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Effect of subchronic lithium carbonate treatment on anxiolytic-like effect of citalopram and MKC-242 in conditioned fear stress in the rat

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Abstract

We investigated the effect of citalopram [selective serotonin (5-HT) reuptake inhibitor] and MKC-242 (5-[3-{(2*S*)-(1,4-Benzodioxan-2-ylmethyl) amino}propoxy]-1, 3-benzo-dioxol hydrochloride; a selective 5-HT_{1A} receptor agonist) on the expression of conditioned freezing, an index of anxiety, following treatment with subchronic lithium carbonate ($LiCO_3$). Male Sprague–Dawley rats were used in these experiments. Acute administration of citalopram (subcutaneously, s.c.) reduced freezing significantly at a high dose (30 mg/kg), while showing no effect at lower doses (3 and 10 mg/kg). Acute administration of MKC-242 (s.c.; 0.1–10 mg/kg) dose dependently reduced freezing. Subchronic $LiCO_3$ treatment (1 week; 0.05% or 0.2% $LiCO_3$ in diet; p.o.) showed no effect on freezing behavior. Acute treatment with both citalopram (3 and 30 mg/kg) and MKC-242 (1 mg/kg) after subchronic treatment with the higher, but not the lower concentration of $LiCO_3$ (1 week), reduced freezing markedly and significantly, as compared with either drug alone. These results suggest that subchronic $LiCO_3$ treatment enhanced the anxiolytic-like effects of these serotonergic drugs by facilitating central 5-HT neurotransmission at clinically therapeutic plasma lithium levels.

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

1. Introduction

Recent clinical evidence has shown that selective serotonin (5-HT) reuptake inhibitors are effective in the treatment of various anxiety disorders, such as panic disorder, obsessive compulsive disorder, social phobia and post-traumatic stress disorder, in addition to depressive disorders (Van der Kolk et al., 1994; Lane et al., 1995). There are reports of an anxiolytic-like effect of selective 5-HT reuptake inhibitors, 5-HT_{1A} receptor agonists and 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 foot-shock) (Hashimoto et al., 1996; Inoue et al., 1996). The effects on the expression of conditioned freezing were consistent with recent clinical evidence showing that a number of serotonergic agents (selective serotonin reuptake inhibitors, 5-HT_{1A} receptor agonists, etc.) are effective in the treatment of human anxiety disorders (Erikkson and Humble, 1990). In vivo microdialysis studies have shown that selective 5-HT reuptake inhibitors increase the output from the 5-HT synapse in the raphe nuclei, hypothalamus and cerebral cortex (Fuller, 1986). Based on clinical and experimental data, the anxiolytic effect of these serotonergic drugs is assumed to be mediated by the facilitation of central 5-HT neurotransmission (Erikkson and Humble, 1990).

Lithium has been shown to alter the dynamics of neurotransmission within serotonergic pathways in the central nervous system (Odagaki et al., 1992). Biochemical studies on the effect of lithium administration in vivo have shown an increased synthesis of 5-HT in whole brain (Sheard and Aghajanian, 1970; Perez-Cruet et al., 1971; Grahame-Smith and Green, 1974; Berggren, 1985), and increased 5-HT turnover in various brain regions in rats (Eroglu and Hizal, 1987; Goshdastidar and Poddar, 1990). Administration of lithium chloride (LiCl) enhances the 5-HT syndrome (such as forepaw treading and flat body posture) produced by the

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full 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-propylamino) tetralin (8-OH-DPAT) in rats, suggesting that lithium administration enhances the behavioral response mediated by postsynaptic 5-HT_{1A} receptors (Goodwin et al., 1986). Therefore, this, together with the mechanism of serotonergic anxiolytic action, makes it possible that the anxiolytic effect of serotonergic drugs might be enhanced by adjunctive treatment with lithium. Clinically, lithium enhances the effects of antidepressant, while the lithium-induced enhancement of anxiolytic effects of tricyclic antidepressants, selective 5-HT reuptake inhibitor or 5-HT_{1A} receptor agonists has not been studied in clinical trials or animal experiments (De Montigny et al., 1981; Austin et al., 1991). The present study examined whether subchronic $LiCO_3$ treatment enhanced the inhibitory effect of citalopram (a selective 5-HT reuptake inhibitor) and MKC-242 (a selective 5-HT_{1A} receptor agonist) on the expression of conditioned freezing behavior, as an index of anxiety or fear.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 230–270 g, were housed in groups of four and maintained under a 12-h light–dark cycle (light phase: 0630–1830 h), temperature-controlled environment (22° ± 1°C). The animals were maintained on a diet of standard laboratory rat chow, or rat chow containing 0.05% or 0.2% of $LiCO_3$ for 7 days. In the lithium experiments, the lithium-treated rats and the control rats were given 10 mM NaCl instead of tap water. The rest of the time, all animals had free access to food and water. Experiments began after a 2-week period of acclimatization. The rats were tested between 0800 and 1300 h.

2.2. Drugs

Citalopram hydrobromide, 1-(3-dimetylaminopropyl)-1-(4-fluoro-phenyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide (H. Lundbeck, Copenhagen, Denmark), dissolved in 0.9% sterile saline. MKC-242, 5-[3-{(2*S*)-(1,4-Benzodioxan-2-ylmethyl) amino}propoxy]-1, 3-benzo-dioxol hydrochloride (Mitsubishi Chemical Yokohama, Japan) suspended in 0.5% sodium carboxymethyl cellulose. All drugs were injected subcutaneously (s.c.) in a volume of 1 ml/kg.

2.3. Procedures

2.3.1. Conditioned fear stress-induced freezing

The total duration of the conditioning session was 5 min. The rats were individually subjected to inescapable electric footshock for 2.5 min (2.5 mA scrambled shock, 10-ms shock every 100 ms; shock duration of 30 s × 5; and variable-interval schedule with a mean intershock interval of 60 s, 35–85 s) in a chamber with a grid floor (19 × 22 × 20 cm; Medical Agent, Japan). Electric shock was produced 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 a setting of 2.5 mA, this generator gives a shock intensity of 0.2 mA. Seven days after footshock, the rats were again placed in the shock chamber, this time without shocks being applied, and observed for 5 min. During the observation period, the duration of freezing behavior was recorded using a time-sampling procedure (Fanselow, 1980). Every 10 s, the behavior in which the animal was currently engaged was classified as either freezing or activity. Freezing was defined as the absence of all 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 either frozen or active according to its behavior throughout the entire 10-s period. The percentage scores for the duration of freezing behavior (% freezing) were calculated for each 5-min observation period. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee, and were in compliance with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.3.2. Effect of acute citalopram and MKC-242 treatment on conditioned fear

Seven days after footshock, the rats received a single injection of citalopram (3, 10 and 30 mg/kg) or MKC-242 (0.1, 1 and 10 mg/kg). Citalopram and MKC-242 were administered at 4 h and at 30 min before testing, respectively.

2.3.3. Effect of subchronic $LiCO_3$ (0.05% and 0.2%) with acute citalopram or MKC-242 treatment on conditioned fear

Immediately after footshock, the rats received standard laboratory rat chow ($LiCO_3$ 0%), or rat chow containing 0.05 or 0.2% of $LiCO_3$ for 7 days. On the eighth day, 1 ml/kg of saline or 3 and 30 mg/kg of citalopram was administered at 4 h before testing, and 1 ml/kg of vehicle or 1 mg/kg of MKC-242 was administered at 30 min before testing, respectively. In the experiments with subchronic $LiCO_3$ treatment with acute citalopram (3 mg/kg), rats were randomly assigned to the following four groups (eight rats per group); saline/$LiCO_3$ 0%, citalopram 3 mg/kg/$LiCO_3$ 0%, saline/$LiCO_3$ 0.05% or saline/$LiCO_3$ 0.2%, citalopram 3 mg/kg/$LiCO_3$ 0.05% or citalopram 3 mg/kg/$LiCO_3$ 0.2%. In the experiments with subchronic $LiCO_3$ treatment with acute citalopram (30 mg/kg), rats

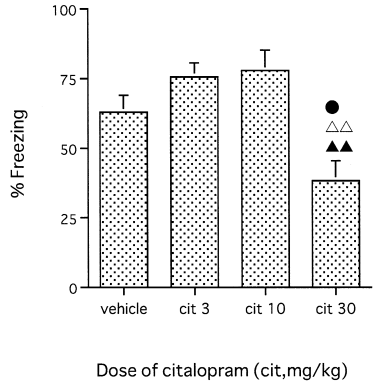

Fig. 1. Effect of acute citalopram treatment on expression of conditioned freezing. Citalopram was subcutaneously administered 7 days after footshock and 4 h before conditioned fear stress. The mean percentages ± S.E.M. for freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. ● $P < 0.05$ vs. vehicle controls; ▲▲ $P < 0.01$ vs. citalopram 3 mg/kg; △△ $P < 0.01$ vs. citalopram 10 mg/kg; $F(3,60) = 6.689$, $P < 0.001$, $N = 16$.

were randomly assigned to the following four groups (eight rats per group); saline/$LiCO_3$ 0%, citalopram 30 mg/kg/$LiCO_3$ 0%, saline/$LiCO_3$ 0.05% or saline/$LiCO_3$ 0.2%, citalopram 30 mg/kg/$LiCO_3$ 0.05% or citalopram 30 mg/kg/$LiCO_3$ 0.2%. In the experiments with subchronic $LiCO_3$ treatment with MKC-242 (1 mg/kg), rats were randomly assigned to the following four groups (eight rats per group); vehicle/$LiCO_3$ 0%, MKC-242 1 mg/kg/$LiCO_3$ 0%, vehicle/$LiCO_3$ 0.05% or vehicle/$LiCO_3$ 0.2%, MKC-242 1 mg/kg/$LiCO_3$ 0.05% or MKC-242 1 mg/kg/$LiCO_3$ 0.2%.

2.3.4. 5-HT syndrome

Whether the 5-HT syndrome was produced by MKC-242 at a dose of 1 mg/kg, with or without subchronic $LiCO_3$ treatment, was examined during the conditioned fear stress for 5 min. Thirty minutes after injection with MKC-242, observation periods of 30 sec per rat were initiated. Observations were repeated every 2.5 min. The signs of 5-HT behavioral syndrome (forepaw treading, head-weaving, hindlimb abduction, flat body posture) were rated for each rat, using a four-point ranked intensity scale (0 = absent, 1 = equivocal, 2 = definite, 3 = intense) (Tricklebamk et al., 1984).

2.3.5. Motor activity

Motor activity was measured for citalopram (3 and 30 mg/kg) and MKC-242 (1 mg/kg), both with and without subchronic $LiCO_3$ treatment. The rats were housed individually for 3 days prior to testing, and their motor activity in their home cages was automatically recorded by an infrared sensor that detected thermal radiation from the animals, as described by Ohmori et al. (1994). Citalopram (3 and 30 mg/kg) and MKC-242 (1 mg/kg) were administered at 4 h and at 30 min before testing for 10 min, respectively. 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 those 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 each rat in all groups. Statistical analysis of differences between the two groups was performed using an unpaired *t*-test (two-tailed). Multiple group comparisons were made using a one-way analysis of vari-

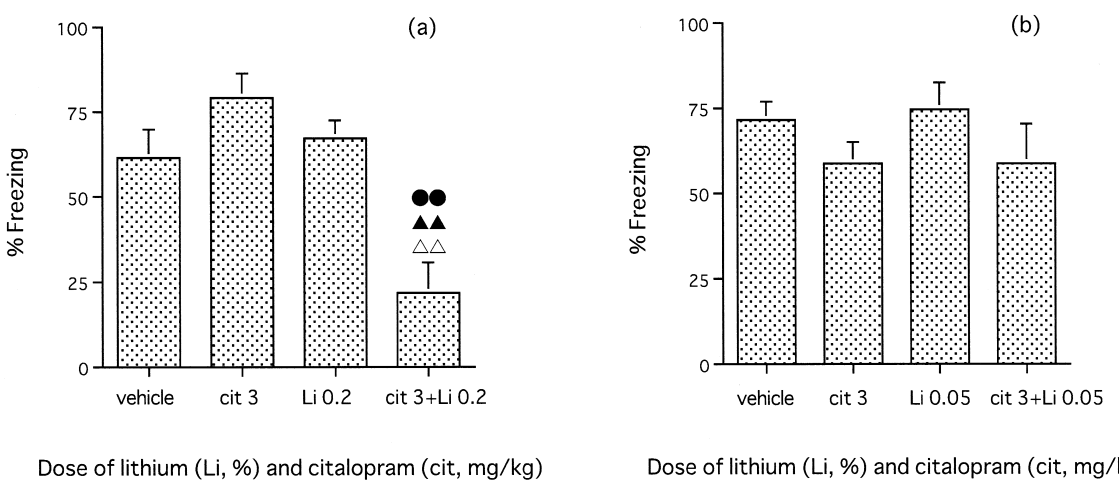

Fig. 2. Effect of subchronic lithium treatment (a, 0.2% $LiCO_3$ in diet; b, 0.05% $LiCO_3$ in diet) on citalopram (3 mg/kg)-induced inhibition of expression of conditioned freezing. Lithium was administered p.o. for 7 days after footshock and citalopram (3 mg/kg) was s.c. administered 4 h before conditioned fear stress. The mean percentages ± S.E.M. for freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. (a) ●● $P < 0.01$ vs. vehicle controls; ▲▲ $P < 0.01$ vs. citalopram; △△ $P < 0.01$ vs. lithium; $N = 8$–16. (b) $N = 8$.

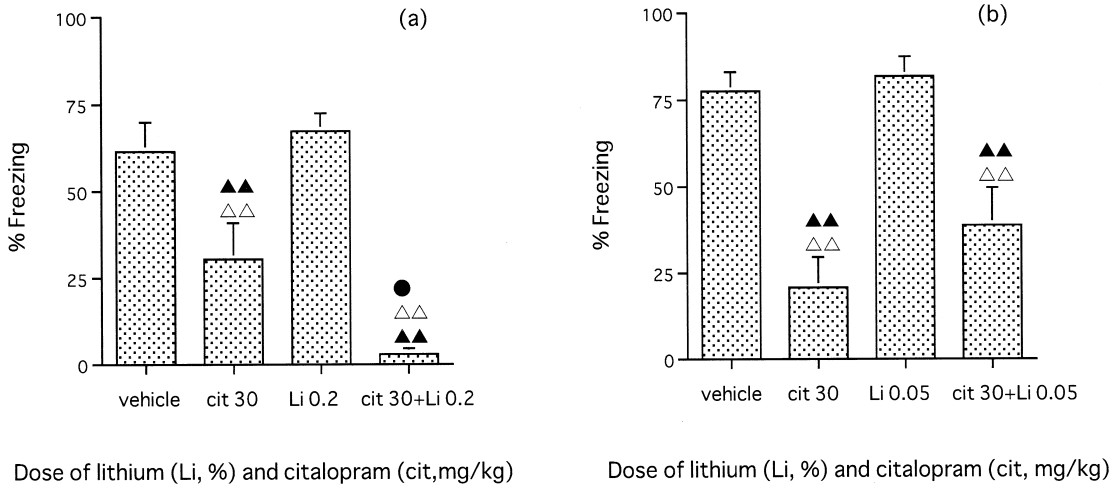

Fig. 3. Effect of subchronic lithium treatment (a, 0.2% $LiCO_3$ in diet; b, 0.05% $LiCO_3$ in diet) on citalopram (30 mg/kg)-induced inhibition of expression of conditioned freezing. Lithium was administered p.o. for 7 days after footshock and citalopram (30 mg/kg) was s.c. administered 4 h before conditioned fear stress. The mean percentages ± S.E.M. for freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. (a) ▲ ▲ $P < 0.01$ vs. vehicle controls; △ △ $P < 0.01$ vs. lithium; ● $P < 0.05$ vs. citalopram; $N = 8$–16. (b) ▲ ▲ $P < 0.01$ vs. vehicle controls; △ △ $P < 0.01$ vs. lithium; $N = 8$.

ance (ANOVA), followed by Duncan's test or two-way ANOVA.

3. Results

3.1. Effect of subchronic $LiCO_3$ treatment on plasma lithium levels

The plasma lithium levels in the rats treated with 0.05% and 0.2% $LiCO_3$ for 7 days were 0.26 ± 0.01 and 0.71 ± 0.05 mEq/l ($N = 8$), respectively.

3.2. Effect of subchronic $LiCO_3$ treatment on citalopram-induced inhibition of expression of conditioned freezing

3.2.1. Effect of acute citalopram treatment

The selective 5-HT reuptake inhibitor, citalopram, significantly reduced the expression of conditioned freezing [one-way ANOVA, $F(3,60) = 6.689$, $P < 0.001$] at a high dose (30 mg/kg), while low doses (3 and 10 mg/kg) of citalopram showed no significant effect (Fig. 1).

3.2.2. Effect of subchronic $LiCO_3$ with acute citalopram (3 and 30 mg / kg) treatment

There was a significant interaction effect between $LiCO_3$ 0.2% and citalopram 3 mg/kg on freezing [two-way ANOVA, $F(1,44) = 15.199$, $P < 0.01$] (Fig. 2a). Post hoc analysis revealed that citalopram 3 mg/kg/$LiCO_3$ 0% and saline/$LiCO_3$ 0.2% had no effect on freezing behavior compared with saline/$LiCO_3$ 0%, while citalopram 3 mg/kg/$LiCO_3$ 0.2% significantly reduced the expression of conditioned freezing compared with saline/$LiCO_3$ 0%, citalopram 3 mg/kg/$LiCO_3$ 0% or saline/$LiCO_3$ 0.2%. Similarly, there was a significant interaction effect between $LiCO_3$ 0.2% and citalopram 30 mg/kg on freezing [two-way ANOVA, $F(1,44) = 4.367$, $P < 0.05$] (Fig. 3a). Post hoc analysis revealed that saline/$LiCO_3$ 0.2% had no effect on freezing behavior compared with saline/$LiCO_3$ 0%, while citalopram 30 mg/kg/$LiCO_3$ 0% significantly reduced the expression of conditioned freezing compared with saline/$LiCO_3$ 0% or saline/$LiCO_3$ 0.2%, and that citalopram 30 mg/kg/$LiCO_3$ 0.2% significantly reduced the expression of conditioned freezing compared with saline/$LiCO_3$ 0%, citalopram 30 mg/kg/$LiCO_3$ 0% or saline/$LiCO_3$ 0.2%.

In contrast to the effect of subchronic treatment with $LiCO_3$ at 0.2% in the diet, subchronic treatment with 0.05% of $LiCO_3$ in the diet did not enhance the inhibitory

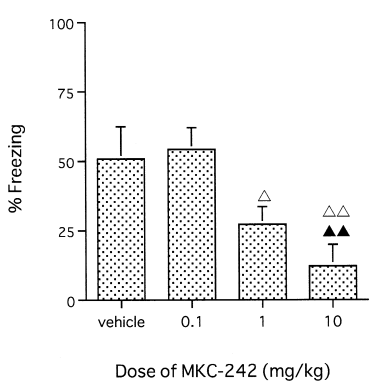

Fig. 4. Effect of acute MKC-242 treatment on expression of conditioned freezing. MKC-242 was administered 7 days after footshock and 30 min before conditioned fear stress. The mean percentages ± S.E.M. for freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. ▲ ▲ $P < 0.01$ vs. vehicle controls; △ △ $P < 0.01$ vs. MKC-242 0.1 mg/kg; △ $P < 0.05$ vs. MKC-242 0.1 mg/kg; $F(3,28) = 5.422$, $P < 0.005$, $N = 8$.

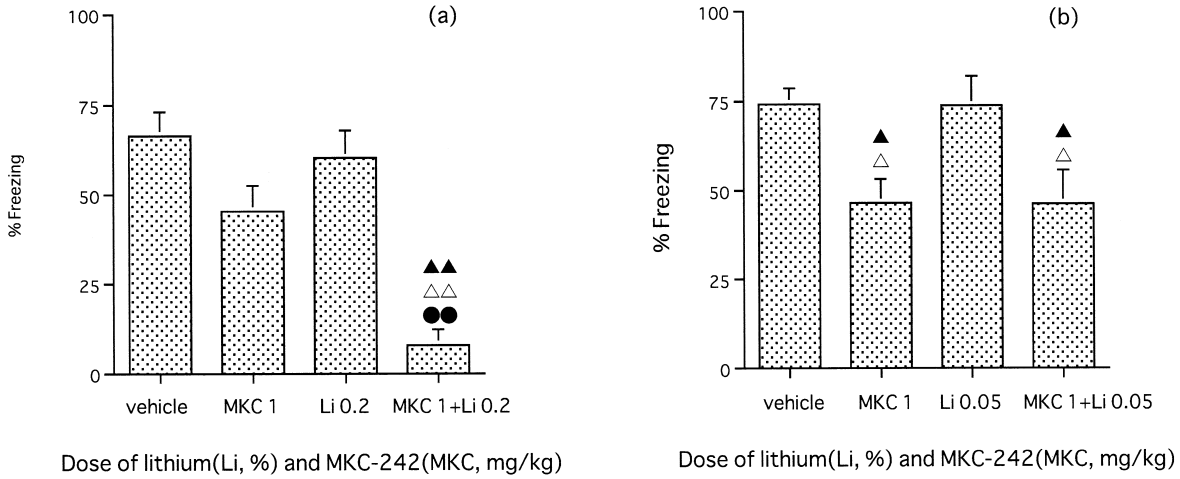

Fig. 5. Effect of subchronic lithium treatment (a, 0.2% $LiCO_3$ in diet; b, 0.05% $LiCO_3$ in diet) with MKC-242 (1 mg/kg) on expression of conditioned freezing. Lithium was administered p.o. for 7 days after footshock and MKC-242 (1 mg/kg) was s.c. administered 30 min before conditioned fear stress. The mean percentages ± S.E.M. for freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. (a) ▲▲ $P < 0.01$ vs. vehicle controls; ●● $P < 0.01$ vs. MKC-242; △△ $P < 0.01$ vs. lithium; $N = 8–16$. (b) ▲ $P < 0.05$ vs. vehicle controls; △ $P < 0.05$ vs. lithium; $N = 8–16$.

effect of citalopram on the expression of conditioned freezing (Figs. 2b and 3b). There was no significant interaction effect between $LiCO_3$ 0.05% and citalopram 3 mg/kg, or between $LiCO_3$ 0.05% and citalopram 30 mg/kg on freezing. There was no main effect of citalopram 3 mg/kg or $LiCO_3$ 0.05% on freezing, but a significant main effect of citalopram 30 mg/kg [two-way ANOVA, $F(1,44) = 35.753$, $P < 0.0001$].

3.3. Effect of subchronic $LiCO_3$ treatment on MKC-242-induced inhibition of expression of conditioned freezing

3.3.1. Effect of acute MKC-242 treatment

The selective 5-HT_{1A} receptor agonist MKC-242 dose dependently reduced the expression of conditioned freezing [one-way ANOVA, $F(3,28) = 5.422$, $P < 0.01$] (Fig. 4). MKC-242 at a dose of 10 mg/kg had a significant effect, compared with the vehicle controls.

3.3.2. Effect of subchronic $LiCO_3$ treatment with acute MKC-242 (1 mg/kg) treatment

Subchronic $LiCO_3$ treatment (0.2%) significantly enhanced the effect of MKC-242 (1 mg/kg) on freezing behavior. There was a significant interaction effect between $LiCO_3$ 0.2% and MKC 1 mg/kg on freezing [two-way ANOVA, $F(1,44) = 4.101$, $P < 0.05$] (Fig. 5a). Post hoc analysis showed that MKC 1 mg/kg/$LiCO_3$ 0% and vehicle/$LiCO_3$ 0.2% had no significant effect on freezing behavior compared with vehicle/$LiCO_3$ 0.2%, while MKC 1 mg/kg/$LiCO_3$ 0.2% significantly reduced freezing compared with the effect of vehicle/$LiCO_3$ 0%, MKC 1 mg/kg/$LiCO_3$ 0% or vehicle/$LiCO_3$ 0.2%.

There was no significant interaction between $LiCO_3$ 0.05% and MKC 1 mg/kg (Fig. 5b). There was only a main effect of MKC 1 mg/kg on freezing [two-way ANOVA, $F(1,44) = 14.723$, $P < 0.01$], but no main effect of $LiCO_3$ 0.05%.

MKC 1 mg/kg/$LiCO_3$ 0.2% did not show any component of 5-HT behavioral syndrome during the conditioned fear stress, as with MKC 1 mg/kg/$LiCO_3$ 0% or vehicle/$LiCO_3$ 0.2% (data not shown).

3.4. Effect of drugs on motor activity

Acute citalopram (3 or 30 mg/kg) and MKC-242 (1 mg/kg) treatment failed to affect motor activity in the home cages (data not shown). Subchronic $LiCO_3$ treatment with citalopram 3 mg/kg [vehicle + vehicle, 13.9 ± 8.8 counts ($N = 8$); citalopram 3 mg/kg + lithium 0.2%, 13.3 ± 8.9 counts ($N = 8$)] and 30 mg/kg [vehicle + vehicle, 0 ± 0 counts ($N = 8$); citalopram 30 mg/kg + lithium 0.2%, 0.6 ± 0.6 counts ($N = 8$)] and with MKC-242 (1 mg/kg) [vehicle + vehicle, 19.3 ± 16.1 counts ($N = 8$); MKC-242 1 mg/kg + lithium 0.2%, 20.1 ± 8.5 counts ($N = 8$)] also failed to affect motor activity in the home cages.

4. Discussion

In the present study, acute treatment with the selective 5-HT reuptake inhibitor, citalopram, and the selective 5-HT_{1A} receptor agonist, MKC-242, significantly inhibited the expression of conditioned freezing. This is consistent with our previous results showing that acute treatment with citalopram and fluvoxamine, another selective 5-HT reuptake inhibitor, and ipsapirone, a selective 5-HT_{1A} receptor agonist, decreased the expression of conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996). Furthermore, subchronic $LiCO_3$ treatment (0.2% $LiCO_3$ in the diet for 1 week) enhanced the inhibitory effect of both citalopram

and MKC-242 on conditioned freezing. Citalopram (3 and 30 mg/kg) and MKC-242 (1 mg/kg), which were effective in the conditioned fear test, did not affect motor activity compared with the vehicle controls. Moreover, subchronic $LiCO_3$ treatment (0.2%) with citalopram (3 and 30 mg/kg) and MKC-242 (1 mg/kg) did not affect motor activity compared with the vehicle controls, either. Therefore, the reduction in freezing observed with these treatments would appear to be independent of any non-specific effect on motor activity at doses required to significantly reduce freezing. The reduction in freezing behavior induced by citalopram (3 and 30 mg/kg) or MKC-242 (1 mg/kg) was not enhanced by a low dose of lithium (0.05%). These results indicate that subchronic $LiCO_3$ treatment (0.2%) enhanced the anxiolytic-like effect of citalopram and MKC-242. The enhancement of the anxiolytic-like effect of these drugs was observed at plasma lithium concentrations (0.71 mEq/l), which had previously been reported to be clinically effective (Schou, 1968; Jerram and McDonald, 1978). Lithium treatment has been shown to enhance 5-HT neurotransmission in several studies (Odagaki et al., 1992). Taking these factors together, the mechanism of lithium action responsible for this enhancement of the effect of citalopram and MKC-242 may involve the facilitation of central 5-HT neurotransmission.

In the present study, the inhibitory effect of MKC-242 on conditioned freezing was enhanced by subchronic $LiCO_3$ treatment. It is suggested that the inhibition of serotonergic impulse flow induced by 5-HT_{1A} receptor agonists may lead to their anxiolytic-like activity (Traber and Glaser, 1987). On the other hand, the postsynaptic 5-HT_{1A} receptor was also assumed to be involved in the anxiolytic-like effect of 5-HT_{1A} receptor agonists in other studies (De Vry et al., 1992). In our previous study, we assumed that it was the stimulation of postsynaptic 5-HT_{1A} receptors that contributed to the inhibitory effect of 5-HT_{1A} receptor agonists on conditioned freezing, as a 5-HT lesion induced with *p*-chloroamphetamine did not alter on this effect (Inoue et al., 1996). In a recent electrophysiological study, lithium treatment enhanced the sensitivity of postsynaptic 5-HT_{1A} receptors (Blier et al., 1987). These findings suggest that lithium enhances the anxiolytic-like effect of 5-HT_{1A} receptor agonists by enhancing postsynaptic action.

One behavioral study showed that LiCl enhanced the postsynaptic 5-HT_{1A} receptor-mediated 5-HT syndrome produced by the full 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-propylamino) tetralin (8-OH-DPAT) (Goodwin et al., 1986). MKC-242 has high affinity for 5-HT_{1A} receptors (K_i values; 0.35 nM) but no appreciable affinity for any other neurotransmitters' receptors and 5-HT transporter, and acts as a partial agonist for 5-HT_{1A} receptors (Matsuda et al., 1995). In a previous study, the maximum effect of MKC-242 on the 5-HT syndrome, which was observed at 2–5 mg/kg (s.c.), was less than that of the full 5-HT_{1A} receptor agonist, 8-OH-DPAT (Matsuda et al., 1995). MKC-242 at a dose of 1 mg/kg has a very slight and equivocal effect on the 5-HT syndrome (Matsuda et al., 1995). Consistently, in the present study, 1 mg/kg of MKC-242 with subchronic $LiCO_3$ treatment produced no components of the 5-HT behavioral syndrome during conditioned fear stress, as did neither MKC-242 nor lithium alone. Therefore, it is unlikely that the inhibition of the expression of conditioned freezing by MKC-242, or subchronic $LiCO_3$ treatment with MKC-242, was due to these non-specific effects on the 5-HT syndrome.

In vivo microdialysis studies have demonstrated that selective 5-HT reuptake inhibitors increase extracellular 5-HT concentrations in various brain regions in rats (Fuller, 1986). In a preliminary study, we showed that subchronic $LiCO_3$ treatment enhanced K^+-induced increases in extracellular 5-HT concentrations (Koyama et al., 1991). These results suggest the possibility that the lithium-induced enhancement of the anxiolytic-like effect of selective 5-HT reuptake inhibitors may be mediated by enhanced increases in extracellular 5-HT concentrations. Further study will be required to clarify whether subchronic $LiCO_3$ enhances the effect of citalopram on extracellular 5-HT levels.

The effect of citalopram at a high dose (30 mg/kg) was enhanced by subchronic $LiCO_3$ treatment, as was the effect of a low dose of citalopram (3 mg/kg). In our dose–response experiments, 30 mg/kg of citalopram produced a maximal inhibitory effect on freezing, similar to that of 100 mg/kg citalopram (unpublished data). Therefore, it is unlikely that subchronic $LiCO_3$ treatment enhanced citalopram's effects by increasing in vivo concentrations.

In conclusion, the selective 5-HT reuptake inhibitor, citalopram, and the selective 5-HT_{1A} receptor agonist, MKC-242, were effective to reduce the expression of conditioned fear stress-induced freezing behavior. Subchronic $LiCO_3$ treatment (1 week) enhanced the inhibitory effect of these drugs on freezing markedly at therapeutic levels of plasma lithium. These results suggest that the lithium-induced enhancement of the anxiolytic-like effect of selective 5-HT reuptake inhibitors and 5-HT_{1A} receptor agonists may be mediated by the facilitation of central 5-HT neurotransmission, e.g., increases in extracellular 5-HT concentrations or enhancement of postsynaptic 5-HT_{1A} receptor function. However, it is not clear that the lithium-induced enhancement of citalopram's effect on freezing was mediated by an enhancement of postsynaptic 5-HT_{1A} receptor function. The present results suggest that the addition of lithium to selective 5-HT reuptake inhibitors and 5-HT_{1A} receptor agonists is a promising new strategy for improving their treatment efficacies in anxiety disorders. Further clinical and experimental studies will be required to clarify the efficacy of subchronic $LiCO_3$ treatment combined with various serotonergic anxiolytics, such as selective 5-HT reuptake inhibitors and 5-HT_{1A} receptor agonists.

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