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Effects of the Co-administration of 5-HT$_{1A}$ Receptor Antagonists with an SSRI in Conditioned Fear Stress-Induced Freezing Behavior

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HASHIMOTO, S., T. INOUE AND T. KOYAMA. *Effects of the co-administration of 5-HT$_{1A}$ receptor antagonists with an SSRI in conditioned fear stress-induced freezing behavior.* PHARMACOL BIOCHEM BEHAV **58**(2) 471–475, 1997.—The effects of the co-administration of the serotonin (5-HT) 1A receptor antagonists NAN-190 or (+)-WAY100135 with a selective 5-HT reuptake inhibitor (SSRI) citalopram on conditioned fear stress (CFS)-induced freezing behavior, which is the animal model of anxiety, were examined. The inhibitory effects of co-administration of NAN-190 (0.1–10 mg/kg) with citalopram on CFS-induced freezing were potent; in particular, at 0.1 and 0.25 mg/kg, NAN-190 significantly enhanced the effect of citalopram alone. At 0.1 mg/kg, (+)-WAY100135 also markedly enhanced the inhibitory effect of citalopram on freezing behavior. These findings suggest that 5-HT$_{1A}$ receptor antagonist, particularly at low doses, enhances the antifreezing effect of citalopram by blocking the autoreceptor-mediated negative feedback mechanisms of the 5-HT neuron. These experimental results concur with clinical findings that 5-HT$_{1A}$ receptor antagonist pindolol potentiates the effect of 5-HT reuptake inhibitors. © 1997 Elsevier Science Inc.

Conditioned fear stress Freezing behavior Serotonin (5-HT) Citalopram NAN-190 (+)-WAY100135, 5-HT$_{1A}$ receptor

THE excess extracellular serotonin (5-HT) produced by 5-HT reuptake inhibitors in the cell body region (raphe nuclei) may activate presynaptic 5-HT$_{1A}$ autoreceptors and slow down the firing rate of 5-HT neurons and their terminal release. Artigas (1) proposed that co-administration of a 5-HT$_{1A}$ receptor antagonist is another way to counteract the increased activation of presynaptic 5-HT$_{1A}$ autoreceptors that limit the increase in terminal 5-HT release after a 5-HT reuptake inhibitor is given.

The postsynaptic mechanism involved in the effects of selective 5-HT reuptake inhibitors (SSRIs) may be the enhancement of 5-HT$_{1A}$ receptor-mediated neurotransmission (5,11). Recent studies in our laboratory also have shown that the anxiolytic effect of the 5-HT$_{1A}$ agonist ipsapirone (26) may be mediated by postsynaptic 5-HT$_{1A}$ receptors (22). Therefore, a 5-HT$_{1A}$ receptor antagonist, by its postsynaptic actions, might block rather than enhance the therapeutic effects of SSRI.

In rodents, exposure to an environment with previously inescapable electric foot shock reliably elicits a response characterized by a period of crouching and complete immobility (4,7-9). This behavior is termed "conditioned fear stress (CFS)-induced freezing behavior," and freezing behavior could be used as a model of anxiety (13,24). In rats, freezing behavior is attenuated by benzodiazepines (13,16,22) and nonbenzodiazepine anxiolytics (10,22,30).

We have reported that acute treatment with the SSRIs citalopram and fluvoxamine (29) and the 5-HT/noradrenaline (NA) mixed reuptake inhibitor milnacipran (27) reduce CFS-

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induced freezing behavior without affecting motor activity. Acute treatment with the NA reuptake inhibitors and dopamine reuptake inhibitors failed to alter CFS-induced freezing. Because SSRIs are effective for the treatment of anxiety disorders such as panic disorder, social phobia and posttraumatic stress disorder (25), these results indicate that CFS-induced freezing is an acceptable means for testing the efficacy of SSRIs and that there appear to be circumstances in which increased 5-HT activity can reduce anxiety (17,22).

Based on these considerations, we studied the effects of the co-administration of multiple doses of the 5-HT_{1A} receptor antagonist NAN-190 (15) with the SSRI citalopram on CFS-induced freezing. We then tried to find out whether the selective and silent 5-HT_{1A} receptor antagonist (+)-WAY100135 (31) could enhance the inhibitory effect of citalopram on freezing behavior.

METHODS

Animals

Male Sprague–Dawley rats were obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan). They weighed 230–270 g, were housed in groups of 4 and maintained in a 12-h light–dark cycle (lights on at 7:00), temperature-controlled environment (22 ± 1°C) with free access to food and water. Experiments began after a 2-week acclimatization period. Rats were tested between 9:00 and 13:00.

Drugs

Citalopram hydrobromide (H. Lundbeck & Co. A/S, Denmark) was dissolved in 0.9% sterile saline and injected subcutaneously. (+)-WAY100135 (Asahi Chemical Industries, Japan) and NAN-190 (Research Biochemicals International, USA) were suspended in 0.5% sodium carboxymethyl cellulose and 1% Tween 80, respectively, and were injected intraperitoneally. All drugs were administered in a volume of 1 ml/kg.

Procedures

CFS-induced freezing. The total duration of the conditioning session was 5 min. Rats were subjected to inescapable electric foot shock 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 30 s, 5–55 s) in a chamber with a grid floor (19 × 22 × 20 cm; Medical Agent, Japan). Electric shock was provided by a Model SGS-02D Shock Generator (Medical Agent). This machine 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 actually produces a shock intensity of 0.2 mA to rats. Twenty-four hours after the shock, the rats were again placed and observed for 5 or 10 min in the shock chamber, but no current was applied to the floor of the chamber. During the observation period, the duration of freezing behavior was recorded by using a modification of a time-sampling procedure (12), as previously described (17). 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 score represented the number of 10-s periods during which the animal froze for the entire 10 s. Behavior was recorded by videotape and scored by two independent observers (one of whom did not know the experimental grouping of the animals). Interscorer reliability by this method was very high (>0.95). Citalopram (1 or 10 mg/kg) was administered 60 min before testing, and NAN-190 (0.1, 0.25, 1, 10 mg/kg) or (+)-WAY100135 (0.1 mg/kg) was given 15 min before the citalopram injection. 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.

Motor activity. Motor activity was measured on co-administration of (+)-WAY100135 (0.1 mg/kg) with citalopram (1 mg/kg). 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 animals, as described by Ohmori et al. (28). Citalopram was administered 30 min before testing, and (+)-WAY100135 was given 15 min before the citalopram injection. Horizontal movements of the rats were digitized and fed into a computer for 60 min. Locomotion predominantly contributed to the count, but repeated rearing, head weaving and other nonspecific body movements also contributed to the count when these movements has substantial horizontal components. Ohmori et al. reported a good dose–response relationship in the count elicited by methamphetamine in the dose range of 0.5–1.5 mg/kg.

Data Analysis

All the data are presented as means ± SEM of individual values of the rats from each group. Statistical differences between the two groups were analyzed by an unpaired *t*-test (two-tailed). Multiple group comparisons were made by using a one-way analysis of variance (ANOVA) followed by Duncan's test or two-way ANOVA.

RESULTS

Effects of the Co-administration of NAN-190 with Citalopram

During a 5-min observation period, while a single 10 mg/kg dose of citalopram tended to inhibition but had no significant effect on CFS-induced freezing as a result of multiple comparisons, co-administration of NAN-190 (0.1–10 mg/kg) with citalopram (10 mg/kg) significantly reduced freezing behavior compared with the vehicle controls [$F(5, 61) = 6.107$, $p < 0.01$]. Because of the potent inhibitory effects of the co-administration of 0.1 and 0.25 mg/kg of NAN-190 with citalopram (10 mg/kg) on freezing behavior, these effects were statistically significant compared with the effect of citalopram alone (Fig. 1). NAN-190 alone did not change conditioned freezing [$F(3, 27) = 1.381$, $p = 0.270$] (Table 1).

Effects of the Co-administration of (+)-WAY100135 with Citalopram

The results of a 10-min observation period (two 5-min blocks) are shown in Fig. 2. The effect of citalopram (1 mg/kg) on CFS-induced freezing behavior was affected by (+)-WAY100135 (0.1 mg/kg) over the 10-min observation period and the first 5-min block [0–5 min: effect of citalopram, $F(1, 47) = 8.421$, $p < 0.01$; effect of (+)-WAY100135, $F(1, 47) = 1.494$, $p = 0.228$; interaction of (+)-WAY100135 with citalopram, $F(1, 47) = 4.383$, $p < 0.05$] [5–10 min: effect of citalopram, $F(1, 47) = 12.790$, $p < 0.01$; effect of (+)-WAY100135, $F(1, 47) = 0.525$, $p = 0.472$; interaction of (+)-WAY100135

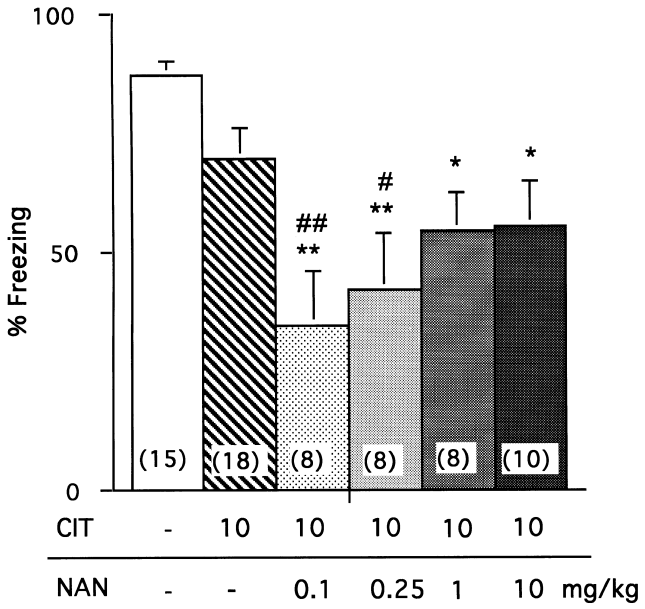

FIG. 1. Effects of the co-administration of the 5-HT_{1A} receptor antagonist NAN-190 with the selective 5-HT reuptake inhibitor citalopram on CFS-induced freezing behavior. Citalopram (CIT; 1 mg/kg) was administered 60 min before testing, and NAN-190 (NAN; 0.1–10 mg/kg) was given 15 min before the citalopram injection. Represented are the mean percentages ± SEM of freezing, scored over a 5-min observation period. The values in parentheses represent the number of animals. Behavior was sampled at 10-s intervals. Difference from vehicle treatment: $^{*}p < 0.05$; $^{**}p < 0.01$. Difference from citalopram treatment: $^{\#}p < 0.05$; $^{\#\#}p < 0.01$.

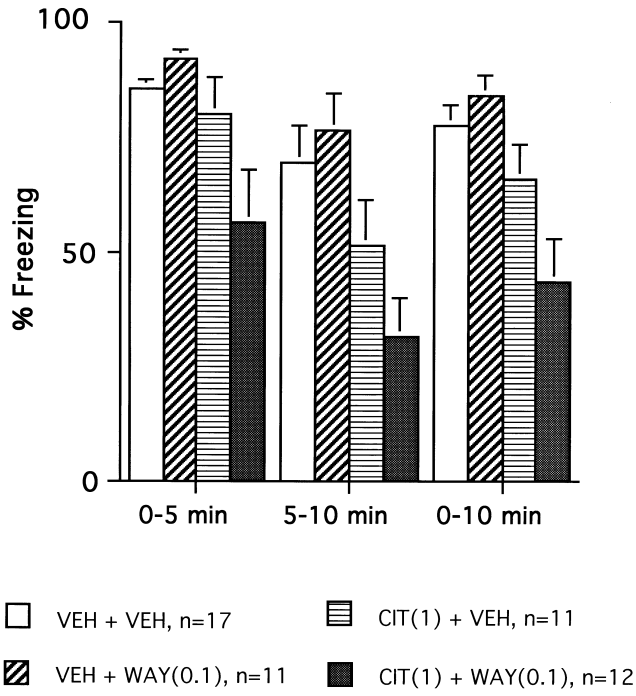

FIG. 2. Effect of the co-administration of the 5-HT_{1A} receptor antagonist (+)-WAY100135 with the selective 5-HT reuptake inhibitor citalopram on CFS-induced freezing behavior. Citalopram (CIT; 1 mg/kg) was administered 60 min before testing, and (+)-WAY100135 (WAY; 0.1 mg/kg) was given 15 min before the citalopram injection. Represented are the mean percentages ± SEM of freezing, scored over a 10-min observation period in two 5-min blocks. Behavior was sampled at 10-s intervals. Significant F values by two-way ANOVA; (0–5 min) effect of citalopram; [$F(1,47) = 8.421$, $p < 0.01$]; interaction of (+)-WAY100135 with citalopram [$F(1,47) = 4.383$, $p < 0.05$]; (0–10 min) effect of citalopram [$F(1,47) = 13.525$, $p < 0.01$]; interaction of (+)-WAY100135 with citalopram [$F(1,47) = 4.100$, $p < 0.05$].

with citalopram, $F(1, 47) = 2.326$, $p = 0.134$] [0–10 min: effect of citalopram, $F(1, 47) = 13.525$, $p < 0.01$; effect of (+)-WAY100135, $F(1, 47) = 1.202$, $p = 0.279$; interaction of (+)-WAY100135 with citalopram, $F(1, 47) = 4.100$, $p < 0.05$]. The possibility that the effect of the co-administration of (+)-WAY100135 with citalopram facilitated motor activity and reduced freezing behavior can be discarded because co-administration of (+)-WAY100135 with citalopram failed to affect motor activity in the home cages [vehicle + vehicle, 8.5 ± 6.7 counts ($n = 6$); citalopram + (+)-WAY100135, 8.2 ± 2.6 counts ($n = 6$)].

DISCUSSION

The present investigation was undertaken to define more clearly the impact of 5-HT_{1A} receptor antagonists on the anxiolytic effect of SSRI. We examined the effects of the co-administration of multiple doses of NAN-190 with citalopram on CFS-induced freezing. The antifreezing effect of citalopram was enhanced by lower doses of NAN-190; thus, we focused on the effects of the co-administration of a low dose (0.1 mg/kg) of the selective and silent 5-HT_{1A} receptor antagonist (+)-WAY100135 with citalopram on freezing behavior. In this study, co-administration of (+)-WAY100135 with citalopram strongly reduced freezing behavior. These findings may provide evidence that 5-HT_{1A} receptor antagonists NAN-190 and (+)-WAY100135, particularly at low doses, enhances the antifreezing effect of the SSRI citalopram.

The 5-HT-mediated transmission in the raphe nuclei is largely enhanced by 5-HT reuptake inhibitors because this is the area with the highest density of 5-HT transporters in rat and human brains (21). Indeed, somatodendrites of 5-HT-containing neurons have a very high density of 5-HT_{1A} autoreceptors that exert negative control on cell firing and release from nerve terminals (32). Hence, low doses of SSRIs usually have no effect on nerve terminal 5-HT output, whereas the same doses induce large increases in 5-HT concentrations in the raphe nuclei (3,23).

Recent studies have shown that co-administration of a 5-HT_{1A} receptor antagonist with an SSRI allows the SSRI to induce an immediate increase in terminal 5-HT release by blocking somatodendritic autoreceptors (18,19). There is evidence indicating that this procedure has clinical advantages. Artigas et al. (2) reported that the combined 5-HT_{1A} antagonist pindolol and SSRI treatment resulted in dramatic and rapid improvement in a group of depressed patients, including those previously considered resistant to therapy. Blier and Bergeron (6) also reported that pindolol effectively accelerated the antidepressant effect of SSRI and had a therapeutic effect on depressed drug-resistant patient. In this study, co-administration of NAN-190 or (+)-WAY100135 with citalopram resulted in a very potent inhibitory effect on CFS-induced freezing behavior, which is an appropriate means for testing the efficacy

TABLE 1
EFFECT OF NAN-190 ON CFS-INDUCED FREEZING BEHAVIOR

Drug	Dose (mg/kg)	No. of Rats	% Freezing
Vehicle + saline		8	87.92 ± 4.63
NAN-190 + saline	0.1	8	71.25 ± 12.39
	1	7	79.52 ± 5.88
	10	8	65.42 ± 8.11

Saline was administered 60 min before testing, and NAN-190 (0.1–10 mg/kg) was given 15 min before the saline injection. Represented are the mean percentages ± SEM of freezing, scored over a 5-min observation period. Behavior was sampled at 10-s intervals. In no cases were statistically significant differences observed.

of SSRIs. Consequently, disinhibition of presynaptic 5-HT_{1A} autoreceptor-mediated negative feedback should further augment the effect of 5-HT reuptake inhibitors on extracellular 5-HT in the terminal.

The postsynaptic mechanism involved in the effects of 5-HT reuptake inhibitors may be the enhancement of 5-HT_{1A} receptor-mediated neurotransmission (11). In this study, it was clear that the antifreezing effect of citalopram also was enhanced by lower doses of NAN-190. Thus, low doses of NAN-190 probably blocked somatodendritic autoreceptors but blocked postsynaptic 5-HT_{1A} receptors at higher doses. These findings suggest that SSRIs induce their antifreezing effects at least in part via the stimulation of postsynaptic 5-HT_{1A} receptor subtypes indirectly. The reason NAN-190 (10 mg/kg) had not returned toward the control level of freezing seems to be that the anxiolytic effect of SSRIs is associated with multiple interactions between 5-HT receptor subtypes.

NAN-190 (20) and (+)-WAY100135 (14) are not pure antagonists, no doubt because these compounds are partial agonists at somatodendritic autoreceptors in addition to acting as antagonists at postsynaptic receptors. Their intrinsic activities, however, are much lower than that of 5-HT. Therefore, when endogenous levels of 5-HT are raised in the raphe nuclei by SSRIs, NAN-190 and (+)-WAY100135 may appear to be antagonists.

In conclusion, these results indicate that 5-HT_{1A} receptor antagonist, particularly at low doses, enhances the antifreezing effect of SSRI by blocking the autoreceptor-mediated negative feedback mechanisms of 5-HT neurons. These experimental results concur with the clinical findings that the 5-HT_{1A} receptor antagonist pindolol potentiates the effect of 5-HT reuptake inhibitors.

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