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Chronic treatment with fluvoxamine desensitizes 5-HT_{2C} receptor-mediated hypolocomotion in rats

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Abstract

The effectiveness of fluvoxamine, a selective serotonin re-uptake inhibitor (SSRI), in the treatment of anxiety disorders, such as obsessive-compulsive, panic and social anxiety disorders, has been confirmed in clinical studies. The hypersensitivity of 5-HT_{2C} receptors has been reported in subjects with these disorders, and SSRIs have been suggested to have therapeutic effects in such cases through the desensitization of the 5-HT_{2C} receptor function. In the present study, we investigated whether chronic administration of fluvoxamine desensitizes 5-HT_{2C} receptors using a putative in vivo rat model of 5-HT_{2C} receptor function. Acute treatment with fluvoxamine or another SSRI, paroxetine, reduced spontaneous locomotion, as observed with the administration of *m*-chlorophenylpiperazine (mCPP). This effect of fluvoxamine was reversed by treatment with a selective 5-HT_{2C} receptor antagonist, SB 242084. On the other hand, chronic treatment with fluvoxamine or paroxetine inhibited mCPP-induced hypolocomotion, while they had no effects in control rats. In addition, chronic treatment with these drugs had no effects on the mCPP concentration in the rat brain. These results suggest that 5-HT_{2C} receptors are desensitized by chronic treatment with fluvoxamine, as well as paroxetine. Thus, the clinical efficacy of fluvoxamine on anxiety disorders might involve the normalization of the 5-HT_{2C} receptor function.

Keywords: Selective serotonin re-uptake inhibitor; Fluvoxamine; Paroxetine; Anxiety disorders; 5-HT_{2C} receptor; *m*-Chlorophenylpiperazine; Locomotor activity; Rat

1. Introduction

Recent clinical evidence suggests that the activation of 5-HT_{2C} receptors is directly related to stress and anxiety. *m*-Chlorophenylpiperazine (mCPP), a 5-HT_{2C} receptor agonist, has anxiogenic effects in healthy controls and in subjects with anxiety disorders, such as obsessive-compulsive disorder (OCD) and panic and social anxiety disorders, and some of these effects are inhibited by nonselective $5HT_2$ receptor antagonist (Zohar et al., 1987; Kahn et al., 1988a; Kahn and Wetzler, 1991; Pigott et al., 1991). Exposure to mCPP also increased the secretion of several stress-related neuropeptides and hormones, like adrenocorticotropic hormone (ACTH), cortisol and prolactin, and this effect was greater in subjects with anxiety disorders than in healthy controls (Kahn et al., 1988b; Kahn and Wetzler, 1991; Hollander et al., 1998). In subjects with anxiety disorders, significant correlation between anxiety and the cortisol response to mCPP was reported (Klaassen et al., 2002). In their study, ipsapirone, a 5-HT_{1A} agonist, also increased the secretion of cortisol, but no significant correlations could be established between the levels of anxiety and cortisol response to ipsapirone. These data suggest that subjects with anxiety disorders may exhibit a hypersensitization of $5HT_{2C}$ receptors. This hypothesis is supported by studies using rats, in which the administration of mCPP induced hypolocomotion and anxiogenic-like responses, such as reduced social interaction in pairs. These effects of mCPP were reversed by a highly selective 5-HT_{2C} antagonist, SB 242084 (Kennett et al., 1997; Bagdy et al., 2001; Gleason et al., 2001; Martin et al., 2002).

Recently, selective serotonin re-uptake inhibitors (SSRIs), including fluvoxamine, have been used to treat anxiety disorders (Kent et al., 1998; Masand and Gupta, 1999; Nutt et al., 1999). They are at least as effective as traditional anxiety medications, and they have a favorable side-effect profile in safety and tolerability. Thus, they are rapidly

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replacing older agents in the treatment of anxiety disorders. Several studies have reported the suppression of mCPP-induced responses in rats through chronic treatment with SSRIs. Studies of fluoxetine, paroxetine, sertraline and citalopram have shown that the repeated administration attenuates mCPP-induced hypolocomotion (Maj and Moryl, 1992; Kennedy et al., 1993; Kennett et al., 1994). Another study reported that chronic treatment with sertraline attenuated the decrease in social interaction induced by mCPP (Kennedy et al., 1993). These data suggest that the therapeutic effects of SSRIs in anxiety disorders involve the normalization of the 5-HT_{2C} receptor function.

In the present study, we investigated whether chronic treatment with fluvoxamine desensitizes 5-HT_{2C} receptors using an mCPP-induced hypolocomotion model as an index of $5HT_{2C}$ receptor function. Another SSRI, paroxetine, was also examined. For the purpose of confirming that the administration of SSRIs indirectly stimulates 5-HT_{2C} receptors, we examined the acute effects of fluvoxamine or paroxetine on spontaneous locomotion in rats. To date, no previous study has demonstrated the attenuation of mCPP-induced responses through chronic treatment with SSRIs showing indirect stimulation of 5-HT_{2C} receptors by acute treatment. Moreover, there has been no report on the chronic effects of fluvoxamine on mCPP-induced hypolocomotion. Thus, the results of the present study may be useful for confirming the desensitization of the 5-HT_{2C} receptor function through chronic treatment with fluvoxamine.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Japan) were housed in a humidity- and temperature-controlled room under a 12-h light/dark cycle (lights on 7:00 a.m.) in standard cages (37 × 25 × 18 cm), with free access to food and water for at least 1 week prior to use. Locomotor activity was measured using 8- or 9-week-old rats following acute or chronic administration of fluvoxamine or paroxetine. All studies were performed according to the guidelines of the Animal Care and Use Committee of the Pharmaceutical Research Center, Meiji Seika Kaisha.

2.2. Drugs

Fluvoxamine maleate (Meiji Seika, Tokyo) and paroxetine hydrochloride (extracted from Paxil tablets, SmithKline Beecham, Pharmaceutical Research Center, Meiji Seika) were given orally, in 1% (v/v) Tween-80 in distilled water, at 5 ml/kg. mCPP (Sigma-Aldrich, St. Louis, MO) was administered intraperitoneally in physiological saline at 1 ml/kg. SB 242084 (Sigma-Aldrich) was administered intraperitoneally, in 10% (w/v) hydroxypropyl-β-cyclodextrin, at 1 ml/kg.

2.3. Experimental protocol

2.3.1. Effect of mCPP on locomotor activity

The rats were acclimatized to handling for 3 days before the test. They were injected with mCPP (2, 4 and 8 mg/kg) or vehicle intraperitoneally and returned to their home cages ($n = 10$ each). Twenty minutes later, they were each placed in an automated locomotor activity box, and locomotion was recorded for 10 min. Locomotor activity was measured with an infrared sensor (NS-AS01, Neuroscience, Tokyo) placed over an open-top plastic box (25 × 30 × 17 cm). To investigate an antagonizing effect on 5-HT_{2C} receptors, SB 242084 (0.03, 0.1 and 0.3 mg/kg) or vehicle was injected intraperitoneally 30 min before the test.

2.3.2. Effect of acute treatment with fluvoxamine or paroxetine on locomotor activity

The rats were acclimatized to handling for 3 days before the test. They were given fluvoxamine (30 and 90 mg/kg) or paroxetine (10 mg/kg) per os and returned to their home cages ($n = 10$ each). One hour later, locomotor activity was measured as described above. To investigate the antagonizing effect on 5-HT_{2C} receptors, SB 242084 (0.3 mg/kg) was given intraperitoneally 30 min before the test.

2.3.3. Effect of chronic treatment with fluvoxamine or paroxetine on mCPP-induced hypolocomotion

Fluvoxamine (10, 30 and 90 mg/kg) or paroxetine (10 mg/kg) was given to rats per os between 9:00 and 12:00 a.m. daily for 21 days prior to the test ($n = 9–10$ each). To investigate the effect of a single administration, vehicle was given for 20 days, and then fluvoxamine (90 mg/kg) or paroxetine (10 mg/kg) was given on Day 21. Control animals were given vehicle only. The rats were injected intraperitoneally with mCPP (4 mg/kg) or vehicle on the day after their last treatment with SSRIs. Twenty minutes later, locomotor activity was measured as described above.

2.3.4. Effect of chronic treatment with fluvoxamine or paroxetine on the brain concentration of mCPP

To determine whether chronic treatment with fluvoxamine or paroxetine altered the brain concentration of mCPP, fluvoxamine (30 and 90 mg/kg) or paroxetine (10 mg/kg) was given to rats per os between 9:00 and 12:00 a.m. daily for 21 days ($n = 5$ each). On Day 22, the rats were injected intraperitoneally with mCPP (4 mg/kg) or vehicle. Thirty minutes later, whole rat brains were isolated and immediately frozen in liquid N_2 prior to storage at −80 °C. The brain concentration of mCPP was measured as described in a previous report (Kennett et al., 1994). The whole rat brains were homogenized in 10 vol. of buffer containing 0.4 M perchloric acid, 0.1% (w/v) sodium metabisulphite, 0.01% (w/v) EDTA and 0.1% (w/v) L-cysteine and *O*-tolyl piperazine, as an internal standard, and were centrifuged to remove protein. Supernatants

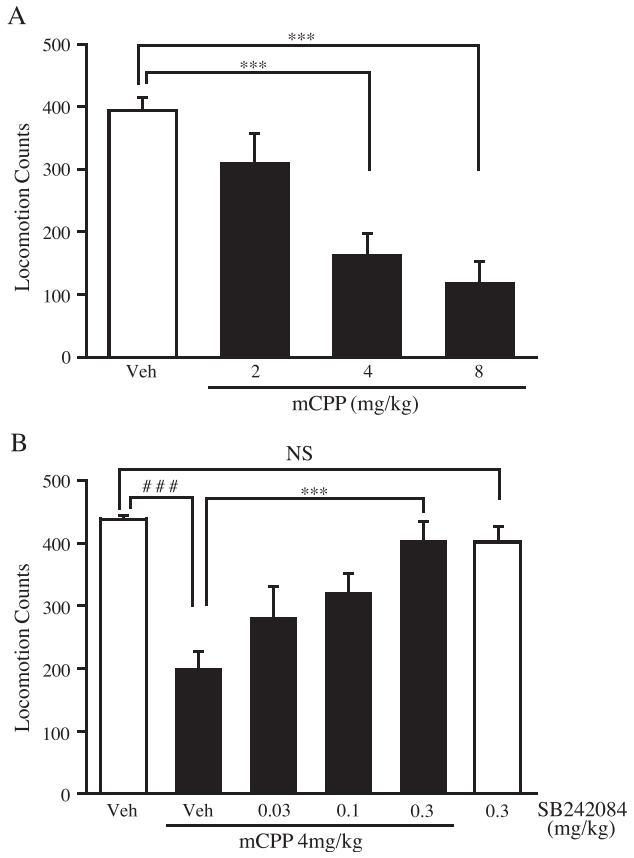

Fig. 1. (A) Effect of mCPP on spontaneous locomotion in rats. (B) Effect of SB 242084 on hypolocomotion induced by mCPP (4 mg/kg) in rats. The results are expressed as mean ± S.E.M. Each group consists of 10 rats. Veh: vehicle. * * * $P < .001$ by Dunnett's multiple comparison test; $^{###}P < .001$ by Student's t test. NS: no significant difference by Student's t test.

were neutralized with buffer containing 0.5 M potassium phosphate, 0.01% (w/v) EDTA and 0.1% (w/v) L-cysteine. These samples were applied to CN Bond Elut sorbent columns (100 mg) that had initially been conditioned with full-column volumes of methanol and water, respectively. Each column was washed with water and fully dried under vacuum. The compounds were eluted with methanol containing 2% (v/v) acetic acid. The eluates were evaporated to dryness under a N_2 stream. The samples were reconstituted in a mobile phase and made ready for injection into the HPLC. The samples were analyzed by an HPLC system with an Xterra RP18 column (150 × 4.6 mm i.d., 3.5 μm, Waters Corporation, Tokyo). The mobile phase consisted of 20% (v/v) acetonitrile and 80% (v/v) 50 mM ammonium acetate containing 0.1% (v/v) triethylamine, and the flow rate was 1 ml/min. The peak of mCPP was detected by UV absorbance at 254 nm.

2.4. Statistics

Data are presented as mean ± S.E.M. The data were analyzed using Dunnett's multiple comparison or Student's t tests.

3. Results

The administration of mCPP to rats resulted in a marked and dose-dependent reduction in locomotor activity (Fig. 1A). Pretreatment with SB 242084, intraperitoneally, 30 min before the test, dose dependently inhibited this effect of mCPP. Moreover, significant and complete attenuation was observed at the dose of 0.3 mg/kg. On the other hand, when SB 242084 (0.3 mg/kg) was given alone to rats, it had no effect on locomotor activity (Fig. 1B).

The acute administration (1 h before the test) of fluvoxamine (30 and 90 mg/kg) or paroxetine (10 mg/kg) reduced locomotor activity in rats, and statistically significant differences were observed with the administration of fluvoxamine at 90 mg/kg and of paroxetine (Fig. 2A). Pretreatment with SB 242084 (0.3 mg/kg), intraperitoneally, 30 min before the test, inhibited the effect of fluvoxamine (90 mg/kg; Fig. 2B).

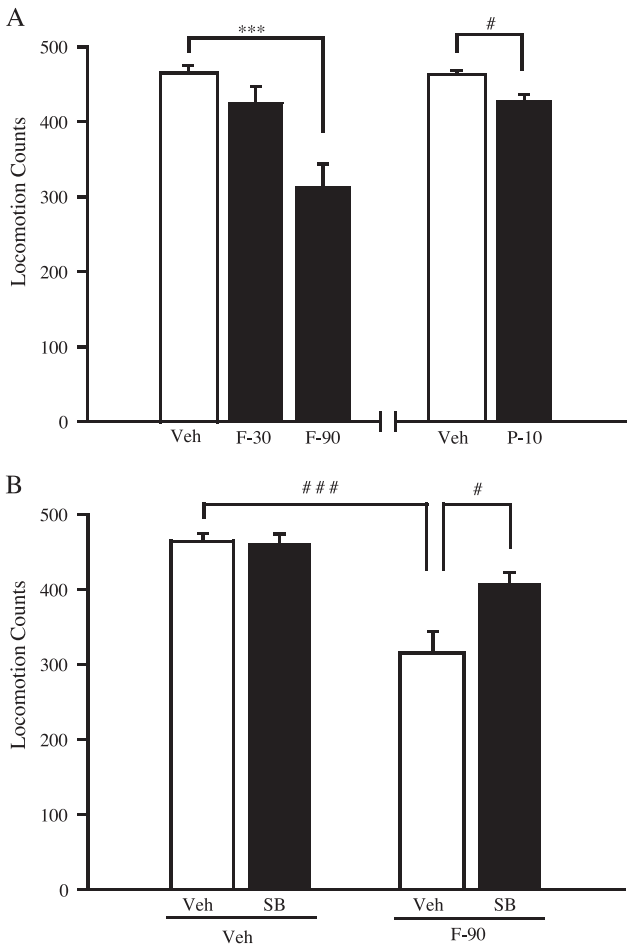

Fig. 2. (A) Acute effects of fluvoxamine and paroxetine on spontaneous locomotion in rats. (B) Effect of SB 242084 (0.3 mg/kg) on hypolocomotion induced by fluvoxamine in rats. The results are expressed as mean ± S.E.M. Each group consists of 10 rats. Veh: vehicle; F-30: fluvoxamine 30 mg/kg; F-90: fluvoxamine 90 mg/kg; P-10: paroxetine 10 mg/kg; and SB: SB 242084 0.3 mg/kg. * * * $P < .001$ by Dunnett's multiple comparison test; $^{#}P < .05$ and $^{###}P < .001$ by Student's t test.

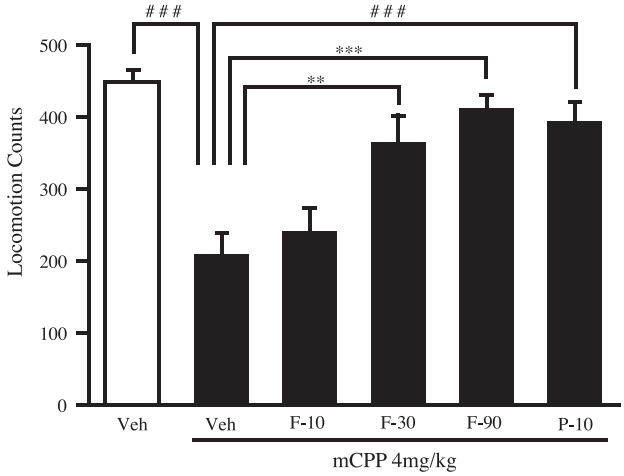

Fig. 3. Effect of repeated administration of fluvoxamine or paroxetine on hypolocomotion induced by mCPP (4 mg/kg) in rats. The results are expressed as mean ± S.E.M. Each group consists of 9–10 rats. Veh: vehicle; F-10: fluvoxamine 10 mg/kg; F-30: fluvoxamine 30 mg/kg; F-90: fluvoxamine 90 mg/kg; and P-10: paroxetine 10 mg/kg. $^{**}P<.01$ and $^{***}P<.001$ by Dunnett's multiple comparison test; $^{\#\#\#}P<.001$ by Student's *t* test.

mCPP-induced hypolocomotion in rats given chronic fluvoxamine at 30 or 90 mg/kg was significantly attenuated. Chronic treatment with paroxetine (10 mg/kg) also attenuated mCPP-induced hypolocomotion in rats (Fig. 3). Chronic treatment with fluvoxamine (90 mg/kg) or paroxetine (10 mg/kg) did not affect spontaneous locomotion in control rats (Table 1). Single administration of fluvoxamine (90 mg/kg) or paroxetine (10 mg/kg) had no effect on mCPP-induced hypolocomotion in rats (Table 2). Chronic treatment with either fluvoxamine (30 and 90 mg/kg) or paroxetine (10 mg/kg) had no effect on the brain levels of mCPP (Table 3).

4. Discussion

The affinity of mCPP for the rat 5-HT_{2C} receptor is only 5- to 15-fold greater than that for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_3 receptors, and mCPP has agonist/partial agonist activity at 5-HT_{1A}, 5HT_{1B}, 5-HT_{1D} and 5-HT_{2B} receptors (Kahn and Wetzler, 1991; Baxter et al., 1995). mCPP also binds strongly to α_2-adrenoceptors (Smith and Suckow, 1985). In the present study, the administration of mCPP to rats induced hypolocomotion, and this response was completely reversed by treatment with a selective 5-HT_{2C} receptor antagonist, SB 242084, as previously reported (Kennett et al., 1997; Bagdy et al., 2001; Gleason et al., 2001; Martin et al., 2002). While mCPP has some affinity for other 5-HT receptors and α_2-adrenoceptors, the present results indicate that mCPP-induced hypolocomotion is mediated through 5-HT_{2C} receptors. Moreover, the present study showed that the acute administration of fluvoxamine or paroxetine induced hypolocomotion in rats, as observed in the administration of mCPP, and this effect of fluvoxamine was also reversed by the treatment with SB 242084. Similar results were obtained in previous studies using citalopram, fluoxetine and sertraline (Dekeyne et al., 2000; Bagdy et al., 2001). Although fluoxetine has weak affinity for 5-HT_{2C} receptors, other SSRIs have little affinity for these receptors (Sánchez and Hyttel, 1999). Thus, these results indicate that the administration of SSRIs indirectly stimulates 5-HT_{2C} receptors.

In our chronic study, the repeated administration of fluvoxamine or paroxetine significantly attenuated mCPP-induced hypolocomotion. Previous studies have reported that chronic treatment with SSRIs decreased mCPP metabolism (Zohar et al., 1988; Hollander et al., 1991). However, in the present study, the brain concentration of mCPP was not affected by chronic treatment with fluvoxamine or paroxetine. Therefore, the effects on the response to mCPP are unlikely to reflect reduced concentration of mCPP in the

Table 1
Effect of repeated administration of fluvoxamine or paroxetine on spontaneous locomotion in rats

Group	Locomotion counts	
Vehicle	446.5 ± 19.5	–
Fluvoxamine, 90 mg/kg	441.9 ± 6.1	NS
Paroxetine, 10 mg/kg	457.1 ± 11.6	NS

The results are expressed as mean ± S.E.M. Each group consists of 10 rats. NS: no significant difference from the vehicle by Student's *t* test.

Table 2
Effect of a single administration of fluvoxamine or paroxetine on hypolocomotion induced by mCPP (4 mg/kg) in rats

Group	Locomotion counts	
Vehicle	446.5 ± 19.5	–
Vehicle + mCPP	209.3 ± 30.6	***
Fluvoxamine 90 mg/kg + mCPP	250.5 ± 47.4	NS
Paroxetine 10 mg/kg + mCPP	252.4 ± 33.5	NS

The results are expressed as mean ± S.E.M. Each group consists of 10 rats.
*** $P<.001$ vs. vehicle group. NS: no significant difference from the vehicle + mCPP group by Student's *t* test.

Table 3
Concentration of mCPP in the whole rat brain after intraperitoneal injection at 4 mg/kg on Day 22

Pretreatment (per os daily, × 21 days)	Concentration of mCPP (mg/g tissue)	
Vehicle	4.45 ± 0.59	–
Fluvoxamine, 30 mg/kg	3.85 ± 0.72	NS
Vehicle	5.32 ± 0.58	–
Fluvoxamine, 90 mg/kg	3.77 ± 0.67	NS
Vehicle	4.45 ± 0.59	–
Paroxetine, 10 mg/kg	5.72 ± 1.49	NS

The results are expressed as mean ± S.E.M. Each group consists of five rats. NS: no significant difference from the vehicle by Student's *t* test.

rat brain. On the other hand, in the control for the chronic study, a single administration of fluvoxamine or paroxetine had no effect on mCPP-induced hypolocomotion. These results indicate that only repeated treatment with SSRIs attenuated the mCPP-induced response. Paroxetine and other SSRIs, such as sertraline, fluoxetine and citalopram, have been reported to reduce mCPP-induced hypolocomotion with chronic treatment (Maj and Moryl, 1992; Kennedy et al., 1993; Kennett et al., 1994). Consequently, our data reproduced the previous data. Moreover, the present findings showed that repeated administration of fluvoxamine reduced mCPP-induced hypolocomotion in rats, as seen with other SSRIs.

Although chronic treatment with fluvoxamine at 30 mg/kg or paroxetine at 10 mg/kg suppressed mCPP-induced hypolocomotion dramatically, the acute administration of these SSRIs induced hypolocomotion only slightly at the same doses as chronic treatment. The acute administration of SSRIs elicits a large increase in the level of extracellular 5-HT in the vicinity of serotonergic cell bodies and dendrites in the midbrain raphe nucrei compared with the terminal areas (Bel and Artigas, 1992; Gardier et al., 1996; Tao et al., 2000). The excess of 5-HT outside the cell bodies stimulates somatodendritic 5-HT_{1A} autoreceptors in the midbrain raphe nuclei and reduces 5-HT cell firing. This action at the cell body is thought to dampen an increase in extracellular 5-HT levels in the terminal areas (Gartside et al., 1995; Artigas et al., 1996). The doses of fluvoxamine or paroxetine effective in our experiment (fluvoxamine 30 or 90 mg/kg po, paroxetine 10 mg/kg po) are considered to increase extracellular 5-HT levels in both the vicinity of the cell body and the terminal areas (Bosker et al., 1995; Gardier et al., 1996). However, the increase in extracellular 5-HT levels in the terminal areas through acute treatment might be smaller than that of chronic treatment. These phenomena may cause the lower efficacy in acute treatment in our study. On the other hand, chronic treatment with SSRIs may desensitize somatodendritic 5-HT_{1A} autoreceptors and elicit marked and persistent increases of 5-HT in the synaptic cleft (Bel and Artigas, 1993; Roberts et al., 2000). Thus, in this study, chronic treatment with fluvoxamine or paroxetine might cause the desensitization of 5-HT_{2C} receptors through the continuous stimulation of these receptors and reduce the response to mCPP. This hypothesis is supported by a previous report, in which cotreatment with a $5HT_{1A}$ antagonist and fluoxetine accelerated the desensitization of 5-HT_{2C} receptors (Bristow et al., 2000).

In clinical studies, mCPP has worsened the symptoms in anxiety disorders. The administration of mCPP exacerbated the symptoms in patients suffering from OCD, and this effect was prevented by pretreatment with the nonselective 5-$HT_{1/2}$ receptor antagonist metergoline (Zohar et al., 1987; Kahn and Wetzler, 1991; Pigott et al., 1991). In subjects with panic disorder, mCPP induced a greater degree of anxiety and panic attacks than in healthy controls (Kahn et al., 1988a). Based on this clinical evidence, the hypersensitivity of 5-HT_{2C} receptors may occur in anxiety disorders. Chronic treatment with clomipramine and fluoxetine diminished the behavioral sensitivity to mCPP in subjects with OCD (Zohar et al., 1988; Hollander et al., 1991). The increase in anxiety or the exacerbation of the symptoms in anxiety disorders also occurred early in the treatment with SSRIs, but these symptoms recovered by chronic treatment with SSRIs (Masand and Gupta, 1999; Nutt et al., 1999). These clinical data are consistent with the present findings that chronic treatment with fluvoxamine or paroxetine reduced the effect of mCPP, while acute treatment with these SSRIs induced the same response as mCPP did. Consequently, SSRIs may have therapeutic effects on anxiety disorders through the normalization of the 5-HT_{2C} receptor function.

The attenuated response after the chronic treatment with fluvoxamine may be brought about by the reduction in the number or the function of 5-HT_{2C} receptors. However, chronic treatment with fluvoxamine did not alter the number of 5-HT_2 binding sites in the rat brain (Ishikane et al., 1994). Similar results were obtained in other studies using citalopram (Arnt et al., 1984) and fluoxetine (Goodnough and Baker, 1994). Thus, chronic administration of SSRIs may not reduce the number of 5-HT_{2C} receptors, but rather, may desensitize their function. 5-HT_{2C} receptors are coupled to G protein, and the stimulation with an agonist induces the activation of phospholipase C, leading to the liberation of Ca^{2+} from the intracellular stores (Julius et al., 1988). The desensitization of 5-HT_2-mediated phosphoinositide hydrolysis through chronic treatment with sertraline has been previously reported (Sanders-Bush et al., 1989). Several recent studies have indicated that the 5-HT_{2C} receptor has been the only G-protein-coupled receptor known to undergo RNA editing. Pre-mRNA editing of 5-HT_{2C} receptors occurs in a region involved in G-protein-coupling and decreases both G-protein-coupling activity and the agonist potency in stimulation for G protein coupling (Sanders-Bush et al., 2003). Four days of treatment with the 5-$HT_{2A/2C}$ agonist (±)-1-(4-iodo-2, 5-dimethoxyphenyl)-2-aminopropane (DOI) significantly increased the editing of 5-HT_{2C} pre-mRNA and led to the increased expression of receptor isoforms that have low efficacy to activate G protein (Gurevich et al., 2002). As a consequence, the repeated administration of SSRIs may cause the RNA editing of 5-HT_{2C} receptors and decrease signaling in the certain brain regions. Further studies are required to elucidate the mechanisms underlying the attenuated response to mCPP after the chronic administration of SSRIs.

In conclusion, this study provides evidence that chronic treatment with fluvoxamine reduced response to mCPP through the desensitization of 5-HT_{2C} receptor function, just like the other SSRIs that were previously reported. The hypersensitization of 5-HT_{2C} receptors has been observed in subjects with anxiety disorders. Thus, our results suggest that fluvoxamine has clinical effects on

anxiety disorders through the normalization of the 5-HT_{2C} receptor function.

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