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Characterization of the anxiolytic-like effects of fluvoxamine, milnacipran and risperidone in mice using the conditioned fear stress paradigm

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Abstract

It has been known that rodents exhibit the immobility when tested in the same environment in which they had been previously exposed to aversive stimuli. This behavior is called conditioned fear stress-induced freezing behavior, and has been used as a model of anxiety. Using this animal model, the present study tried to characterize the anxiolytic-like effects of fluvoxamine, a selective serotonin reuptake inhibitor, milnacipran, a serotonin noradrenaline reuptake inhibitor and risperidone, an atypical antipsychotic in mice. Fluvoxamine (1.25–10 mg/kg, intraperitoneally (i.p.)) and milnacipran (0.5–4 mg/kg, i.p.) each dose-dependently and significantly suppressed the conditioned fear stress-induced freezing behavior in mice, an indicator of anxiety, and milnacipran had a weaker effect than fluvoxamine. While risperidone also significantly suppressed freezing behavior at a low dose (0.01 mg/kg, i.p.), a high dose (0.04 mg/kg, i.p.) decreased spontaneous motor activity. On the contrary, sulpiride, a typical antipsychotic (2–8 mg/kg, i.p.), did not affect freezing behavior. In a combination study, the suppressive effect of a low dose of risperidone (0.01 mg/kg, i.p.) on freezing behavior was significantly antagonized by the co-administration of low/middle doses of fluvoxamine (1.25 and 2.5 mg/kg, i.p.), whereas a high dose of fluvoxamine (10 mg/kg, i.p.) was unaffected. Additionally, the co-administration of milnacipran (0.5–2 mg/kg, i.p.) also tended to inhibit the suppressive effect of risperidone (0.01 mg/kg, i.p.). These findings indicate that fluvoxamine, milnacipran and risperidone may each be clinically effective at treating anxiety disorders, but their effects may be attenuated in combination with other medications.

Keywords: Fluvoxamine; Milnacipran; Risperidone; Anxiety; Conditioned fear stress; (Mouse)

1. Introduction

According to the Diagnostic and Statistical Manual of Mental Disorder (DSM)-IV (American Psychiatric Association, 1994), which is frequently used in the clinical setting, anxiety disorders can be divided into various subtypes; e.g. panic disorders, generalized anxiety disorders, social phobias, obsessive-compulsive disorders and post-traumatic stress disorders. Over the past few years, there has been dramatic progress in the development of medications for treating these anxiety disorders; various types of drugs that act at different molecules in the central nervous system are used clinically as therapeutic agents. For example, although benzodiazepine anxiolytics as well as some tricyclic antidepressants and antipsychotics have conventionally been used for the treatment of anxiety disorders, selective serotonin reuptake inhibitors are now also used as first-line drugs (van der Linden et al., 2000; Blanco et al., 2002). Also, the effectiveness of serotonin noradrenaline reuptake inhibitors on anxiety disorders has recently been established in clinical trials (Denys et al., 2003; Hollander et al., 2003a,b). Furthermore, the effectiveness of some antipsychotics in the combined therapy for treatment-resistant anxiety disorders has also been reported (Saxena et al., 1996; Stein et al., 1997; Hollander

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et al., 2003a,b). However, it is also known that the therapeutic effects of these medications vary depending on the type of anxiety disorder (Vaswani et al., 2003). These facts strongly indicate that the pathology of anxiety disorders is diverse from the perspective of not only clinical diagnostics but also neurobiology (Connor and Davidson, 1998; Stein et al., 2002). Therefore, the accurate diagnosis of pathology and the subsequent selection of suitable medication have become critical issues in the treatment of anxiety disorders. To achieve these goals, accumulation of preclinical studies using animal models of anxiety may be helpful in examining the characteristics of the efficacy as well as the mechanism of action of the therapeutic agents that are now used clinically for anxiety disorders.

A growing body of evidence suggests that most animal models of anxiety that are now commonly used in preclinical studies, including the conflict test or elevated plus-maze test, are very useful to evaluate the efficacy of benzodiazepines. However, it is difficult to evaluate the anxiolytic-like effects of non-benzodiazepine compounds such as selective serotonin reuptake inhibitors using these classical methods (Chopin and Briley, 1987; Handley and McBlane, 1993; Rodgers et al., 1997). Rodents exhibit a response that is characterized by a period of crouching and complete immobility when tested in the same environment in which they had been previously exposed to aversive stimuli such as inescapable footshock. This behavior is called conditioned fear stress-induced freezing behavior, and can be used as a model of anxiety (Fanselow and Helmstetter, 1988). This freezing behavior is attenuated by both benzodiazepine (Kitaichi et al., 1995; Inoue et al., 1996; Miyamoto et al., 2000) and non-benzodiazepine anxiolytics (Hashimoto et al., 1996; Inoue et al., 1996; Miyamoto et al., 2000). Therefore, this animal model may be useful for evaluating the efficacy of various therapeutic agents for anxiety disorders.

In the present study, we examined the anxiolytic-like effects of fluvoxamine, a selective serotonin reuptake inhibitor, milnacipran, a serotonin noradrenaline reuptake inhibitor, and risperidone, an atypical antipsychotic, which are commonly used for the clinical treatment of anxiety disorders, using the conditioned fear stress paradigm in mice. Furthermore, the changes in the anxiolytic-like effects of these drugs produced by their co-administration were also evaluated.

2. Materials and methods

The present studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Tokyo Medical University and the Japanese Pharmacological Society.

2.1. Animals

Male ICR mice (Charles River, Japan) weighing 30–35 g were housed at six per cage (20×30×12 cm high) at a room temperature of 23±1 °C with a 12-h light/dark cycle (light on at 0600 to 1800 h). Food and water were available ad libitum.

2.2. Apparatus and procedure for the conditioned fear stress paradigm

For the experiments, we used a wooden box divided into three compartments by walls (10×30×25 cm high) with a stainless steel grid floor. Intermittent inescapable electric foot shocks (intensity: 1.2-mA interval: 10-s duration: 1 s) were delivered through the grid floor by an isolated shock generator (Muromachi Kikai, Japan). The durations of freezing behavior and motor activity of mice were recorded automatically by an activity-monitoring system (SUPER-MEX, Muromachi Kikai).

The conditioned fear stress procedure was performed over 2 days; i.e., a day for the conditioning session and a day for the test session, as previously described (Miyamoto et al., 2000; Takeda et al., 2002, 2003). In the conditioning session, mice were subjected to inescapable electric foot shocks for a total of 6 min in each compartment of the box. Non-stressed mice were placed in the box for 6 min, but were not subjected to electric foot shocks. Twenty-four hours later, mice were used in the test session. In the test session, the mice were again placed in the same compartment and exposed to only a single electric foot shock (intensity: 1.2-mA duration: 1 s), and the durations of freezing behavior as well as motor activity were recorded for 6 min. In the preliminary study, we have confirmed that the exposure to single foot shock did not affect the motor activity in non-stressed mice. Drugs were injected 30 min prior to the start of the test session. In the combination study, risperidone was co-injected with either fluvoxamine or milnacipran.

2.3. Drugs

The drugs used in the present study were fluvoxamine (Solvay-Meiji, Japan), milnacipran (Asahi Kasei Pharma, Japan), risperidone (Sigma, USA) and sulpiride (Sigma). Each drug was dissolved in saline and injected intraperitoneally (i.p.) in a volume of 10 ml/kg.

2.4. Statistical analysis

The data are presented as the mean±S.E.M. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparisons test was used for the statistical evaluation ($P<0.05$ and 0.01).

3. Results

3.1. Effects of fluvoxamine, milnacipran, risperidone, and sulpiride on the conditioned fear stress-induced freezing behavior in mice

The effects of fluvoxamine, milnacipran, risperidone, and sulpiride on the conditioned fear stress-induced freezing behavior in mice are shown in Figs. 1–4. While fluvoxamine, a selective serotonin reuptake inhibitor, (1.25–10 mg/kg, i.p.) and milnacipran, a serotonin noradrenaline reuptake inhibitor, (0.5–4 mg/kg, i.p.) each dose-dependently and significantly suppressed the freezing behavior in mice (fluvoxamine: $F(4,40)=12.019$, $P<0.01$; milnacipran: $F(4,39)=5.408$, $P<0.01$), milnacipran had a weaker effect than fluvoxamine (the maximum percent suppression produced by fluvoxamine (10 mg/kg, i.p.) and milnacipran (2 mg/kg, i.p.) was 71.1% and 50.3%, respectively) (Figs. 1A and 2A). Risperidone, an atypical antipsychotic (0.005–0.04 mg/kg, i.p.), produced a U-shaped dose–response curve; i.e., while a low dose (0.01 mg/kg, i.p.) significantly suppressed the freezing behavior ($P<0.05$), this effect was not observed at high dosages (0.04 mg/kg, i.p.) (Fig. 3A). On the contrary, sulpiride, a typical antipsychotic, (2–8 mg/kg, i.p.) did not affect freezing behavior at any dose (Fig. 4A). With respect to spontaneous motor activity, only a high dose of risperidone (0.04 mg/kg, i.p.) produced a significant suppressive effect ($P<0.01$) (Fig. 3B), and none of the other medications had any effects ((Figs. 1B, 2B and 4B)).

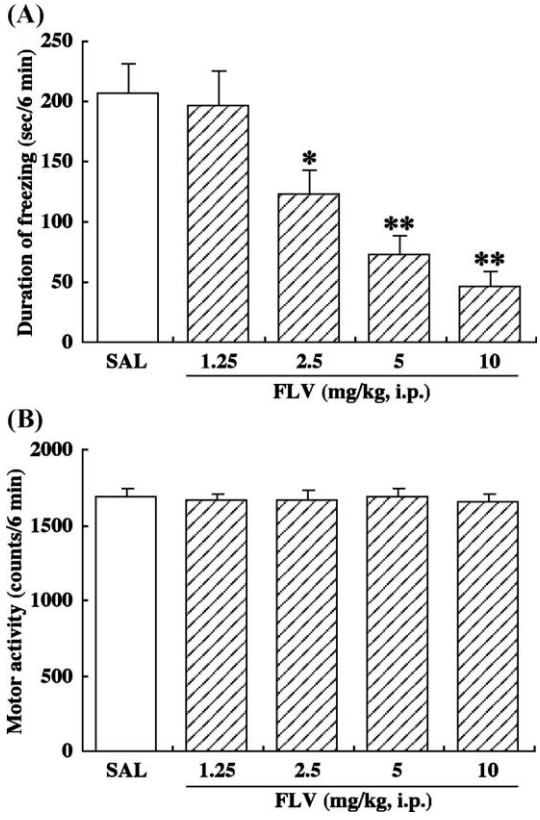

Fig. 1. (A) Effects of fluvoxamine (FLV) on conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. *$P<0.05$, **$P<0.01$ vs. saline (SAL)-treated group. (B) Effects of FLX on spontaneous motor activity in non-stressed mice. Each column represents the mean with S.E.M. of six mice.

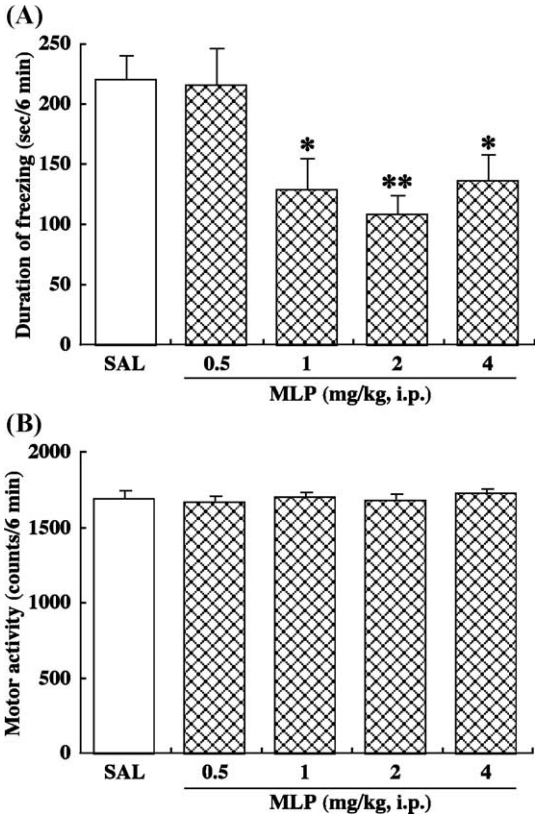

Fig. 2. (A) Effects of milnacipran (MLP) on conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. *$P<0.05$, **$P<0.01$ vs. saline (SAL)-treated group. (B) Effects of MLP on spontaneous motor activity in non-stressed mice. Each column represents the mean with S.E.M. of six mice.

3.2. Effects of the co-administration of risperidone with sub-effective doses of fluvoxamine or milnacipran on the conditioned fear stress-induced freezing behavior in mice

The effects of the co-administration of risperidone with sub-effective doses of fluvoxamine or milnacipran on the conditioned fear stress-induced freezing behavior in mice are shown in Figs. 5 and 6. When risperidone, at a dose that did not significantly affect freezing behavior (0.005 mg/kg, i.p.), was co-administered with a sub-effective dose of fluvoxamine (1.25 mg/kg, i.p.) or milnacipran (0.5 mg/kg, i.p.), the freezing behavior was unaffected (Figs. 5A and 6A). On the other hand, although risperidone (0.01 mg/kg, i.p.) produced a significant decrease in freezing behavior ($P<0.05$), this effect disappeared with the co-administration of a sub-effective dose of fluvoxamine (1.25 mg/kg, i.p.) or milnacipran (0.5 mg/kg, i.p.) (Figs. 5A and 6A). None of these medications affected spontaneous motor activity in mice (Figs. 5B and 6B).

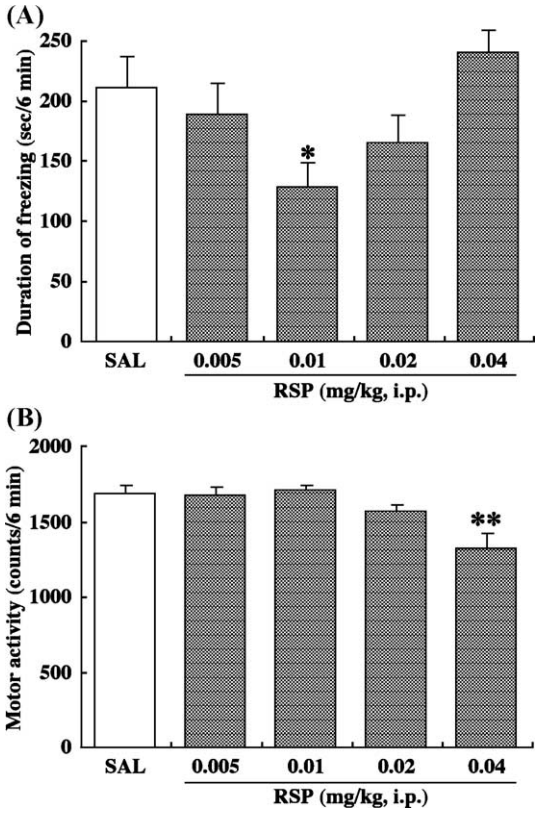

Fig. 3. Effects of risperidone (RSP) on conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. *P<0.05 vs. saline (SAL)-treated group. (B) Effects of RSP on spontaneous motor activity in non-stressed mice. Each column represents the mean with S.E.M. of six mice. **P<0.01 vs. saline (SAL)-treated group.

3.3. Effects of the co-administration of fluvoxamine or milnacipran on the risperidone-induced decrease in freezing behavior in mice

The effects of the co-administration of fluvoxamine or milnacipran on the risperidone-induced decrease in freezing behavior in mice are shown in Figs. 7 and 8. The suppressive effect on freezing behavior induced by risperidone (0.01 mg/kg, i.p.) was significantly antagonized by the co-administration of a low/medium dose of fluvoxamine (1.25, 2.5 mg/kg, i.p.) (P<0.01), whereas a high dose of fluvoxamine (10mg/kg, i.p.) had no effect (Fig. 7A). Furthermore, the co-administration of milnacipran (0.5–2 mg/kg, i.p.) also tended to antagonize the suppressive effect of risperidone (0.01 mg/kg, i.p.) (Fig. 8A). None of these medications affected spontaneous motor activity in mice (Figs. 7B and 8B).

4. Discussion

In the present study, we examined the anxiolytic-like effects of fluvoxamine and milnacipran, antidepressants that are currently used to treat anxiety disorders, using a conditioned fear stress paradigm in mice. Each drug dose-dependently and significantly suppressed the conditioned fear stress-induced freezing behavior in mice, an indicator of anxiety, without affecting general motor activity. These results are in good agreement with previous reports (Hashimoto et al., 1996; Inoue et al., 1996; Miyamoto et al., 2000; Mochizuki et al., 2002), and thus confirm that fluvoxamine and milnacipran exert anxiolytic-like activity. Furthermore, we also found that milnacipran was less effective at suppressing freezing behavior than fluvoxamine. A previous report showed that the suppressive effect of selective serotonin reuptake inhibitor on freezing behavior is inhibited by the co-administration of noradrenaline reuptake inhibitor (Inoue et al., 2002). Therefore, serotonin reuptake inhibition may play an important role in suppressing the expression of freezing behavior, and noradrenaline reuptake inhibition may possibly counteract this effect. Furthermore, the difference in the efficacy of fluvoxamine and milnacipran observed in the present study implies that these drugs may have different therapeutic value in the clinical treatment of anxiety disorders.

The present study also demonstrated that risperidone, an atypical antipsychotic, produced an anxiolytic-like effect at a low dose; i.e., while a low dose of risperidone significantly suppressed the freezing behavior, this effect was not observed at a high dose. It is important to note that

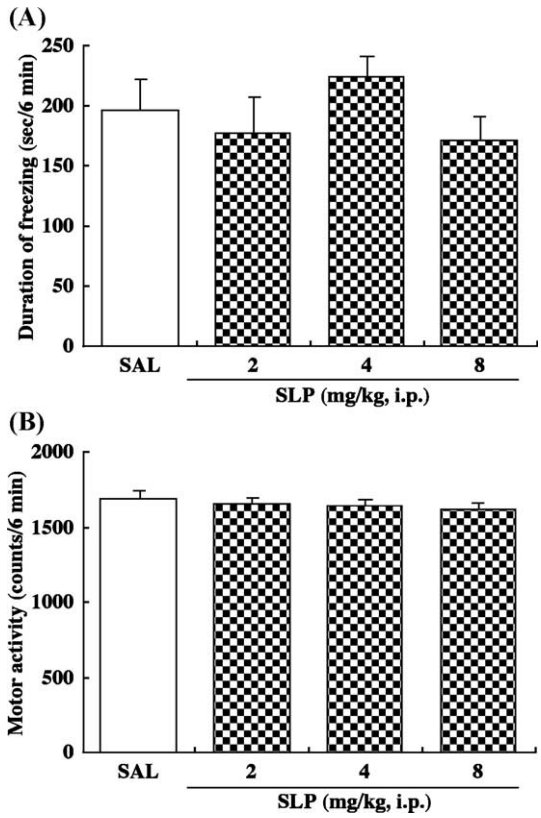

Fig. 4. Effects of sulpiride (SLP) on conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. (B) Effects of SLP on spontaneous motor activity in non-stressed mice. Each column represents the mean with S.E.M. of six mice.

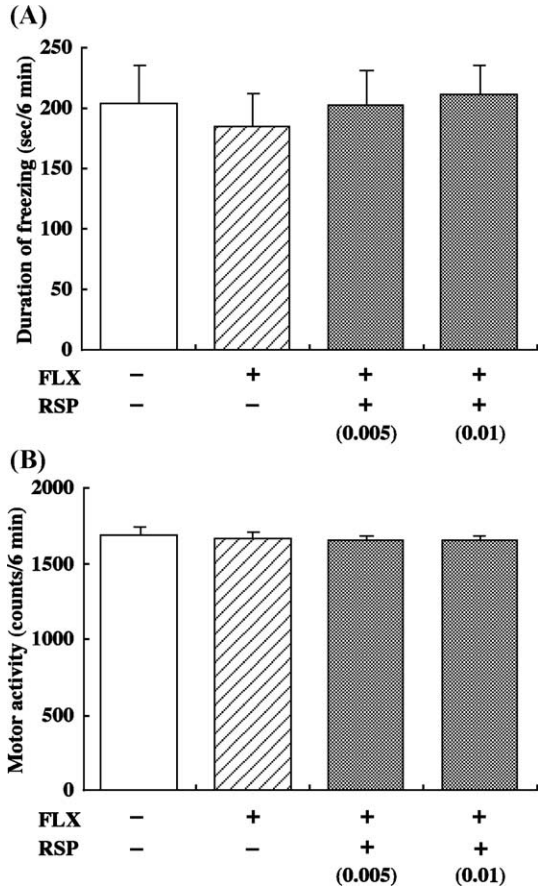

Fig. 5. Effects of the co-administration of risperidone (RSP) with a sub-effective dose of fluvoxamine (FLV) on conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. (B) Effects of the co-administration of RSP with a sub-effective dose of FLV on spontaneous motor activity in non-stressed mice. Each column represents the mean with S.E.M. of six mice.

the lack of an effect with a high dose of risperidone reflects global changes in motor activity. Indeed, the present study confirmed that a high dose of risperidone significantly reduced general motor activity in the non-stressed condition. Therefore, a decrease in general motor activity may counteract the potential reduction in freezing behavior by a high dose of risperidone. To our knowledge, few preclinical studies have investigated the effects of risperidone on anxiety (Ishida-Tokuda et al., 1996). Therefore, the present findings may constitute important behavioral evidence that risperidone has anxiolytic-like activity, and also support the validity of the recent clinical use of risperidone for the treatment of anxiety disorders (Schweitzer, 2001).

Similar to risperidone, it has been previously suggested that sulpiride, a typical antipsychotic, may also has an anxiolytic properties (Wall et al., 2003; Rodgers et al., 1994). Thus, the present study investigated whether sulpiride also suppresses the freezing behavior in mice. However, unlike risperidone, sulpiride did not affect freezing behavior. It is well known that risperidone has antagonistic activities toward both dopamine D2 and 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptors (Leysen et al., 1994), even though sulpiride is a selective dopamine D2 receptor antagonist (Caley and Weber, 1995). Thus, the present findings suggest that the blockade of 5-HT_{2A} receptors may play an important role in the mechanism of the suppressive effect of risperidone on freezing behavior. Furthermore, the different effects of risperidone and sulpiride on freezing behavior may explain how the therapeutic clinical efficacies of these drugs can vary depending on the type of anxiety disorder.

It has recently been indicated that the co-administration of selective serotonin reuptake inhibitors with some antipsychotics is an effective combined therapy for treatment-resistant anxiety disorders (Saxena et al., 1996; Stein et al., 1997; Hollander et al., 2003a,b). This clinical evidence implies that risperidone may act synergistically with the anxiolytic-like effect of selective serotonin reuptake inhibitors. To verify this hypothesis, we examined whether the co-administration of risperidone with fluvoxamine would more efficiently suppress the freezing behavior in mice. However, our results were contrary to our expectation that the effects of both drugs on freezing behavior would disappear when they were co-administered. Furthermore,

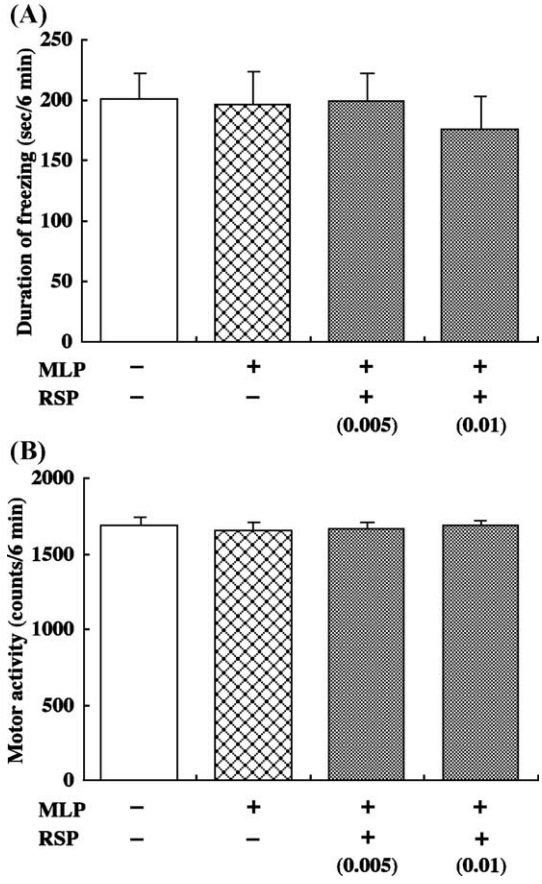

Fig. 6. Effects of co-administration of the risperidone (RSP) with a sub-effective dose of milnacipran (MLP) on conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. (B) Effects of the co-administration of RSP with a sub-effective dose of MLP on spontaneous motor activity in non-stressed mice. Each column represents the mean with S.E.M. of six mice.

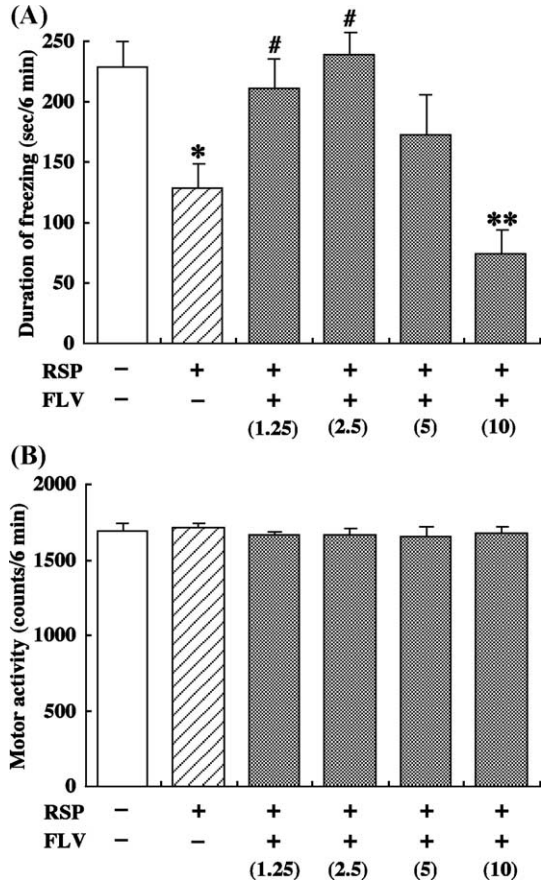

Fig. 7. (A) Effects of fluvoxamine (FLV) on the risperidone (RSP)-induced decrease in conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. *P<0.05, **P<0.01 vs. saline-treated group (open column). $^{\#}P$<0.05 vs. RSP-treated group (solid column). (B) Effects of RSP alone or when co-administered with FLV on spontaneous motor activity in mice. Each column represents the mean with S.E.M. of six mice.

the same interaction was also observed between risperidone and milnacipran. These findings suggest that such medications may not be wholly beneficial in the treatment of anxiety disorders. The mechanisms of these drug interactions are not yet completely understood. However, 5-HT_{2A} receptors could be possible substrates underlying the interaction between risperidone and either fluvoxamine or milnacipran. As noted above, the present findings suggest that the blockade of 5-HT_{2A} receptors may be critical for the expression of the suppressive effect of risperidone on freezing behavior. On the other hand, since it has been shown that 5-HT_{2A} receptors are localized postsynaptically (Hamada et al., 1998; Miner et al., 2003), both fluvoxamine and milnacipran are thought to indirectly activate 5-HT_{2A} receptors by increasing the amount of endogenous 5-HT at nerve terminals. These opposite effects of risperidone and fluvoxamine or milnacipran on 5-HT_{2A} receptors might be involved, at least in part, in the loss of their efficacy at reducing freezing behavior when they are co-administered. Further studies are necessary to clarify these contentions.

Although the effectiveness of combined therapy with some antipsychotics and antidepressants on anxiety disorders has recently been the subject of clinical investigations (Saxena et al., 1996; Stein et al., 1997; Hollander et al., 2003a,b), the present findings, in which the suppressive effects of risperidone and either fluvoxamine or milnacipran on freezing behavior disappeared upon their co-administration, suggest that such medications may not always have a beneficial effect. However, it is also important to note that the present findings do not completely negate previous clinical reports that the therapeutic effect on anxiety disorders is increased by combined therapy with risperidone and selective serotonin reuptake inhibitors, since a high dose of fluvoxamine suppressed freezing behavior regardless of the co-administration of risperidone. The present findings may reflect the diversity of the therapeutic effects of medications on anxiety disorders, and also suggest that it may be necessary to characterize the efficacy of several anti-anxiety agents that are currently used clinically.

In conclusion, the present findings constitute behavioral evidence that the antipsychotic risperidone as well as the

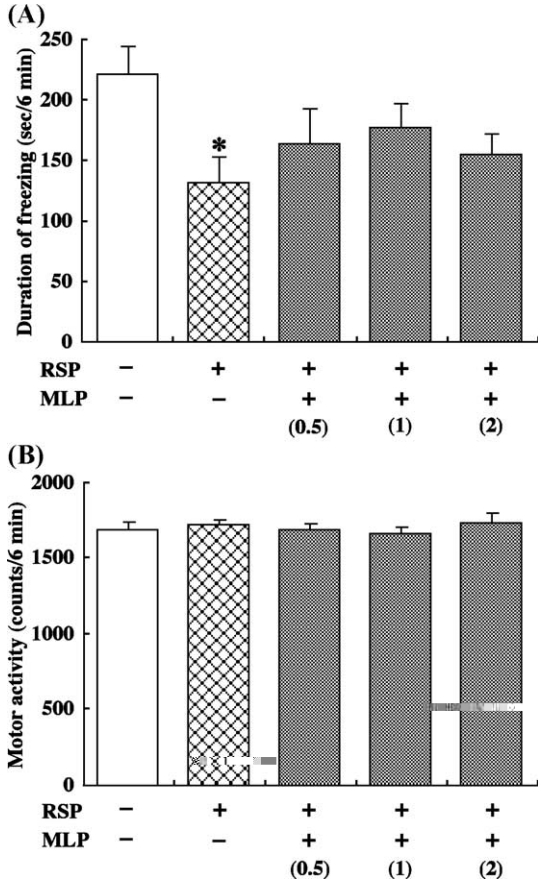

Fig. 8. (A) Effects of milnacipran (MLP) on the risperidone (RSP)-induced decrease in conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. *P<0.05 vs. saline-treated group (open column). (B) Effects of RSP alone or when co-administered with MLP on spontaneous motor activity in mice. Each column represents the mean with S.E.M. of six mice.

antidepressants fluvoxamine and milnacipran are useful for treating patients suffering from anxiety disorders, whereas combined therapy with these drugs may not always produce desirable therapeutic effects.

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