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Chronic Citalopram and Fluoxetine Treatments Upregulate 5-HT_{2C} Receptors in the Rat Choroid Plexus

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The effects of chronic (for 14 days) citalopram and fluoxetine treatments with three doses (2.5, 10, and 20 mg/kg) and withdrawal times (24 hours, 68 hours, and 14 days) on 5-HT_{2C} (formerly 5-HT_{1C}) receptors in the rat brain choroid plexus were studied with quantitative receptor autoradiography in two separate experiments. Chronic citalopram treatment caused a consistent and dose-related increase in the density of 5-HT_{2C} receptors (up to 90%). This effect was slightly more pronounced when measured with an antagonist ligand ([^{3}H]mesulergine) than with an agonist ligand [(±)-1-(2,5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I]DOI)]. The upregulation was most evident 24 hours after the last dose and disappeared thereafter rather rapidly. Chronic fluoxetine treatment also increased the density of 5-HT_{2C} receptors 24 hours from the last dose, but the increase was accompanied by a reduced affinity and was less marked than that observed with citalopram. The changes in receptor characteristics were not observed consistently after the 68-hour withdrawal from fluoxetine. Furthermore, the upregulation of fluoxetine appeared not to be dose related or reflected by an increase in agonist binding. In conclusion, the results show that chronic citalopram and fluoxetine treatments induce an increase of choroid plexus 5-HT_{2C} receptor density, but the effect is more marked with citalopram. These differences in the regulation of the 5-HT_{2C} receptors may lead to pharmacodynamic differences between chronic citalopram and fluoxetine treatments. ***[Neuropsychopharmacology 15:143–151, 1996]***

KEY WORDS: *5-HT_{2C} receptor; Fluoxetine; Citalopram; Serotonin; Choroid plexus; Rat*

Selective serotonin reuptake inhibitors (SSRIs) are a class of clinically effective antidepressants that inhibit the uptake of serotonin (5-hydroxytryptamine, 5-HT) with a much higher specificity than the older tricyclic antidepressants and whose usage for depression and other psychiatric disorders is growing because of a favorable side effect profile. Their mechanism of action probably lies in their ability to block the uptake of 5-HT to neuronal synaptosomes and glial cells and thus to increase the amount of extracellular 5-HT in the synaptic cleft. Because the time needed for the clinical state of a depressive patient to improve is generally several weeks from the beginning of the uptake blockade, general opinion assumes that the therapeutic action of these drugs is the result of adaptive changes in the central nervous system, which take place only after a long-term treatment. Indeed, chronic treatment with SSRIs has been reported to modulate several functional parameters in the brain, including changes in the brain content of 5-HT and its main metabolite 5-hydroxyindole acetic acid (5-HIAA), 5-HT synthesis, density and function of several neurotransmitter receptors, electrical neuronal activity (for reviews, see Johnson 1991; Beasley et al.

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1992) and the extracellular concentration of 5-HT as detected by *in vivo* microdialysis (for review, see Fuller 1994).

The 5-HT_{2C} receptor belongs to the 5-HT_2 receptor family, which consists of three known G-protein-coupled receptors that regulate the phospholipase C-coupled phosphoinositide hydrolysis (Humphrey et al. 1993; Hoyer et al. 1994). 5-HT_{2C} receptors have been suggested to play a role in many emotional, behavioral, and neuroendocrine responses (Hoyer et al. 1994). The effects of several antidepressive treatments, including SSRIs, on the density and function of 5-HT_{2C} receptors have been studied to some extent with various radioligand binding, molecular biological, and physiological response studies. Chronic fluoxetine, citalopram, paroxetine, and sertraline treatments are suggested to attenuate certain putative 5-HT_{2C} receptor-mediated behavioral responses (Maj and Moryl 1992, 1993; Kennett et al. 1994). Chronic fluoxetine treatment has also been reported to potentiate neuroendocrine responses proposedly mediated by 5-HT_{2C} receptors (Li et al. 1993). Chronic treatments with citalopram and fluvoxamine, in turn, had no effect on the levels of 5-HT_{2C} receptor mRNA in the rat brain in the study by Spurlock et al. (1994). However, there are no published studies on radioligand binding concerning the effects of either chronic or even acute treatment with SSRIs on 5-HT_{2C} receptor binding sites that would have used binding conditions selective for the 5-HT_{2C} receptor subtype. In this study, we investigated the effects of chronic treatment with two widely used SSRIs, citalopram and fluoxetine, on the density and affinity of the 5-HT_{2C} receptor subtype using the rat brain choroid plexus as a model.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing between 200 and 270 g at the beginning of the study were used. Rats were housed in groups of three under standard laboratory conditions (temperature 21°C, humidity 55 ± 5%, lights on from 6:00 A.M. to 6:00 P.M.). Standard pelleted food and tap water were available *ad libitum*.

Drug and Treatments

Citalopram hydrobromide was a gift from Dr. J. Arnt, Lundbeck, Copenhagen, Denmark; fluoxetine hydrochloride was a gift from Orion, Helsinki, Finland. All drugs were dissolved in 0.9% saline containing a few drops of Tween 20. Tween 20 was also added in the saline received by the control group. All doses refer to the free base of a drug.

Experiment 1. Seven groups of rats (n = 6 per treatment group) received subcutaneous injections of various doses of citalopram (2.5, 10, or 20 mg/kg) or fluoxetine (2.5, 10, or 20 mg/kg) or an equal volume of saline (1 ml/kg) once a day for 14 days. 68 hours after the last injection, rats were decapitated and brains were dissected.

Experiment 2. Three groups of rats (n = 17 to 18 per treatment group) received subcutaneous injections of citalopram (10 mg/kg), fluoxetine (10 mg/kg), or an equal volume of saline (1 ml/kg) once a day for 14 days. All treatment groups were further divided into three subgroups (n = 5 to 6 per subgroup) to study the effects of various withdrawal times. Rats in different subgroups were then decapitated 24 hours, 68 hours, or 14 days after the last injection and brains were dissected.

Autoradiographic Procedures

12-μm-thick coronal sections containing choroid plexi from the lateral ventricles [between levels A 6860-6570 μ according to König and Klippel (1963)] were cut on a cryostat microtome and thaw-mounted on to gelatin-coated slides for radioligand binding studies. Thoroughly dried slides were stored at −80°C until used for receptor autoradiography.

5-HT_{2C} Receptor Autoradiography with [3H]Mesulergine. 5-HT_{2C} receptor autoradiography with [^{3}H]mesulergine was performed as previously described (Kuoppamäki et al. 1994). Slide-mounted tissue sections were preincubated for 15 minute at room temperature in 170 mM Tris-HCl buffer (pH 7.4) and allowed to dry for 1 hour. Thereafter, the sections were drop-incubated for 2 hours at room temperature with 100 μl of Tris-HCl buffer containing 5 nM [^{3}H]mesulergine (78 Ci/mmol, Amersham, Little Chalfont, UK). 100 nM spiperone was used to prevent binding to 5-HT_{2A} sites. Nonspecific binding was determined by incubating the adjacent sections in the presence of 5 μM methysergide. The sections were then washed twice for 10 minutes in ice-cold buffer, dipped in ice-cold deionized water to remove salts, and dried thoroughly before being apposed to film. To measure possible variations in K_d values of 5-HT_{2C} receptor populations between different treatment groups, a Scatchard analysis was performed on tissue sections obtained from Experiment 2. Conditions identical to those described were used, except that five different [^{3}H]mesulergine concentrations (0.5, 1, 2, 3, and 5 nM) were used and tissue sections were incubated in a large volume of buffer. One example of a typical Scatchard plot obtained from a rat in the control group is presented in Figure 1.

5-HT_{2C} Receptor Autoradiography with [^{125}I]DOI. The agonist radioligand (±)-1-(2,5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane ([^{125}I]DOI) was used to measure the agonist binding of choroid plexus tissue sections ob-

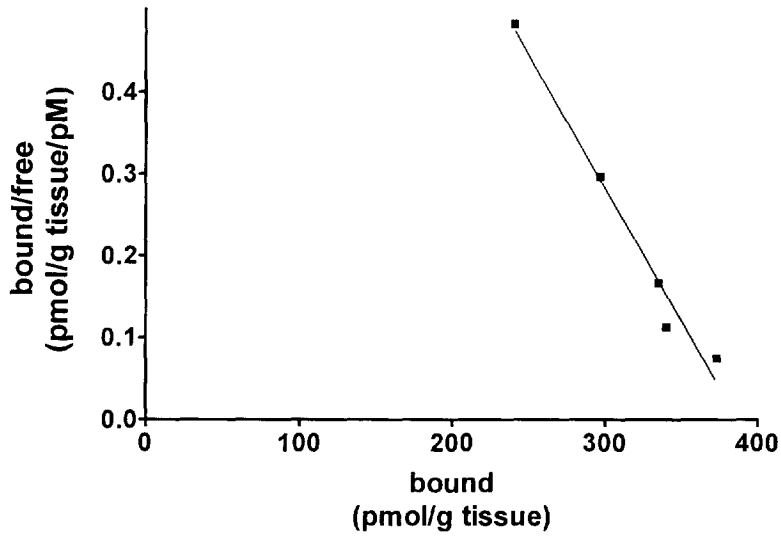

Figure 1. Scatchard analysis of [^{3}H]mesulergine binding to choroid plexus 5-HT$_{2C}$ receptors in one rat in control treatment group, decapitated 24 hours after withdrawal. Concentrations of [^{3}H]mesulergine used varied from 0.5 to 5 nM. B_{max}, and K_d values were 388 pmol/g tissue and 310 pM, respectively, for this specimen. Nonspecific binding was below detection level with all [^{3}H]mesulergine concentrations.

tained from Experiment 2. 5-HT$_{2C}$ receptor autoradiography with [^{125}I]DOI was done as previously described (Kuoppamäki et al. 1994), with some modifications. Slide-mounted tissue sections were preincubated for 10 minutes at room temperature in 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgSO_4$ and allowed to dry for 30 minutes. Thereafter, the sections were drop-incubuated for 1 hour at room temperature with 100 µl of Tris-HCl buffer containing a saturating concentration (5 nM) of [^{125}I]DOI [original activity 2200 Ci/mmol, (New England Nuclear, Boston, MA), diluted to the specific activity of 240 Ci/mmol with nonradioactive DOI (Research Biochemicals International, Natick, MA)]. 100 nM spiperone was used to prevent binding to 5-HT$_{2A}$ sites. Nonspecific binding was determined by incubating adjacent sections in the presence of 5 µM methysergide. The sections were then washed three times for 20 minutes in ice-cold buffer, dipped in ice-cold deionized water to remove salts, and dried overnight at room temperature before being apposed to film.

Autoradiography. All slides were dried throughly and apposed to Kodak XAR-5 X-ray films in Sigma X-ray film cassettes together with plastic ^{3}H or ^{14}C standards (American Radiolabelled Chemicals, St. Louis, MO). Films were exposed for 32 or 4 days for [^{3}H]mesulergine and [^{125}I]DOI autoradiograms, respectively, at +4°C. Films were developed with an automated Fuji RG II X-ray film processor. For each autoradiogram, all sections from all treatment groups with similar withdrawal times were apposed to the same sheet of film.

Image Analysis. Images were initially digitized using a CCD camera (Hamamatsu C3077, Hamamatsu Photonics K.K., Hamamatsu City, Japan) into an array of 640 × 480 pixels, each with a gray value in the range of 0 to 255. Shading correction was applied to compensate for variations in illuminations and light transmission through the optical system. Images were analyzed with a computerized image analysis system (MCID, M4 1.12, Imaging Research Inc., St. Catharines, Ontario, Canada). A standard curve was generated by measuring and plotting the optical densities of the standards versus their radioactivity. Areas of interest were traced with a mouse-controlled cursor and the radioactivity interpolated. All measurements were done on both hemispheres and on two consecutive sections of each brain. The results, given as nCi/mg by the measuring device, were further converted into pmol ligand bound per gram.

The ^{3}H autoabsorption (quenching) of the choroid plexus tissue is minimal (Geary and Wooten 1985). To avoid overestimation of the number of 5-HT$_{2C}$ sites labeled by [^{3}H]mesulergine, a conversion method described by Kuoppamäki et al. (1994) was used.

Statistical Analysis. Statistical analysis of the data was carried out by one-way analysis of variance (ANOVA) followed by Tukey's or Newman-Keul's test for post hoc analyses. Commercially available statistical software (Systat, Evanston, IL) was used for this purpose. A p value lower than .05 was considered statistically significant. Scatchard analysis was performed by plotting specific binding values for different radioligand concentrations ("bound") against specific binding values divided by free radioligand concentrations ("bound/free"). Curve fitting and linear regression analysis were done using commercial software (GraphPad, San Diego, CA).

RESULTS

Effects of Chronic Treatment with Citalopram and Fluoxetine on 5-HT$_{2C}$ Receptor Binding Sites Labeled with an Antagonist Radioligand [^{3}H]Mesulergine in the Rat Choroid Plexus

In experiment 1, we studied the effects of chronic (14 days) citalopram and fluoxetine treatments with several

Table 1. Effects of Chronic Citalopram and Fluoxetine Treatments with Various Doses on the Binding of 5 nM [^{3}H]Mesulergine to 5-HT$_{2C}$ Receptors in the Rat Choroid Plexus, as Studied 68 Hours after the Last Injection

Treatment	Specific Binding (pmol/g)
Control	258 ± 20
Citalopram	
2.5 mg/kg/day	291 ± 15
10 mg/kg/day	338 ± 24[b]
20 mg/kg/day	504 ± 95[c]
Fluoxetine	
2.5 mg/kg/day	275 ± 14
10 mg/kg/day	313 ± 22[a]
20 mg/kg/day	278 ± 17

Values represent mean ± SD of 6 rats. Significantly different from control groups: [a] $p < .05$; [b] $p < .005$; [c] $p < .001$.

drug doses (2.5, 10, and 20 mg/kg) on 5-HT$_{2C}$ receptor binding sites labeled by [^{3}H]mesulergine. Results of this experiment are summarized in Table 1. Chronic citalopram treatment increased antagonist binding to 5-HT$_{2C}$ receptors in the rat choroid plexus in a dose-related manner. [^{3}H]Mesulergine binding was increased significantly by 31% and 95% after chronic citalopram treatment with doses of 10 and 20 mg/kg, respectively, as studied after a 68-hour withdrawal. 2.5 mg/kg of citalopram did not cause a significant upregulation. Chronic fluoxetine treatment with the dose of 10 mg/kg caused a significant upregulation of 5-HT$_{2C}$ receptor antagonist binding sites by 21%, but fluoxetine at the doses of 2.5 and 20 mg/kg was without an effect.

In Experiment 2, rats were treated chronically (for 14 days) with 10 mg/kg of drug, and the state of 5-HT$_{2C}$ receptor regulation at the different withdrawal time points was studied. Animals were killed 24 hours, 68 hours, or 14 days after the last injection. In addition, the antagonist binding properties of the rat choroid plexus 5-HT$_{2C}$ receptors were studied using several [^{3}H]mesulergine concentrations to allow a Scatchard analysis to be performed. Thus, in addition to 5-HT$_{2C}$ receptor density (B_{max} value of the Scatchard analysis), possible changes in the affinity (K_d value of the Scatchard analysis) of 5-HT$_{2C}$ receptors for the antagonist radioligand [^{3}H]mesulergine could be estimated. Results of this experiment are summarized in Table 2. Chronic treatment with 10 mg/kg of citalopram caused a significant increase of 66% and 19% in B_{max} values of 5-HT$_{2C}$ receptors as studied 24 and 68 hours after the last injection, respectively. After 14 days of withdrawal, the upregulation was no longer significant. These changes were not accompanied with any significant alterations in respective K_d values. Chronic fluoxetine treatment with the dose of 10 mg/kg caused an increase of 35% in 5-HT$_{2C}$ B_{max} values as studied 24 hours after the last injection, but was without an effect after longer withdrawal times. In addition, a significant increase in the K_d value of 5-HT$_{2C}$ receptors in the fluoxetine treatment group decapitated 24 hours after the last injection was found. Other treatment groups had no significant alterations in the K_d values of 5-HT$_{2C}$ receptors.

Effects of Chronic Treatment with Citalopram and Fluoxetine on 5-HT$_{2C}$ Receptor Binding Sites Labeled with an Agonist Radioligand [^{125}I]DOI in the Rat Choroid Plexus

To find out whether chronic citalopram and fluoxetine treatments would modulate agonist and antagonist binding properties of the rat choroid plexus 5-HT$_{2C}$ receptors differentially, we performed quantitative receptor autoradiography using an agonist radioligand [^{125}I]DOI. Results of this experiment are summarized in Table 3. Chronic treatment (for 14 days) with 10 mg/kg

Table 2. Effects of Chronic Citalopram and Fluoxetine Treatments with Various Withdrawal Times on the Binding of [^{3}H]Mesulergine to 5-HT$_{2C}$ Receptors in the Rat Choroid Plexus

		Scatchard Analysis	
Withdrawal	Treatment	B_{max} (pmol/g)	K_d (pM)
24 Hours	Control	394 ± 37	321 ± 49
	Citalopram (10 mg/kg/day)	653 ± 74[c]	376 ± 75
	Fluoxetine (10 mg/kg/day)	518 ± 58[b]	602 ± 122[c]
68 Hours	Control	459 ± 16	355 ± 86
	Citalopram (10 mg/kg/day)	544 ± 75[a]	356 ± 53
	Fluoxetine (10 mg/kg/day)	474 ± 57	324 ± 53
14 Days	Control	421 ± 36	222 ± 45
	Citalopram (10 mg/kg/day)	474 ± 45	310 ± 48
	Fluoxetine (10 mg/kg/day)	429 ± 35	327 ± 102

Values represent mean ± SD of 5 to 6 rats. Significantly different from control groups: [a] $p < .05$; [b] $p < .01$; [c] $p < .001$.

Table 3. Effects of Chronic Citalopram and Fluoxetine Treatments with Various Withdrawal Times on the Binding of 5 nM [^{125}I]DOI to 5-HT$_{2C}$ Receptors in the Rat Choroid Plexus

Withdrawal	Treatment	Specific Binding (pmol/g)
24 Hours	Control	11.3 ± 0.73
	Citalopram (10 mg/kg/day)	16.5 ± 1.70[a]
	Fluoxetine (10 mg/kg/day)	11.2 ± 1.60
68 Hours	Control	11.5 ± 1.32
	Citalopram (10 mg/kg/day)	13.3 ± 1.03
	Fluoxetine (10 mg/kg/day)	12.2 ± 1.63
14 Days	Control	10.4 ± 1.20
	Citalopram (10 mg/kg/day)	11.1 ± 1.45
	Fluoxetine (10 mg/kg/day)	11.7 ± 0.80

Values represent means ± SD of 5 to 6 rats. Significantly different from control groups: [a] $p < .001$.

of citalopram caused a significant upregulation of 46% of 5-HT$_{2C}$ receptor agonist binding sites as studied 24 hours after the last injection. No significant change was found after longer withdrawal times. Chronic fluoxetine treatment with the dose of 10 mg/kg did not cause any significant changes in the agonist binding properties of 5-HT$_{2C}$ receptors in the rat choroid plexus.

DISCUSSION

There is increasing evidence that 5-HT$_{2C}$ receptors may play a role in the actions of many psychoactive agents, including hallucinogenic drugs (Sanders-Bush and Breeding 1991), some antipsychotics (Hietala et al. 1992; Roth et al. 1992; Canton et al. 1990), and some antidepressants (Jenck et al. 1993). Therefore, we wanted to investigate how a chronic treatment with the most widely used SSRI, fluoxetine, and the most selective SSRI marketed, citalopram, would modulate the density and affinity of these receptors in the brain (Hyttel et al. 1984). The choroid plexus tissue was used as a model because of its high expression of 5-HT$_{2C}$ receptors. In two separate studies, chronic citalopram treatment caused a significant upregulation of 5-HT$_{2C}$ receptor antagonist binding sites. This effect was also shown to be dose-dependent, and the density of these binding sites increased nearly twofold with the citalopram dose of 20 mg/kg. This increase also disappeared quite rapidly. According to the Scatchard analysis, this upregulation was not accompanied with change in receptor affinity. Since 5-HT$_{2C}$ receptors exist in two agonist affinity states that cannot be distinguished using the antagonist [^{3}H]mesulergine (Havlik and Peroutka 1992; Leonhardt et al. 1992), we performed autoradiography using the agonist radioligand [^{125}I]DOI, which selectively labels the guanyl nucleotide-sensitive high agonist affinity state of this receptor (Appel et al. 1990; Leonhardt et al. 1992). Receptors in the high agonist affinity state were regulated much in the same manner as the antagonist binding sites after chronic citalopram treatment. The effects of chronic fluoxetine treatments on the 5-HT$_{2C}$ receptors were less marked than those of citalopram. Fluoxetine treatment with the dose of 10 mg/kg was shown to cause an upregulation of the 5-HT$_{2C}$ antagonist binding sites, and this effect also disappeared rapidly. This increase was also accompanied with a significant reduction in the affinity of these receptors for the antagonist [^{3}H]mesulergine, the K_d value being significantly higher in the fluoxetine treatment group than in the control group. However, in contrast to citalopram, treatment with the fluoxetine dose of 20 mg/kg seemed to have no effect, and the number of high agonist affinity sites of the 5-HT$_{2C}$ receptor was not increased either.

No other published reports on the modulation of 5-HT$_{2C}$ receptor density by SSRI treatment exist. Spurlock et al. (1994) did not find any changes in 5-HT$_{2C}$ receptor mRNA levels in the rat brain after chronic citalopram and fluvoxamine treatments (although region-specific changes may not have been detected because of the use of whole-brain homogenates). However, Lesch et al. (1992) found an increase in 5-HT$_{2C}$ receptor mRNA levels in the rat neostriatum after chronic treatment with clomipramine, a tricyclic serotonin reuptake inhibitor. They also found a trend toward increase after chronic treatment with a low dose (2.5 mg/kg/day) of fluoxetine. In addition, transcriptional changes are not the only mechanism for receptor upregulation, as many translational and posttranslational factors may also be able to affect 5-HT$_{2C}$ receptor levels (for review, see Roth et al. 1990). Results from behavioral tests with putative 5-HT$_{2C/2B}$ agonist m-CPP (Curzon and Kennett 1990) suggest that chronic SSRI treatment may attenuate the function of these receptors (Maj and Moryl 1992, 1993; Kennett et al. 1994). In contrast, Li et al. (1993) reported that chronic fluoxetine treatment enhanced neuroendocrine responses to DOI, a 5-HT$_{2C/2A}$ agonist. Fluoxetine given acutely seems to have characteristics of an indirect 5-HT$_{2C}$ agonist in some behavioral test paradigms, and tolerance to this action may develop during chronic treatment (Cesana et al. 1993; Berendsen and Broekkamp 1994), suggesting desensitization of these receptors. However, the 5-HT$_{2C}$ receptor specificity of the agents used in these tests is questionable and makes definite conclusions difficult.

Contrary to the general presumption that receptors are downregulated after agonist and upregulated after antagonist treatments, 5-HT$_{2C}$ receptors have been shown to be downregulated after both antagonist and agonist treatments (Sanders-Bush and Breeding 1988, 1990; Barker and Sanders-Bush 1993; Pranzatelli et al. 1993). However, serotonergic denervation has been shown to upregulate both the function (Conn et al.

1987) and density (Rocha et al. 1993) of 5-HT_{2C} receptors in rat brain. The mechanism by which these two SSRIs cause an upregulation of 5-HT_{2C} receptors in the rat choroid plexus is not clear. Because the density of these receptors is known to increase after serotonergic denervation, it could be suggested that continued administration of SSRIs would decrease the amount of serotonin available to stimulate these receptors.

Trouvin et al. (1993) studied *ex vivo* 5-HT levels of rat brain homogenates after chronic fluoxetine treatment and found marked decreases in 5-HT levels in all brain regions studied. However, several studies investigating the effects of SSRI treatment on serotonergic neurotransmission suggest that serotonergic activity is increased during SSRI treatment. *In vivo* microdialysis studies have shown that acute SSRI administration increases extracellular 5-HT levels in several brain areas (reviewed by Fuller 1994). The increase in serotonergic neurotransmission has been suggested to enhance even more during chronic treatment with SSRIs because of the desensitization of somatodendritic 5-HT_{1A} and terminal 5-HT_{1B} autoreceptors. Most studies concerning the chronic effects of these drugs are, however, electrophysiological (Chaput et al. 1986; Blier et al. 1988; de Montigny et al. 1990; Chaput et al. 1991). *In vivo* microdialysis studies have shown that chronic SSRI treatment may regulate extracellular 5-HT levels differently in various brain areas. Available data are not conclusive about how basal 5-HT levels are regulated after chronic exposure to SSRIs or how electrophysiologically observed autoreceptor desensitization affects actual transmitter release. Invernizzi et al. (1994) and Rutter et al. (1994) reported that chronic citalopram and fluoxetine treatments, respectively, reduced the response to 8-OH-DPAT, a 5-HT_{1A} autoreceptor agonist, and thus resulted in facilitation of serotonergic neurotransmission. In contrast, Hjorth and Auerbach (1994) did not detect significant desensitization after chronic citalopram treatment. Invernizzi et al. (1994) and Hjorth and Auerbach (1994) did not find any differences in basal 5-HT levels in frontal cortex, dorsal hippocampus, or dorsal raphe between citalopram and saline groups, and Rutter et al. (1994) reported sixfold differences in basal 5-HT levels in diencephalon between fluoxetine and saline treatment groups. Bel and Artigas (1993) reported that chronic fluvoxamine treatment increased basal 5-HT in frontal cortex sixfold, but was without effect in raphe nuclei. Even though a decrease in 5-HT levels stimulating 5-HT_{2C} receptors in choroid plexus seems not likely, it is a possibility that cannot be excluded. In any case, the upregulation of the 5-HT_{2C} receptors caused by these SSRIs *resembles* very much of that observed after the serotonergic denervation, and more extensive studies are needed to find out the precise mechanism.

It is not clear whether choroid plexus 5-HT_{2C} receptors are "postsynaptic" in a classical sense and thus under the influence of 5-HT released by neuronal activity. It is not known for sure whether choroid plexus receives serotonergic innervation (Napoleone et al. 1982; Moskowitz et al. 1979; Aghajanian and Gallagher 1975; Lidov et al. 1980; Steinbusch 1981). The 5-HT_{2C} receptors in the choroid plexus may be stimulated just by the 5-HT released into cerebrospinal fluid (CSF) by serotonergic supraependymal fibers (Aghajanian and Gallagher 1975; Chan-Palay 1976; Moore et al. 1978; Ternaux et al. 1976, 1977; Nilsson et al. 1992). Thus, one possible explanation for our results would be that the 5-HT content of CSF would be regulated differently from the neuronal 5-HT release in other brain areas after the chronic SSRI treatment, and the observed upregulation of the 5-HT_{2C} receptors would be due to decreased levels of 5-HT in the CSF. However, Salter et al. (1995) have reported that acute fluoxetine treatment (10 mg/kg) increases 5-HT levels in CSF fivefold. The effects of chronic SSRI treatment are not known. If choroid plexus receives serotonergic innervation, one cannot exclude the possibility that this innervation would be regulated differently from the innervation to brain regions studied so far. Possible receptor density changes in these areas are currently being explored in our laboratory.

The effects of citalopram and fluoxetine appeared different. This difference may be explained by 5-HT_{2C} receptor occupancy, as suggested by reduced affinity of the 5-HT_{2C} receptors for [^{3}H]mesulergine in the fluoxetine treatment group 24 hours after the last injection. One explanation is that in rats treated with fluoxetine, but not with citalopram (because of the shorter elimination half-life of citalopram, see later), the concentration of extracellular 5-HT is still increased 24 hours after the last injection, and the decreased affinity of 5-HT_{2C} receptors would be due to endogenous 5-HT bound to these receptors. This possibility is supported by observations (Rutter and Auerbach 1993) according to which the extracellular levels of 5-HT are increased 24 hours after single fluoxetine or sertraline (10 mg/kg) injection. Another possibility is a direct interaction between fluoxetine and the 5-HT_{2C} receptors. According to Wong et al. (1991, 1993), especially R-enantiomers of fluoxetine and its active primary metabolite, norfluoxetine, have nanomolar affinities for the bovine 5-HT_{2C} receptor, inhibiting the binding of [^{3}H]mesulergine to the bovine choroid plexi, with K_i values of 155 and 180 nM for R-fluoxetine and R-norfluoxetine, respectively. In contrast, citalopram has markedly lower affinity for this receptor in bovine and pig choroid plexi, inhibiting the [^{3}H]mesulergine binding to the bovine choroid plexi, with a K_i value of 3.3 μM (Wong et al. 1991) and to pig choroid plexi, with a K_i value of 3.9 μM (Jenck et al. 1993). Another factor supporting this explanation concerns the pharmacokinetic differences between the two drugs. According to Hyttel et al. (1984), brain concen-

trations of citalopram and its demethylated metabolite are negligible 24 hours after chronic treatment with 40 mg/kg (p.o.) of citalopram. Thus, it does not accumulate significantly even during chronic administration. In contrast, considerable amounts of R-norfluoxetine (about 5 μmol/kg) are found in the rat brain 24 hours after a single 10 mg/kg injection of fluoxetine (Torok-Both et al. 1992). Therefore, it is not unreasonable to assume that fluoxetine treatment indeed does cause a similar upregulation to that caused by citalopram, but this effect is masked by the occupancy of the 5-HT_{2C} receptors by residual drug, namely, R-norfluoxetine, in choroid plexus tissue. This would be the first time when a direct *in vivo* interaction between a SSRI and a receptor other than the 5-HT transporter is reported. Because of the kinetic properties of fluoxetine, this interaction may become evident only during chronic administration. Studies investigating the affinities of citalopram, fluoxetine, and norfluoxetine for the 5-HT_{2C} receptor have used bovine and pig choroid plexi. To further evaluate the relevance of direct receptor interactions, the affinities of these drugs for rat and human 5-HT_{2C} receptors must be measured.

The 5-HT_{2C} receptor, characterized in 1984 by Pazos et al., is found from various brain regions, such as certain limbic and thalamic structures and basal ganglia (Molineaux et al. 1989; Hoffman and Mezey 1990; Mengod et al. 1990; Pompeiano et al. 1994), but the most abundantly it is expressed in choroid plexus epithelial cells (Yagaloff and Hartig 1985). 5-HT_{2C} receptors have been suggested to be involved in the number of psychiatric disorders, such as anxiety, obsessive-compulsive behavior, and eating disorders (Hoyer et al. 1994). Control of eating behavior indeed seems to be an important function of these receptors, as demonstrated by recent studies with transgenic animals (Tecott et al. 1995). It is of interest that anxiety and weight loss are common side effects of SSRI treatment. Fluoxetine is also efficient in the treatment of bulimia nervosa (Fluoxetine Bulimia Nervosa Collaborative Study Group 1992) and obsessive-compulsive disorders (Tollefson et al. 1994), and higher doses of fluoxetine are required for these disorders than those used for depression. In addition, the therapeutic response appears somewhat slower than that required for depression, corresponding to the development of steady state for fluoxetine and especially norfluoxetine in man. The efficacy of citalopram in these disorders is not known. Our results suggests that SSRI treatment-induced changes in the 5-HT_{2C} receptor density may contribute to their clinical properties. Fluoxetine also may have direct 5-HT_{2C} receptor-modulating properties in addition to 5-HT reuptake blockade.

In conclusion, our results show that chronic citalopram and fluoxetine treatments induce an upregulation of choroid plexus 5-HT_{2C} receptors, but the effect is more marked with citalopram. These differences in the regulation of the 5-HT_{2C} receptor may lead to pharmacodynamic differences between chronic citalopram and fluoxetine treatments.

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