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Effect of co-administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 and selective 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 on anxiolytic effect of citalopram in conditioned fear stress in the rat

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Abstract

This study investigated the effect of co-administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 and selective 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 with a subactive dose of citalopram [selective serotonin (5-HT) reuptake inhibitor (SSRI)] on the expression of conditioned freezing, an index of fear. In the present study, acute administration of citalopram (s.c.) reduced freezing significantly at high doses (10, 30 and 100 mg/kg), while showing no significant effect at low doses (1 and 3 mg/kg). Co-administration of WAY 100,635 (0.15 mg/kg) with citalopram (3 mg/kg) reduced freezing markedly and significantly, as compared with either drug alone. However, the addition of GR 127,935 (4 mg/kg) did not potentiate the effects of citalopram (3 mg/kg) on freezing and did not enhance the effect of WAY 100,635 (0.15 mg/kg) with citalopram (3 mg/kg). Co-administration of WAY 100,635 (0.15 mg/kg) or GR 127,935 (4 mg/kg) gave no effect on high-dose citalopram (30 mg/kg)-induced inhibition of freezing behavior. These results suggest that co-administration of WAY 100,635 (0.15 mg/kg) strengthens the anxiolytic effect of citalopram (3 mg/kg) by facilitating central 5-HT neurotransmission. Since GR 127,935 (4 mg/kg) failed to accelerate the inhibition of freezing induced by citalopram (3 mg/kg) with WAY 100,635 (0.15 mg/kg) or citalopram (3 mg/kg) alone, it is suggested that blocking 5-HT_{1A} receptors is more effective in facilitating the anxiolytic effect of citalopram than blocking 5-$HT_{1B/1D}$ receptors.

Keywords: SSRI [selective serotonin (5-HT) reuptake inhibitor]; Selective 5-HT_{1A} receptor antagonist; Selective 5-$HT_{1B/1D}$ receptor antagonist; Anxiety; Conditioned fear stress

1. Introduction

Although it is established that selective serotonin (5-HT) reuptake inhibitors (SSRIs) are effective in the treatment of depression, recent clinical evidence has shown that SSRIs are also effective in the treatment of various anxiety disorders (Zohar and Westenberg, 2000). Numerous placebo-controlled studies have demonstrated the efficacy of SSRIs in most anxiety disorders, such as panic disorder, obsessive–compulsive disorder, generalized anxiety disorder, post-traumatic stress disorder and social phobia (Zohar and Westenberg, 2000). From in vivo brain microdialysis studies, Fuller (1994) reviewed that selective 5-HT reuptake inhibitors increase the output from the 5-HT synapse in the raphe nuclei, hypothalamus and cerebral cortex. Based on clinical and experimental data, the mechanism of anxiolytic action of SSRIs is supposed to involve selective increases in the availability of 5-HT.

SSRIs only exert their therapeutic effects after chronic administration and it takes usually 2 to 4 weeks to produce their full therapeutic effects (Blier and de Montigny, 1994; Gardier et al., 1996). Delayed effects of SSRIs suggest that their mechanism of action involves adaptive modifications in serotonergic neurotransmission induced by chronic treatment (Blier and de Montigny, 1994), such as the desensitization of somatodendritic 5-HT_{1A} autoreceptors and terminal 5-HT_{1B} autoreceptors (Goodwin et al., 1996; Leonard, 1995, 1996). The somatodendritic 5-HT_{1A} autoreceptors and 5-$HT_{1B/1D}$

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autoreceptors modulate the 5-HT release, and their activation leads to decreased 5-HT release (Sharp et al., 1989; Hoyer and Middlemiss, 1989). The reduced feedback inhibition of 5-HT neurons obtained with chronic treatment with SSRI and associated elevation of extracellular 5-HT concentrations in the brain, may be due to functional desensitization of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (Invernizzi et al., 1994; Rutter et al., 1994). However, the role of 5-HT_{1B} autoreceptors for functional desensitization following chronic SSRIs is controversial. Although the evidence for desensitization of 5-HT_{1B} receptors was reported in an early electrophysiological study (Chaput et al., 1986), subsequent microdialysis studies could not verify this finding (Auerbach and Hjorth, 1995; Bosker et al., 1995a,b; Moret and Briley, 1996; Davidson and Stamford, 1997).

Previous clinical studies have reported that addition of (±) pindolol, which possesses antagonistic properties at 5-HT_{1A} and 5-HT_{1B} autoreceptors, to an SSRI in the treatment of depression demonstrates a more rapid onset of antidepressant activity in double blind placebo-controlled studies (Bordet et al., 1998; Zanardi et al., 1997, 1998). Moreover, there are clinical studies showing that (−)-pindolol shortens the latency to onset of clinical antidepressant action of SSRIs and improves their efficacy in refractory patients (Artigas et al., 1994; Blier and Bergeon, 1995). In vivo microdialysis studies have demonstrated that (−)-pindolol enhances SSRI-induced 5-HT release in the forebrain (Dreshfield et al., 1996; Hjorth, 1996). Recent in vivo microdialysis studies showed that the addition of 5-HT_{1A} or 5-$HT_{1B/1D}$ receptor antagonists potentiates the effect of SSRIs on extracellular 5-HT concentrations (Hjorth, 1993; Dreshfield et al., 1996; Hjorth and Auerbach, 1996; Gobert et al., 1997; Sharp et al., 1997). These effects are probably associated with the blockade of somatodendritic 5-HT_{1A} receptor and/or terminal 5-$HT_{1B/1D}$ autoreceptors. Moreover, these results showed that the combined antagonism of 5-HT_{1A} and 5-HT_{1B} autoreceptors was more effective than their individual blockade in potentiating SSRI-induced increases in 5-HT levels (Gobert et al., 1997; Sharp et al., 1997).

From basic and clinical findings mentioned above, we hypothesized that the combination of 5-HT_{1A} and/or 5-$HT_{1B/1D}$ receptor antagonists with SSRIs may strengthen the anxiolytic effect of SSRIs and shortens the latency to onset of clinical anxiolytic action of SSRIs. Our previous reports showed acute anxiolytic-like effects of selective 5-HT reuptake inhibitors, 5-HT_{1A} receptor agonists and 5-HT precursor on freezing behavior, an index of fear induced by conditioned fear stress (re-exposure to an environment previously paired with inescapable electric footshock) (Hashimoto et al., 1996; Inoue et al., 1996; Muraki et al., 1999). Little is known about the anxiolytic properties of SSRI in healthy human after acute administration, but the recent findings suggest that acute treatment with SSRIs could affect brain activation and emotional processing associated with fear in human (Harmer et al., 2003; Takahashi et al., 2005; Grillon et al., 2007). In the present study, to clarify the role of the combined antagonism of 5-HT_{1A} and 5-$HT_{1B/1D}$ autoreceptors for the anxiolytic effects, we examined the acute effect of co-administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 and selective 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 on citalopram (SSRI)-induced inhibition of conditioned freezing, as an index of 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: 06:30–18:30), temperature-controlled environment (22±1 °C). The animals were maintained on a diet of standard laboratory rat chow. 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 8:00 and 13:00 h.

2.2. Drugs

Citalopram hydrobromide, 1-(3-dimetylaminopropyl)-1-(4-fluoro-phenyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide (H. Lundbeck A/S, Copenhagen, Denmark), dissolved in 0.9% sterile saline. WAY 100,635, {*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide} (Wyeth Ledaerle), dissolved in distilled water. GR 127,935, *N*-[4-methoxy-3-(4-methylpiperadine-1-yl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carboxamide (GlaxoSmithKlein), dissolved in distilled water with gentle (70 °C) heating. All drugs were injected subcutaneously (s.c.) in a volume of 1 ml/kg. Drugs were administered at the following doses, which were referred to the previous in vivo microdialysis studies (Gartside et al., 1995; Rollema et al., 1996; Sharp et al., 1997; Gobert et al., 1997; Muraki et al., 2001): citalopram (1, 3, 10, 30 and 100 mg/kg), WAY 100,635 (0.15 mg/kg) and GR 127,935 (4 mg/kg). At these doses, WAY 100,635 and GR 127,935 potentiated extracellular 5-HT increases induced by SSRIs significantly. From the inhibitory potency (ED_{50}) of 5-HT reuptake of citalopram in human (3.4 mg/day in vivo) (Meyer et al., 2004) and rats (0.7 mg/kg s.c. in vivo and 5.9 mg/kg p.o. *ex vivo*) (Thomas et al., 1987; Sánchez and Hytell, 1999), 3 and 30 mg/kg of citalopram in rats are estimated to be equivalent to 14.6 (or 1.7) mg/day and 146 (or 17) mg/day in human, respectively (clinical daily doses of citalopram are 20–60 mg/day) (Trivedi et al., 2006).

2.3. Procedures

2.3.1. Conditioned fear stress-induced freezing

For contextual fear conditioning, rats individually underwent inescapable electric footshocks for a total of 2.5 min in a shock chamber with a grid floor (19×22×20 cm; Medical Agent Co., Japan) (Hashimoto et al., 1996). Electric shocks were applied by using a Model SGS-02D Shock Generator (Medical Agent Co., Japan). Five footshocks (2.5 mA scrambled shock, each of 30 s

duration) were delivered at intershock intervals of 35–85 s (mean 60 s). On the next day 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 freezing 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 complied with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.3.2. Effect of acute citalopram treatment on conditioned fear

Twenty-four hours after footshock, the rats received a single injection of citalopram (1, 3, 10, 30 and 100 mg/kg) at 4 h before testing.

2.3.3. Effect of co-administration of WAY 100,635 and/or GR 127,935 with acute citalopram (3 and 30 mg/kg) treatment on conditioned fear

Twenty-four hours after footshock, 1 ml/kg of vehicle, WAY 100,635 (0.15 mg/kg) or GR 127,935 (4 mg/kg) was administered to rats (5 h before testing) at 1 h prior to citalopram (3 and 30 mg/kg), and then 1 ml/kg of saline or 3 mg/kg of citalopram was administered at 4 h before testing. In the same procedure, WAY 100,635 (0.15 mg/kg) plus GR 127,935 (4 mg/kg) was administered prior to citalopram (3 mg/kg).

2.4. Motor activity

Motor activity was measured for citalopram 30 mg/kg and citalopram 3 mg/kg with or without co-administration of WAY 100,635 (0.15 mg/kg) and GR 127,935 (4 mg/kg) in non-shocked animals. The motor activity in Plexiglas boxes (38×33×17 cm) was recorded as described by Ohmori et al. (1994) automatically for 10 min by electronic digital counters with infrared cell sensors between 8:00 and 13:00. WAY 100,635 (0.15 mg/kg) or GR 127,935 (4 mg/kg) was administered at 5 h before testing, and citalopram (3 and 30 mg/kg) was administered at 4 h 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 those movements contained substantial horizontal components.

2.5. 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). We performed an analysis of variance (ANOVA) when a normal distribution was observed in every subgroup; one-way ANOVA with a factor, citalopram doses for the acute citalopram alone experiments; two-way ANOVA with factors, citalopram and WAY 100,635 or GR 127,935 (2×2) for the acute citalopram plus WAY 100,635 or GR 127,935 experiments; three-way ANOVA with factors, citalopram, WAY 100,635 and GR 127,935 (2×2×2) for the acute citalopram plus WAY 100,635 and GR 127,935 experiments, followed by Duncan's test for multiple comparison as a post hoc test.

3. Results

3.1. Effect of acute citalopram treatment on conditioned freezing

The selective 5-HT reuptake inhibitor, citalopram, reduced the expression of conditioned freezing significantly [1-way ANOVA, $F(5,42)=11.138$, $P<0.001$] at high doses (10, 30 and 100 mg/kg), while low doses (1 and 3 mg/kg) of citalopram showed no significant effect (Fig. 1).

3.2. Effect of co-administration of WAY 100,635 and GR 127,935 with acute citalopram (3 mg/kg) treatment on conditioned freezing (Fig. 2)

Three-way ANOVA [citalopram (0 or 3 mg/kg)×WAY 100,635 (0 or 0.15 mg/kg)×GR 127,935 (0 or 4 mg/kg)] indicated a significant interaction effect between citalopram and WAY 100,635 [$F(1,88)=7.085$, $P=0.0092$] on freezing. There was no significant interaction effect between citalopram and GR 127,935 [$F(1,88)=0.171$, $P=0.6799$], between WAY 100,635 and GR 127,935 [$F(1,88)=2.173$, $P=0.1441$], or between

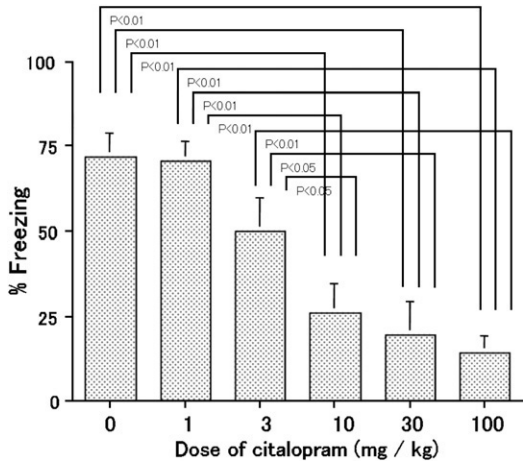

Fig. 1. Effect of acute citalopram treatment on the expression of conditioned freezing. Citalopram was subcutaneously administered 24 h after footshock and 4 h before conditioned fear stress. The mean percentages±S.E.M. for freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. *P* values indicate the results of a post hoc test (Duncan's test). $N=8$. cit, citalopram.

citalopram, WAY 100,635 and GR 127,935 [$F(1,88)=1.167$, $P=0.2830$]. It also indicated significant main effects of citalopram [$F(1,88)=19.939$, $P<0.0001$] and WAY 100,635 [$F(1,88)=6.277$, $P=0.0141$]. There was no significant main effect of GR 127,935 [$F(1,88)=0.146$, $P=0.7037$]. Post hoc analysis revealed that citalopram, WAY 100,635, GR 127,935, WAY 100,635 plus GR 127,935 and citalopram with GR 127,935 had no effect on freezing behavior compared with vehicle, while citalopram with WAY 100,635 and citalopram with WAY 100,635 plus GR 127,935 significantly reduced the expression of conditioned freezing, compared with either drug alone. Moreover, citalopram with WAY 100,635 plus GR 127,935 significantly reduced the expression of conditioned freezing, compared with citalopram with GR 127,935. There was no significant difference between citalopram with WAY 100,635 and citalopram with WAY 100,635 plus GR 127,935 (Fig. 2).

3.3. Effect of co-administration of WAY 100,635 (0.15 mg/kg) with acute citalopram (30 mg/kg) treatment on conditioned freezing (Fig. 3)

Two-way ANOVA [citalopram (0 or 3 mg/kg)×WAY 100,635 (0 or 0.15 mg/kg)] indicated a significant main effect of citalopram [$F(1,28)=20.080$, $P=0.0001$] but no significant interaction effect between citalopram and WAY 100,635 [$F(1,28)=2.467$, $P=0.1275$] or no significant main effect of WAY 100,635 [$F(1,28)=0.146$, $P=0.7049$]. Post hoc analysis showed that WAY 100,635 had no significant effect on freezing behavior compared with vehicle, while citalopram and citalopram with WAY 100,635 significantly reduced freezing, compared with either vehicle or WAY 100,635. There was no significant difference between citalopram alone and citalopram with WAY 100,635 (Fig. 3).

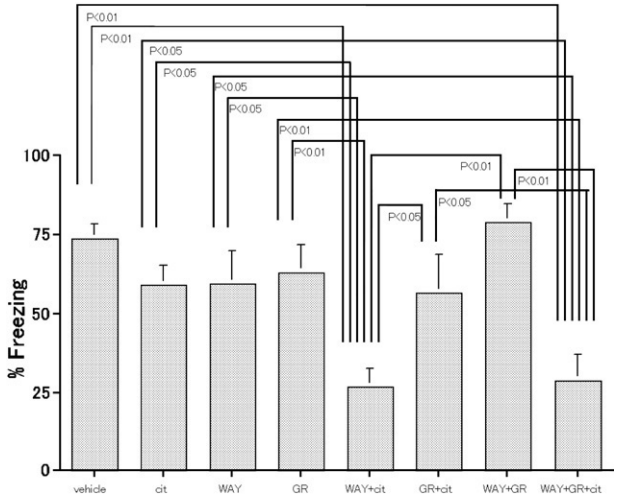

Fig. 2. Effect of co-administration of WAY 100,635 and GR 127,935 with acute citalopram (3 mg/kg) treatment on conditioned freezing. Twenty-four hours after footshock, 1 ml/kg of vehicle, WAY 100,635 (0.15 mg/kg), GR 127,935 (4 mg/kg) or WAY 100,635 (0.15 mg/kg) plus GR 127,935 (4 mg/kg) was administered at 1 h prior to citalopram (5 h before testing), and then 1 ml/kg of saline or 3 mg/kg of citalopram was administered at 4 h before testing. The mean percentages±S.E.M. of freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. *P* values indicate the results of a post hoc test (Duncan's test). $N=24$ (vehicle, cit), $N=8$ (WAY, GR, WAY+cit, GR+cit, WAY+GR, WAY+GR+cit). cit, citalopram; WAY, WAY 100,635; GR, GR 127,935.

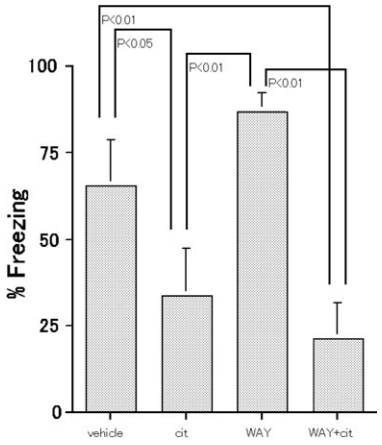

Fig. 3. Effect of co-administration of WAY 100,635 (0.15 mg/kg) with acute citalopram (30 mg/kg) treatment on conditioned freezing. On the next day after footshock, 1 ml/kg of vehicle or WAY 100,635 (0.15 mg/kg) was administered at 1 h prior to citalopram (5 h before testing), and then 1 ml/kg of saline or 30 mg/kg of citalopram was administered at 4 h before testing. The mean percentages±S.E.M. of freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. *P* values indicate the results of a post hoc test (Duncan's test). $N=8$. cit, citalopram; WAY, WAY 100,635.

3.4. Effect of co-administration of GR 127,935 (4 mg/kg) with acute citalopram (30 mg/kg) treatment on conditioned freezing (Fig. 4)

Two-way ANOVA [citalopram (0 or 3 mg/kg)×GR 127,935 (0 or 4 mg/kg)] indicated a significant main effect of citalopram [$F(1,28)=21.820$, $P<0.0001$] but no significant interaction effect between citalopram and GR 127,935 [$F(1,28)=0.594$,

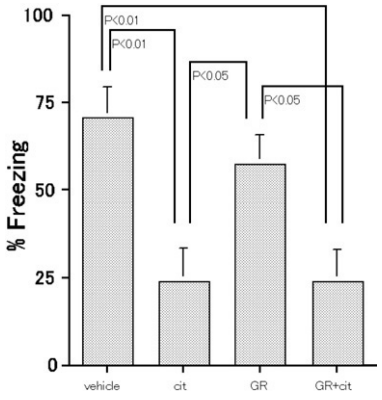

Fig. 4. Effect of co-administration of GR 127,935 (4 mg/kg) with acute citalopram (30 mg/kg) treatment on conditioned freezing. On the next day after footshock, 1 ml/kg of vehicle or GR 127,935 (4 mg/kg) was administered at 1 h prior to citalopram, and then 1 ml/kg of saline or 30 mg/kg of citalopram was administered at 4 h before testing. The mean percentages±S.E.M. of freezing scored for a 5-min observation period are given. Behavior was sampled at 10-s intervals. *P* values indicate the results of a post hoc test (Duncan's test). $N=8$. cit, citalopram; GR, GR 127,935.

Table 1
Motor activity in treatment groups

Treatment	Motor activity			
	Unpaired *t*-test (two-tailed)			
Vehicle	13.9±8.7	N.S.		
Citalopram 3 mg/kg	0±0			
Vehicle	4.8±4.2	N.S.		
Citalopram 30 mg/kg	0±0			
Vehicle+vehicle	4.8±4.2	N.S.	N.S.	
Citalopram 3 mg /kg+WAY 100,635	0±0			
Citalopram 3 mg /kg+GR 127,935	23.8±23.2			

Each number represents the means±S.E.M. (counts) of the individual values for the group of an administered drug. N.S., not significant.

$P=0.4475$] or no significant main effect of GR 127,935 [$F(1,28)=0.591$, $P=0.4483$]. Post hoc analysis showed that GR 127,935 had no significant effect on freezing behavior compared with vehicle, while citalopram and citalopram with GR 127,935 reduced freezing significantly, compared with either vehicle or GR 127,935. There was no significant difference between citalopram alone and citalopram with GR 127,935 (Fig. 4).

3.5. Motor activity (Table 1)

Acute citalopram (3 and 30 mg/kg) treatment failed to affect motor activity (data not shown). Citalopram 3 mg/kg with WAY 100,635 (0.15 mg/kg) and citalopram 3 mg/kg with GR 127,935 (4 mg/kg) also failed to affect motor activity (Table 1).

4. Discussion

In the present study, acute administration of citalopram reduced freezing significantly at high doses (10, 30 and 100 mg/kg), while showing no significant effect at low doses (1 and 3 mg/kg). We previously reported, by administering a single systemic dose of citalopram (3 and 30 mg/kg), that extracellular 5-HT levels are increased and sustained as such for hours after citalopram dosing and that 30 m/kg produced more increases than 3 mg/kg (Muraki et al., 2001). Ten to 30 mg/kg of citalopram produced a maximal inhibitory effect on freezing, which was comparable to the inhibitory effect of an extremely high dose (100 mg/kg) of citalopram. These results are well consistent with our previous results showing that acute treatment with citalopram and fluvoxamine, another selective 5-HT reuptake inhibitor, decreased the expression of conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996; Muraki et al., 1999). Moreover, the effects of SSRIs on the expression of conditioned freezing are consistent with recent clinical evidence showing that a number of serotonergic agents (selective 5-HT reuptake inhibitors, 5-HT_{1A} receptor agonists, etc) are effective in the treatment of human anxiety disorders (Zohar and Westenberg, 2000). The mechanism of action of SSRIs presumably involves the facilitation of serotonergic neurotransmission, and an increase in postsynaptic receptor activation after SSRIs plays a functional role in the reduction of conditioned freezing (Inoue et al., 1996; Hashimoto et al., 1999). In support of this theory, the microinjection of citalopram into the amygdala reduced the expression of conditioned freezing (Inoue et al., 2004). Thus, high doses of citalopram would yield a higher degree of postsynaptic 5-HT receptor activation than low doses as shown by previous in vivo microdialysis studies (Invernizzi et al., 1994; Muraki et al., 2001), so that high doses produce a more anxiolytic effect.

Little is known about the anxiolytic properties of SSRI in healthy human after acute administration. Acute citalopram increased the fear related response in healthy subjects, but was not anxiogenic in the absence of danger (Grillon et al., 2007). Acute citalopram facilitates the recognition of fear and happiness from facial expressions (Harmer et al., 2003). Acute fluvoxamine attenuated the amygdala activity, despite no significant differences being found in the subjective ratings of affective pictures (Takahashi et al., 2005). These human data suggest that even acute treatment could affect brain activation and emotional processing associated with fear in human and are consistent with our experimental data of acute anxiolytic effects of SSRIs. Further studies are needed to examine acute anxiolytic effects of SSRIs in human.

Acute administration of WAY 100,635, GR 127,935 and WAY 100,635 plus GR 127,935 showed no effect on freezing behavior. The selective 5-HT_{1A} receptor antagonist WAY 100,635 (Sharp et al., 1997) and the selective 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 (Skingle et al., 1995; Sharp et al., 1997) produced no significant changes in extracellular 5-HT levels in the rat brain. These in vivo microdialysis findings explain lack of anxiolytic effects of these autoreceptor antagonists alone.

WAY 100,635 enhanced the anxiolytic effect of a subactive dose of citalopram (3 mg/kg) on conditioned freezing markedly, consistent with our previous finding that other 5-HT_{1A} receptor antagonists enhanced the anxiolytic effect of citalopram in conditioned freezing (Hashimoto et al., 1997). A number of previous in vivo microdialysis studies have demonstrated that acute treatment with WAY 100,635 produced a significant potentiation of SSRI-induced changes in extracellular 5-HT levels in various brain regions of rats (Gartside et al., 1995; Romero et al., 1996; Invernizzi et al., 1996; Hjorth et al., 1997; Dawson and Nguyen, 1998). Since the mode of action of SSRIs as anxiolytic drugs is assumed to be postsynaptic 5-HT receptor activation as mentioned above (Inoue et al., 1996; Muraki et al., 1999; Inoue et al., 2004), it is likely that WAY 100,635 potentiates the anxiolytic effect of citalopram by enhancing citalopram-induced increases in extracellular 5-HT levels through the blockade of presynaptic 5-HT_{1A} autoreceptors.

On the other hand, WAY 100,635 did not enhance the effect of very high-dose citalopram (30 mg/kg). High doses of citalopram (10–30 mg/kg) produced a maximal inhibitory effect on freezing behavior but did not inhibit freezing completely in contrast to the effect of dose-dependent inhibition by 5-HT_{1A} receptor agonists (Inoue et al., 1996). In our previous study, no-shock rats, which were placed in the shock chamber and were placed again without shocks in a different day, showed only a low level (8.3%) of freezing (Inoue et al., 2006), suggesting that incomplete inhibition by high-dose citalopram cannot be explained by freezing levels in no-shock controls, i.e. limitation

of reduction in freezing. One might account for this mechanism by the inhibition of 5-HT nerve firing by autoreceptor stimulation. However, no effect of combined autoreceptor antagonists with a high dose of citalopram in this study suggests that incomplete inhibition by high-dose citalopram is related to other unknown factors except for autoreceptor stimulation. High-dose citalopram (30 mg/kg) produced a maximal inhibitory effect on freezing, similar to that of 100 mg/kg citalopram (Fig. 1). Hence, the behavioral effect of citalopram (30 mg/kg) may not be enhanced by WAY 100,635 because of a ceiling effect on increased extracellular 5-HT. Future studies are needed to examine whether WAY 100,635 enhances the increases in extracellular 5-HT levels induced by high-dose citalopram (30 mg/kg).

Recent data have reported that WAY 100,635 possesses high affinity for dopamine D_4 receptors and behaved as a full agonist (Chemel et al., 2006). In contrast to this study, Martel et al. (2007) showed clear pharmacological differences between the high potency of WAY 100,635 for blocking serotonin 5-HT_{1A} receptors and much lower potency for interacting with dopamine D_4 receptors, and its weak partial agonism at dopamine D_4 receptors. In a behavioral study, the absence of dopamine D_4 receptors increased avoidance behavior to unconditioned stimuli but did not impair behavioral reactions to conditioned fear (Falzone et al., 2002). Taken together, WAY 100,635 might have an influence on dopamine D_4 receptors, but at appropriate doses or concentrations, this compound shows a high selectivity for 5-HT_{1A} receptors, suggesting little influence via dopamine D_4 receptors on conditioned fear.

Only a few behavioral experiments concerning the anxiolytic effect of combined SSRI and 5-HT_{1A} receptor antagonist have been reported, and one report showed a similar finding in rat high□light social interaction, a model of anxiety (Duxon et al., 2000). WAY 100,635 (1 mg/kg) enhanced the anxiolytic effect of paroxetine, an SSRI, in this test. On the other hand, WAY 100,635 (3 mg/kg) did not enhance the anxiolytic effect of paroxetine in the rat ultrasonic vocalization test, another model of anxiety (Schreiber et al., 1998). A high dose of WAY 100,635 can block not only presynaptic but also postsynaptic 5-HT_{1A} receptors. The differences in doses or models may be associated with some inconsistencies, since the different responses to SSRIs in various animal models of anxiety are usual (Borsini et al., 2002).

In contrast to WAY 100,635, the 5-$HT_{1B/1D}$ receptor antagonist GR 127,935 did not enhance the effects of citalopram or citalopram with WAY 100,635 on freezing. In vivo microdialysis studies reported that GR 127,935 enhances SSRI-induced increases in extracellular 5-HT levels, although there is conflicting data (Sharp et al., 1997; Gobert et al., 1997; Rollema et al., 1996). The degree of enhancement of SSRI-induced 5-HT increases is similar between WAY 100,635 and GR 127,935 (Gobert et al., 1997), and this cannot account for the behavioral difference in this study. The addition of GR 127,935 to SSRI with WAY 100,635 produces more increases in extracellular 5-HT levels than SSRI with WAY 100,635 does (Sharp et al., 1997; Gobert et al., 1997), and this result suggests that GR 127,935 may enhance the anxiolytic effect of citalopram that is potentiated by WAY 100,635. However, co-administration of GR 127,935 with WAY 100,635 plus citalopram did not show such enhancement in this study. It should be noted that the previous in vivo microdialysis studies have been conducted in non-stressed rats (Gobert et al., 1997; Rollema et al., 1996). Furthermore, the possibility that GR, 127,935 may enhance the effect of citalopram with a lower or subeffective dose of WAY 100,635 cannot be excluded. In future experiments, the effect of WAY 100,635 and GR 127,935 in combination with SSRIs on extracellular 5-HT needs to be confirmed in rats receiving conditioned fear.

Citalopram (30 mg/kg) and citalopram (3 mg/kg) with WAY 100,635, which were effective in the conditioned fear test, did not affect motor activity compared with the vehicle controls. Furthermore, each treatment did not show any component of 5-HT behavioral syndrome, using four-point ranked intensity scale (Tricklebamk et al., 1984), during conditioned fear stress (data not shown). Therefore, the reduction in freezing observed with this treatment appears to be independent of any non-specific effect on motor activity or 5-HT behavioral syndrome at doses required to significantly reduce freezing.

The importance of 5-HT_{2C} receptors in the mechanism of action of citalopram has also been reported. For example, it has been recently shown that acute citalopram treatment enhances the expression of auditory fear conditioning, which is blocked by systemic administration of a 5-HT_{2C} receptor antagonist (Burghardt et al., 2007). However, the effect of citalopram on contextual conditioned fear in our study is opposite and the effect of 5-HT_{2C} receptor antagonist combination has not been reported. These findings suggest that the effect of co-administration of a 5-HT_{2C} receptor antagonist with SSRIs on contextual conditioned fear should be examined in future.

In conclusion, the present study shows that WAY 100,635, a selective 5-HT_{1A} receptor antagonist, potentiates citalopram (an SSRI)-induced inhibition of freezing behavior in conditioned fear stress. However, the addition of GR 127,935, a selective 5-$HT_{1B/1D}$ receptor antagonist, did not enhance the effect of citalopram or the effect of the combination of citalopram and WAY 100,635 on freezing. These results suggest that co-administration of WAY 100,635 facilitated the anxiolytic effects of citalopram by more increases of the extracellular 5-HT concentrations, blocking the presynaptic 5-HT_{1A} receptors. Since the blockade of 5-$HT_{1B/1D}$ receptors failed to accelerate the inhibition of freezing by citalopram or citalopram with WAY 100,635, it is supposed that 5-HT_{1A} receptor blockade is sufficient and critical to facilitate the anxiolytic effect of SSRIs in contrast to 5-$HT_{1B/1D}$ receptor blockade. These results suggest that the 5-HT_{1A} receptor blockade, but not 5-$HT_{1B/1D}$ receptor blockade, is a promising strategy to augment or accelerate the clinical effect of SSRIs on anxiety disorders.

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