 5-HT_{1A} receptor agonists and selective 5-HT reuptake inhibitors) have been shown in the conditioned fear model by our research group (Hashimoto et al., 1996; Inoue et al., 1996a; Muraki et al., 1999). The anxiolytic effects of 5-HT_{1A} receptor agonists on conditioned fear are suggested to be mediated by the activation of postsynaptic 5-HT_{1A} receptors rather than of presynaptic 5-HT_{1A} receptors, because the 5-HT lesion induced with *p*-chloroamphetamine did not modify the anxiolytic effect of a selective 5-HT_{1A} receptor agonists (Inoue et al., 1996a). However, the brain regions where selective 5-HT neurotransmission enhanced by selective 5-HT reuptake inhibitors act have not been yet determined, although the 5-HT is suggested to mediate the anxiolytic effects (Hashimoto et al., 1997). These ideas are consistent with the additive effects of acute co-administration of flesinoxan and fluvoxamine in this study.

In the present study, chronic fluvoxamine treatment enhanced the acute inhibitory effect of fluvoxamine at a dose of 30 mg/kg, but not 60 mg/kg, on conditioned freezing. In the previous study, brain and plasma levels of fluvoxamine after chronic fluvoxamine treatment were estimated and were below the detection limit 24 h after the final administration (Bosker et al., 1995). Therefore, it is unlikely that accumulation of the drug had taken place upon repeated administration. In the forced swimming test, the suppression of immobility time was slightly potentiated by repeated administration of selective 5-HT reuptake inhibitors (Egawa et al., 1995; Detke et al., 1997). These results of behavioral studies are consistent with the slow onset of the effect of 5-HT reuptake inhibitors clinically. However, the effect of high-dose fluvoxamine was not enhanced by chronic treatment in this study, indicating that chronic fluvoxamine enhanced the sensitivity of the animals to fluvoxamine in the context of conditioned fear, but did not change the maximal effect of fluvoxamine.

In a previous in vivo microdialysis study, chronic fluvoxamine (30 mg/kg) treatment for 14 days did not change basal 5-HT levels in the dorsal hippocampus 24 h after the final administration and did not enhance the increase in extracellular 5-HT levels after acute administration of fluvoxamine in this region (Bosker et al., 1995). Chronic treatment with other selective 5-HT reuptake inhibitors was reported to enhance or fail to change the increase in extracellular 5-HT levels after acute challenge with 5-HT reuptake inhibitors (Invernizzi et al., 1994; Hjorth and Auerbach, 1999).

In these studies, the effect of the low-dose, but not high-dose, selective 5-HT reuptake inhibitors on extracellular 5-HT levels was enhanced in the frontal cortex after chronic treatment, although there were still the inconsistencies in the results from in vivo microdialysis studies. It should be noted that the experimental animals in these studies were not placed in a situation eliciting fear or anxiety. The effect of chronic selective 5-HT reuptake inhibitors on extracellular 5-HT levels must be tested during anxiety or fear, as reported about acute effects of selective 5-HT reuptake inhibitors by the authors (Hashimoto et al., 1999). An electrophysiological study showed that chronic administration of 5-HT reuptake inhibitors induced desensitization of somatodendritic 5-HT autoreceptors, while it failed to modify the sensitivity of postsynaptic 5-HT_{1A} receptors (Chaput et al., 1986). These neurochemical and electrophysiological findings may partly account for the results of this study that chronic fluvoxamine increased its acute inhibitory effect on conditioned freezing.

Regarding postsynaptic 5-HT_{1A} receptor function, other studies, using other behavioral indices, showed that chronic treatment with the selective 5-HT reuptake inhibitors, citalopram and sertraline, did not change postsynaptic 5-HT_{1A} receptor function (5-HT syndrome induced by a 5-HT_{1A} receptor agonist) (Maj and Moryl, 1992). However, chronic treatment with another selective 5-HT reuptake inhibitor, paroxetine, reduced the 5-HT syndrome induced by a 5-HT_{1A} receptor agonist, but increased the electrophysiological response to a 5-HT_{1A} receptor agonist in the hippocampal slices ex vivo (Maj et al., 1996). Thus, various behavioral and electrophysiological indices gave inconsistent results for postsynaptic 5-HT_{1A} receptor function after chronic treatment with selective 5-HT reuptake inhibitors. Concerning fear or anxiety behavior, there has been no study of postsynaptic 5-HT_{1A} receptor function after chronic treatment with selective 5-HT reuptake inhibitors. The present results suggested that the acute effect of flesinoxan on conditioned freezing were enhanced by chronic fluvoxamine treatment. Our previous findings suggested that the inhibitory effect of a selective 5-HT_{1A} receptor agonist on conditioned freezing was mediated by activation of postsynaptic 5-HT_{1A} receptors, as mentioned above (Inoue et al., 1996a). Accordingly, the present result suggests that chronic fluvoxamine enhanced the funtion of postsynaptic 5-HT_{1A} receptors in relation to fear or anxiety states.

Previous electrophysiological studies showed that chronic treatment with 5-HT_{1A} receptor agonists desensi-

tized presynaptic 5-HT_{1A} receptor, but did not change the responsiveness of postsynaptic 5-HT_{1A} receptor (Blier and De Montigny, 1987; Dong et al., 1998; Haddjeri et al., 1999). Consistent with these data, the present results showed that chronic flesinoxan treatment did not enhance the acute inhibitory effect of flesinoxan on conditioned freezing. The desensitization of presynaptic 5-HT_{1A} receptors may account for the enhanced efficacy of fluvoxamine following chronic flesinoxan in conditioned fear, as blockade of somatodendritic autoreceptors was suggested to enhance the inhibitory effect of a selective 5-HT reuptake inhibitor on conditioned freezing (Hashimoto et al., 1997).

An interaction between 5-HT_{1A} and $5HT_2$ receptors, both of which are suggested to be closely related to anxiety and depressive disorders, has been reported, based on other behavior indices (Glennon et al., 1991). Results of clinical and experimental studies suggest a putative anxiolytic action of 5-HT2 receptor antagonists, although this anxiolytic action has not been ascertained (Chopin and Briley, 1987; Erikkson and Humble, 1990). As we reported, 5-HT2 receptor antagonists had no effect on conditioned freezing (Inoue et al., 1996a). In our previous studies, low-dose, but not high-dose, 5-HT_{1A} antagonists enhanced the anxiolytic effect of selective 5-HT reuptake inhibitors in the conditioned fear test (Hashimoto et al., 1997), while co-administration of 5-HT2 antagonists did not change the anxiolytic effect of selective 5-HT reuptake inhibitors (our unpublished data). Repeated administration of 5-HT_{1A} receptor agonists was reported to induce down-regulation of 5-HT2 receptors in the brain (De Vry, 1995), but repeated fluvoxamine did not affect 5-HT2 receptor binding sites (Ishikane et al., 1994). Thus, it seems unlikely that functional changes in 5-HT2 receptors are involved in the enhanced anxiolytic effects of fluvoxamine and flesinoxan following chronic fluvoxamine or flesinoxan treatment.

In conclusion, the present study showed that chronic flesinoxan treatment enhanced the anxiolytic effect of acute fluvoxamine and that chronic fluvoxamine treatment enhanced the anxiolytic effect of acute fluvoxamine treatment and acute flesinoxan treatment. The desensitization of presynaptic 5-HT_{1A} receptor function may partly account for the mechanism of action of the enhanced anxiolytic effects following chronic treatment with a selective 5-HT reuptake inhibitor and a selective 5-HT_{1A} receptor agonist, although further research is necessary to clarify this mechanism action. This interactive effect between a selective 5-HT reuptake inhibitor and a selective 5-HT_{1A} receptor agonist might provide a useful reference for clinical anti-anxiety therapy.

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