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European Journal of Pharmacology 409 (2000) 81–84

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Short communication

Pretreatment with diazepam suppresses the reduction in defensive freezing behavior induced by fluvoxamine in the conditioned fear stress paradigm in mice

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Received 6 July 2000; accepted 14 September 2000

Abstract

The effects of the selective serotonin (5-hydroxytryptamine (5-HT)) reuptake inhibitor fluvoxamine, given alone or in combination with the benzodiazepine anxiolytic diazepam on the defensive freezing behavior of mice in the conditioned fear stress paradigm were examined. Fluvoxamine (5–20 mg/kg, i.p.) induced a dose-dependent reduction in freezing behavior. In contrast, while low doses of diazepam (0.125 and 0.25 mg/kg, i.p.) reduced the freezing behavior, such effects were not observed with high doses of diazepam (0.5 and 1 mg/kg, i.p.). In the combination study, fluvoxamine (20 mg/kg, i.p.) did not reduce the freezing behavior in mice that had been pretreated with diazepam (0.125–1 mg/kg, i.p.). None of the doses of fluvoxamine and diazepam used in the present study had any effects on motor activity under non-stressed conditions. These results suggest that benzodiazepines may negatively influence the clinical efficacy of selective 5-HT reuptake inhibitors in the treatment of anxiety disorders.

Keywords: Fluvoxamine; Diazepam; Anxiety; Conditioned fear stress; Defensive freezing behavior; (Mouse)

1. Introduction

Benzodiazepines and antidepressants are commonly used together to clinically treat affective disorders such as major depressive disorder, neurotic depression or anxious–depressive reactions. One reason for this drug combination may be the frequent comorbidity of depression and anxiety. Benzodiazepines are usually coadministered with antidepressants to reduce symptoms associated with depression, which do not respond sufficiently to antidepressants alone. Moreover, benzodiazepines are also given to control the release of psychomotor inhibition, which may occur at the onset of the action of antidepressants. However, there have been few studies on the consequences of such an association on their efficacy in reducing the anxiogenic and/or depressive symptoms. Thus, preclinical studies in animals may be necessary as a first step in examining the possible interaction between anxiolytics and antidepressants in the management of anxiety and/or depression.

Previous studies have demonstrated that benzodiazepines counteract the reduction in immobility induced by tricyclic antidepressants or monoamine oxidase inhibitors in the forced swimming test in mice (Van Der Meersch-Mougeot et al., 1993), an experimental procedure that is widely accepted for its value to predict the anti-depressive activity of antidepressants in humans (Porsolt et al., 1991). Similarly, it has been recently reported that the anti-immobility effects of selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors are also suppressed by benzodiazepines (Da Rocha et al., 1997). These reports indicate that benzodiazepines may be able to disturb the therapeutic utility of antidepressants in depression. In contrast, a growing body of evidence suggests that selective 5-HT reuptake inhibitors are therapeutically effective not only in depressive but also in anxiety states, including panic disorders (Van Der Kolk et al., 1994; Van Vliet et al., 1994; Katzelnick et al., 1995). However, the effects of benzodiazepines on the anti-anxiety effects of selective 5-HT reuptake inhibitors are unclear.

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00722-6

Rodents exhibit a response characterized by a period of crouching and complete immobility when tested in the same environment where 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). Thus, freezing behavior is attenuated by both benzodiazepine (Kitaichi et al., 1995; Inoue et al., 1996) and non-benzodiazepine anxiolytics (Hashimoto et al., 1996; Inoue et al., 1996). The aim of the present study was to investigate whether the typical benzodiazepine anxiolytic diazepam would interfere with the action of the 5-HT reuptake inhibitor fluvoxamine in the conditioned fear stress paradigm in mice.

2. Materials and methods

2.1. Animals

Male ddY mice (Tokyo Experimental Animals, Tokyo, Japan) weighing 30–40 g were housed 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) (Masuo et al., 1997).

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. 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 without being exposed to an electric foot shock, and the durations of freezing behavior as well as motor activity were recorded for 6 min. Fluvoxamine (5–20 mg/kg, i.p.) and diazepam (0.125–1 mg/kg, i.p.) were injected 30 and 60 min prior to the start of the test session, respectively. In the combination study, diazepam (0.125–1 mg/kg, i.p.) was injected 30 min before fluvoxamine (20 mg/kg, i.p.), and the test session was performed 30 min after fluvoxamine injection.

2.3. Drugs

The drugs used in the present study were fluvoxamine maleate (Solvay-Meiji, Japan) and diazepam (Wako, Japan). Fluvoxamine was dissolved in saline. Diazepam was dissolved in Tween 20 until it produced a clear solution, and then was diluted with saline to reach the proper concentrations. The final concentration of Tween 20 in the solution was 1%.

2.4. Statistical analysis

The data are presented as the mean ± S.E.M. One-way repeated measures analysis of variance (ANOVA) fol-

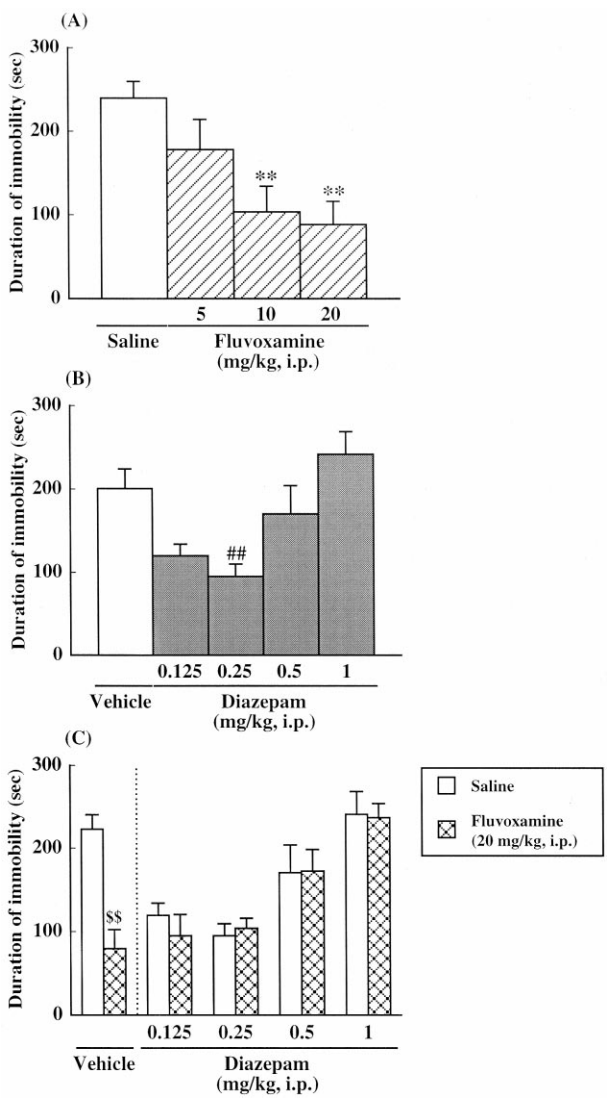

Fig. 1. The effects of fluvoxamine (A) or diazepam (B) and the influence of pretreatment with diazepam on the effects of fluvoxamine (C) on conditioned fear stress-induced freezing behavior in mice. Each column represents the mean with S.E.M. of nine mice. (A) $^{**}P < 0.01$ vs. control group. (B) $\#\#P < 0.01$ vs. vehicle group. (C) $\$\$P < 0.01$ vs. saline group.

lowed by Dunnett's test was used for the statistical evaluation ($P < 0.05$ and 0.01).

3. Results

The effects of fluvoxamine or diazepam and the influence of pretreatment with diazepam on the effects of fluvoxamine in the conditioned fear stress-induced freezing behavior in mice are shown in Fig. 1. Fluvoxamine (5–20 mg/kg, i.p.) produced a dose-dependent reduction in freezing behavior ($F(3,32) = 5.849$, $P < 0.01$), and the differences were statistically significant at 10 and 20 mg/kg ($P < 0.01$) (Fig. 1A). In contrast, while low doses of diazepam (0.125 and 0.25 mg/kg, i.p.) reduced the freezing behavior, such effects were not observed at high doses of diazepam (0.5 and 1 mg/kg, i.p.) (Fig. 1B). In the combination study, the suppression of freezing behavior induced by fluvoxamine (20 mg/kg, i.p.) was not observed in mice that had been pretreated with diazepam (0.125–1 mg/kg, i.p.) (Fig. 1C).

The effects of diazepam on spontaneous motor activity in non-stressed mice are shown in Fig. 2. None of the doses of diazepam (0.125–1 mg/kg, i.p.) used in the present study had any effects on motor activity under non-stressed conditions (Fig. 2). Similarly, fluvoxamine (5–20 mg/kg, i.p.) also did not modify spontaneous motor activity in non-stressed mice (data not shown).

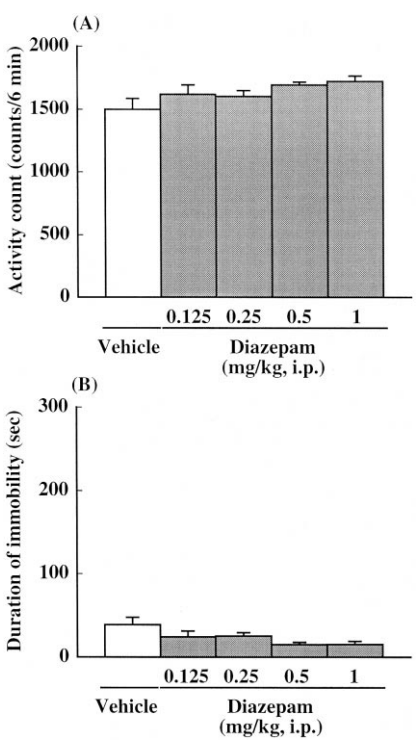

Fig. 2. Effects of diazepam on motor activity ((A) activity count; (B) duration of immobility) in non-stressed mice. Each column represents the mean with S.E.M. of six mice.

4. Discussion

The present study clearly demonstrated that the selective 5-HT reuptake inhibitor fluvoxamine dose-dependently suppressed conditioned fear stress-induced freezing behavior, an index of anxiety, without affecting general motor activity in mice. The results of the present study are in good agreement with previous reports in rats (Hashimoto et al., 1996; Inoue et al., 1996), suggesting that enhancing the availability of 5-HT in the brain by inhibiting the reuptake of 5-HT is beneficial in anxiety. This supposition may be supported by recent clinical findings that drugs that are assumed to facilitate 5-HT neurotransmission, such as 5-HT reuptake inhibitors, monoamine oxidase inhibitors and 5-HT precursors, are all effective in the treatment of anxiety disorders (Eriksson and Humble, 1990; Van Der Kolk et al., 1994; Van Vliet et al., 1994; Katzelnick et al., 1995).

Interestingly, in contrast to fluvoxamine, while low doses of diazepam suppressed the freezing behavior, high doses were ineffective. It is important to note that the lack of an effect by high doses of diazepam in the conditioned fear stress paradigm did not reflect global changes in motor activity. Indeed, although the sedative properties of diazepam are well defined (Woods et al., 1992), the present study demonstrated that none of the doses of diazepam used in the present study affected motor activity under non-stressed conditions. Therefore, it can hardly be proposed that changes in non-specific motor activity may counteract a potential reduction in freezing behavior by high doses of diazepam. Although the reason why diazepam does not show a dose-dependent effect is unclear, one possibility is that there might be a critical range of benzodiazepine receptor activity in modulating changes in emotionality. Thus, an optimal range of benzodiazepine receptor activation is beneficial for controlling anxiety states, whereas too little or too much benzodiazepine activity is ineffective. Further experiments are necessary to clarify the relationship between the benzodiazepine receptor system and the regulation of emotionality under stressful conditions.

Among the various paradigms proposed as experimental models of depression, the forced swimming test (Porsolt et al., 1991) is one of the most commonly used. It has been previously reported that, in mice subjected to the forced swimming test, the anti-immobility effects of tricyclic antidepressants, monoamine oxidase inhibitor and selective 5-HT inhibitors were all antagonized by the coadministration of benzodiazepines (Van Der Meersch-Mougeot, 1993; Da Rocha et al., 1997). These reports suggest that benzodiazepines may reduce the anti-depressive efficacy of antidepressants. In contrast, although the therapeutic effects of selective 5-HT reuptake inhibitors as well as benzodiazepines in anxiety disorders have been well documented (Van Der Kolk et al., 1994; Van Vliet et al., 1994; Katzelnick et al., 1995), there are no reports dealing with

the effects of the combination of benzodiazepines and 5-HT reuptake inhibitors in animals subjected to stressful conditions. The present results may suggest that diazepam inhibits not only the anti-depressive but also the anxiolytic effects of fluvoxamine: the suppressive effects of fluvoxamine on conditioned fear-induced freezing behavior, an index of anxiety, were not observed in mice that had been pretreated with diazepam. This finding implies that benzodiazepines may disturb the therapeutic efficacy of selective 5-HT reuptake inhibitors in anxiety disorders.

The mechanisms by which diazepam suppresses the anxiolytic activity of fluvoxamine in the conditioned fear stress paradigm are not yet completely understood. However, under the present conditions, 5-HT neurons could be possible substrates based on the interactions between fluvoxamine and diazepam. A variety of clinical results have indicated that 5-HT processes might be critically involved in anxiety and in the therapeutic activity of fluvoxamine (Eriksson and Humble, 1990; Van Der Kolk et al., 1994; Van Vliet et al., 1994; Katzelnick et al., 1995). Accordingly, a variety of animal studies using the conditioned fear stress paradigm have provided evidence that facilitation of 5-HT neurotransmission in the brain, especially in the prefrontal cortex, may be involved in the suppression of freezing behavior by fluvoxamine (Jordan et al., 1994; Yoshioka et al., 1995; Inoue et al., 1996; Hashimoto et al., 1999). In contrast, diazepam reduces the activity of 5-HT in the prefrontal cortex (Cheng et al., 1993; Rex et al., 1993; Yoshioka et al., 1995). Although the present study did not induce a detailed neurochemical analysis, these reports imply that the reduction of the effects of fluvoxamine in the conditioned fear stress paradigm might result from a decrease in the activity of prefrontal cortical 5-HT neurons by diazepam.

In conclusion, the present results demonstrate that pretreatment with diazepam suppresses the anxiolytic activity of fluvoxamine in the conditioned fear stress paradigm. These findings suggest that benzodiazepines may negatively influence the therapeutic efficacy of selective 5-HT reuptake inhibitors in patients suffering from anxiety disorders.

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