ELSEVIER

Chronic Fluoxetine Reduces Serotonin Transporter mRNA and 5-HT_{1B} mRNA in a Sequential Manner in the Rat Dorsal Raphe Nucleus

John F. Neumaier, M.D., Ph.D., Daniel C. Root, B.A., and Mark W. Hamblin, M.D., Ph.D.

In major depression in humans and in animal models of depression, there is a defect in serotonergic neurotransmission that can be relieved by chronic antidepressant treatment. One possibility is that this pathologic state is caused by excessive presynaptic autoreceptor activity in serotonergic neurons, and that antidepressants down-regulate the number of these inhibitory receptors, allowing more normal serotonin release to occur. To evaluate this hypothesis, we measured the effects of the antidepressant fluoxetine on neuronal levels of 5-HT_{1B} receptor mRNA, the putative serotonin terminal autoreceptor in rat brain, and on serotonin transporter mRNA, the direct site of fluoxetine binding. Fluoxetine reduced serotonin transporter mRNA briefly, but this was not sustained after 21 days of treatment. However, fluoxetine reduced dorsal raphe 5-HT_{1B} mRNA levels in a time-dependent and washout-reversible manner. This reduction in 5-HT_{1B} mRNA was specific to dorsal raphe nucleus and was not found in several postsynaptic (nonserotonergic) regions. These results suggest that chronic fluoxetine may increase serotonin release from axonal terminals by down-regulating the messenger RNA coding for presynaptic 5-HT_{1B} autoreceptors while causing only transient effects on serotonin transporter mRNA.

[Neuropsychopharmacology 15:515–522, 1996]

KEY WORDS: *Serotonin; Depression; Antidepressive agents; Fluoxetine; Receptors Serotonin; Serotonin uptake inhibitors; 5-HT_{1B}; Dorsal raphe*

Depression is associated with diminished serotonin neurotransmission, and clinical efficacy of some antidepressant medications has been correlated with their ability to augment serotonergic function (Maes and Meltzer 1995). Recent studies have addressed how antidepressants affect serotonergic function in rat brain with particular attention focused on how chronic administration of serotonin selective reuptake inhibitor (SSRI) antidepressants such as fluoxetine, paroxetine, and fluvoxamine augment serotonin release in rat forebrain. The immediate action of these drugs involves binding to serotonin transporters (SERT) and inhibition of serotonin reuptake, but the antidepressant effect requires weeks to be manifest (Fuller and Wong 1987). Presumably, this involves subsequent adaptations of other mechanisms that regulate serotonergic neurotransmission.

Because 5-HT_{1B} autoreceptors in serotonin terminals suppress the synthesis (Hjorth et al. 1995) and release (Hjorth and Tao 1991; Hoyer and Middlemiss 1989; Martin et al. 1992; O'Connor and Cruk 1992) of serotonin in rat brain, they tonically inhibit serotonin neu-

From the Departments of Psychiatry and Behavioral Sciences (JFN, DCR, MWH) and Harborview Medical Center (JFN, DCR), University of Washington; and Geriatric Research Education and Clinical Center (MWH) at Seattle VA Medical Center, Seattle, WA.

Address correspondence to: John F. Neumaier, M.D., Ph.D., Harborview Medical Center, Psychiatry, Box 359911, 325 Ninth Avenue, Seattle, WA 98104-2499.

Received January 22, 1996; revised March 13, 1996; accepted April 2, 1996.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/96/$15.00
PII S0893-133X(96)00095-3

rotransmission and may be important in the mechanism of antidepressant action. In fact, several recent articles have described how chronic SSRI treatments increased serotonin release, perhaps by down-regulating terminal autoreceptors (Blier et al. 1987, 1990; Briley and Moret 1993; Mansari et al. 1995). Additional lines of evidence connect 5-HT_{1B} autoreceptor function with the physiology of depression. Learned helplessness is an animal model of depression that is associated with diminished serotonin release in frontal cortex (Petty et al. 1992); 5-HT_{1B} agonists may interfere with the prevention of learned helplessness by antidepressants (Martin and Puech 1991). 5-HT_{1B}-like radioligand binding is increased in some forebrain regions in learned helpless rats (Edwards et al. 1991, 1992). Furthermore, 5-HT_{1B} mRNA levels are elevated in dorsal raphe nucleus of learned helpless rats (Neumaier et al. 1996a). Together, these suggest that learned helplessness may be associated with elevated presynaptic 5-HT_{1B} autoreceptor activity in the terminals of dorsal raphe neurons. Because serotonin reuptake sites and 5-HT_{1B} autoreceptors are closely juxtaposed on serotonergic terminals, interactions between SERT blockade by antidepressants and 5-HT_{1B} receptor activity are favored and have been detected experimentally (Briley and Moret 1993; Davidson and Stamford 1995; Johanning et al. 1992; O'Connor and Kruk 1994). In fact, Briley and Moret (1993) suggested that down-regulation of 5-HT_{1B} autoreceptors may be an important feature of the chronic adaptations involved in the therapeutic response to antidepressant treatment.

5-HT_{1B} receptors are located both presynaptically in raphe neurons and postsynaptically in nonserotonergic neurons. In either case they appear to be localized in axonal terminals (Boschert et al. 1994) where they inhibit the release of neurotransmitters (Boeijinga and Boddeke 1993; Bolanos Jim'enez et al. 1994; Johnson et al. 1992; Maura and Raiteri 1986; Raiteri et al. 1986). Chemical lesioning has shown that only a small proportion of 5-HT_{1B} binding sites are localized presynaptically (Frankfurt et al. 1994; Offord et al. 1988). However, this comparatively small population of presynaptic 5-HT_{1B} receptors is quite important to the overall action of the serotonin projections to the forebrain. It is critical to address whether antidepressants modify presynaptic or postsynaptic 5-HT_{1B} receptor levels because the functional consequence of 5-HT_{1B} receptor activation in presynaptic (serotonergic and postsynaptic (nonserotonergic) neurons on a particular neuronal circuit may be quite different. Because the presynaptic and postsynaptic 5-HT_{1B} binding sites are mixed together throughout the forebrain, radioligand binding is of very limited utility in identifying presynaptic 5-HT_{1B} receptors. In order to distinguish presynaptic from postsynaptic 5-HT_{1B} receptor regulation, we have used in situ hybridization histochemistry to detect the mRNA coding for 5-HT_{1B} receptors in the cell bodies of origin. Because the therapeutic effects of antidepressants develop slowly, it is reasonable that changes in gene expression may be involved. Our hypothesis is that chronic SSRI treatment down-regulates presynaptic 5-HT_{1B} mRNA and receptor levels. Because SSRI antidepressants act through SERT and may regulate 5-HT_{1B} receptors, we have examined how fluoxetine affects the level of SERT and 5-HT_{1B} mRNAs in rat brain, with particular attention to serotonergic neurons in the dorsal raphe nucleus.

METHODS

Animal Procedures

Male Sprague-Dawley rats (Simonson, 200 g average weight) were housed in groups using methods approved by the animal research committee at this institution. Animals were treated with fluoxetine (3mg/kg/day, a gift from Lilly) or saline vehicle IP (4- and 7-day treatments) or by using osmotic minipumps (Alzet) implanted subcutaneously (21 days). Twenty-four hours after the last injection (4- and 7-day groups, rats were stunned by CO_2 inhalation, decapitated, and the brains were rapidly removed and frozen on dry ice and then stored at −70°C. Animals treated for 21 days were killed on day 21 in a similar manner. Some of the animals treated with fluoxetine for 7 days were allowed to washout for an additional 7 days before being killed.

In Situ Hybridization Histochemistry. 5-HT_{1B} and serotonin transporter (SERT) mRNA levels were measured as previously described (Neumaier et al. 1996b). Tissue sections (20 μm) were prepared in the frontal plane using a cryostat, then thaw mounted onto gelatin subbed glass slides. The sections were stored at −70°C until processed for in situ hybridization. In brief, tissue sections were thawed at room temperature and fixed in cold 4% paraformaldehyde. After washing in phosphate buffered saline, sections were treated with acetic anhydride (0.25% in 0.1 mol ≠ L triethanolamine), dehydrated, delipidated, and air dried.

Oligonucleotide probes complementary to portions of the 5-HT_{1B} mRNA were designed on the basis of low sequence homology to other known receptor mRNA sequences. Because of the low level of receptor mRNA expression, we used a "cocktail" of three oligonucleotide probes for the receptor. Oligonucleotide probes corresponded to residues 1343 to 1382, 1630 to 1668, and 1790 to 1829 of the rat 5-HT_{1B} clone, MG11B (Hamblin et al. 1992). The probes were individually labeled with [^{33}P]-dATP (Dupont NEN Research Products), using terminal deoxyribonucleotidyl transferase (Gibco) and purified on NEN-sorb columns (Dupont NEN Research Products). The specific activity of each oligonucleotide probe was approximately 7 μCi/pmol.

The labeled probes were diluted (2 pmol/ml) in a hybridization buffer containing 50% formamide, 10% dextran sulfate, 0.3 mol ≠ L sodium chloride, 10 mmol ≠ L Tris (pH 8.0), 1 mmol ≠ L EDTA, 1 × Denhart's (0.2% each bovine serum albumin, Ficoll, and polyvinylpyrollidine), 0.4 mg/ml yeast tRNA, and 10 mmol ≠ L dithiothreitol. Then 50 μl of the hybridization mixture was applied to each slide, and the sections were covered with silanized coverslips. The slides were incubated in moist, covered trays at 37°C overnight. After the hybridization reaction, coverslips were removed and the slides were washed in SSC (150 mmol ≠ L NaCl and 15 mmol ≠ L sodium citrate) for 1 hour at 55°C, and again in SSC at room temperature for 1 hour. The slides were rinsed in distilled water, dehydrated through a series of graded alcohol rinses containing 300 mmol ≠ L ammonium acetate, and air dried. Autoradiography was performed using Hyperfilm bmax (Amersham) with 4- to 6-day exposures. The distribution of 5-HT_{1B} in situ hybridization signal detected agrees with previously published reports (Boschert et al. 1994; Bruinvels et al. 1994; Neumaier et al. 1996b) and is abolished either by adding excess unlabeled oligonucleotides or by using labeled sense probes (Neumaier et al. 1996b).

In situ hybridization to SERT mRNA was also evaluated. Two oligonucleotide probes complementary to nucleotides 101 to 149 and 570 to 618 of the cloned rat serotonin transporter sequence (Blakely et al. 1991) were a gift from Dr. Patricia Szot and were labeled and used in an identical assay procedure to that described above, except the post-hybridization wash was performed at 58°C and films were exposed overnight. The distribution of SERT hybridization signal agreed with previously published reports (Fujita et al. 1993; Lesch et al. 1993; Lopez et al. 1994; Neumaier et al. 1996b).

Data Analysis

In situ hybridization signal was quantified using a computer-based densitometry system (MCID, Imaging Research, Inc., St. Catherines, ON); optical density (OD) was defined by using commercial film standards (Kodak). The rater was blinded to treatment condition of brains in most of the assays. Cresyl violet–stained

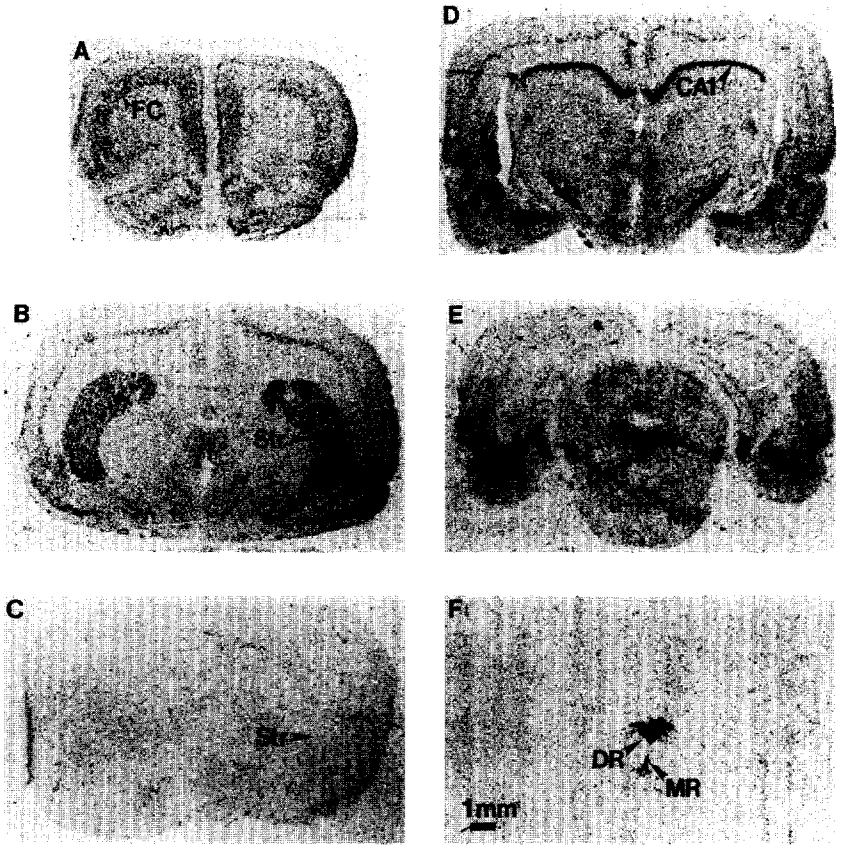

Figure 1. Regional distribution of 5-HT_{1B} and SERT mRNA in situ hybridization signal. Tissue sections were processed and hybridized with either 5-HT_{1B}-specific (**A–E**) or SERT-specific (**F**) oligonucleotides as described in the Methods section. Sections were then exposed to film yielding autoradiographic images, examples of which are shown here. Hybridization signal in a rectangle within layers 3 to 4 of frontal cortex (**A**) was quantified densitometrically. Within the striatum (**B**), and oval region just within the outer margins was quantified. Sections hybridized using sense oligonucleotide probes produced no specific signal above background in striatum (**C**) and elsewhere (not shown). Hybridization signal in the hippocampal CA1 pyramidal layer (**D**) and midbrain dorsal raphe nucleus (**E,F**) were measured using the image analysis software's automatic edge identification. Note that 5-HT_{1B} hybridization signal is restricted to the ventromedial portion of the dorsal raphe nucleus (**E**) and was faint in median raphe nucleus. SERT mRNA hybridization was strong in the ventromedial, dorsal, and lateral portions of the dorsal raphe nucleus as well as in the median raphe nucleus (**F**). The calibration bar in (**F**) applies to each image. Abbreviations: FC, frontal cortex area 1; Str, striatum; DR, dorsal raphe nucleus; MR, median raphe nucleus.

sections from each brain were matched anatomically to correspond to plates 8 (frontal cortex, area 1), 23 (striatum), 33 (hippocampus, CA1), and 49 (dorsal raphe nucleus) from the rat brain atlas by Paxinos and Watson (1986). OD was determined in the regions of interest of three consecutive autoradiographs corresponding to these coordinates. These were averaged, and film background OD was subtracted. After the treatment condition of the brains was uncoded, regional mean values were calculated for each treatment group. Treatment group averages were evaluated for statistical significance using the two-tailed Student's *t* test.

RESULTS

5-HT_{1B} mRNA

The distribution of 5-HT_{1B} mRNA hybridization signal agreed with previously published reports from this and other laboratories (Boschert et al. 1994; Bruinvels et al. 1994; Neumaier et al. 1996b). The frontal cortex, striatum, hippocampus, and dorsal raphe regions were studied in detail (Figure 1 *A–E*). The OD of 5-HT_{1B} hybridization signal was quantified for each experiment and is summarized in Table 1. Animals treated with fluoxetine for 4 days showed no changes in 5-HT_{1B} hybridization signal in any brain region studied. After 7 days of fluoxetine treatment, 5-HT_{1B} hybridization signal was slightly increased in frontal cortex and the CA1 layer of hippocampus. However, there was a near significant reduction in 5-HT_{1B} mRNA level in the dorsal raphe nucleus (87.6% of control, $p = .058$). Animals treated with fluoxetine for 7 days, and then allowed 7 days of drug washout, returned to 101.2% control levels of dorsal raphe 5-HT_{1B} hybridization signal (Figure 2 *A*). Animals treated for 21 days with fluoxetine again showed modest, nonsignificant increased 5-HT_{1B} hybridization signal in the postsynaptic regions studied, but 5-HT_{1B} signal in the dorsal raphe nucleus was reduced to 83.3% of control ($n = 12$, $p = .021$).

SERT mRNA

SSRI antidepressants interact directly with the serotonin transporter to block the reuptake of serotonin into axonal terminals and may be regulated by SSRI treatment. To evaluate whether SSRI alter the levels of mRNA coding for this serotonergic terminal protein, we measured SERT mRNA hybridization signal in dorsal raphe neurons after fluoxetine treatment. Four days of fluoxetine treatment produced no changes in SERT mRNA hybridization signal in dorsal raphe nucleus (Figure 2 *B*). Animals treated for 7 days with fluoxetine had significantly reduced SERT hybridization signal (62.2% of saline-treated controls, $p = .017$); in animals

Table 1. Regional 5-HT_{1B} mRNA Levels After Fluoxetine Treatment

Brain Region	4 Days OD (*n*) (% of control)	7 Days OD (*n*) (% of control)	21 Days OD (*n*) (% of control)
Dorsal raphe nucleus:			
control	0.214 ± 0.147 (8)	0.314 ± 0.013 (6)	0.204 ± 0.004 (12)
fluoxetine	0.213 ± 0.009 (8)	0.275 ± 0.013 (6)	0.170 ± 0.012 (12)
	99.5%	87.6%[a]	83.3%[b]
Frontal cortex:			
control	0.121 ± 0.015 (6)	0.121 ± 0.014 (6)	0.075 ± 0.007 (9)
fluoxetine	0.116 ± 0.016 (8)	0.134 ± 0.009 (5)	0.083 ± 0.011 (8)
	95.9%	110.7%	110.7%
Striatum:			
control	0.151 ± 0.009 (8)	0.193 ± 0.021 (6)	0.110 ± 0.010 (12)
fluoxetine	0.142 ± 0.012 (8)	0.189 ± 0.013 (5)	0.124 ± 0.014 (12)
	94.0%	97.9%	112.7%
CA1:			
control	0.598 ± 0.028 (8)	0.176 ± .011 (6)	0.415 ± 0.016 (13)
fluoxetine	0.596 ± 0.029 (8)	0.191 ±.019 (5)	0.448 ± 0.013 (11)
	99.5%	108.1%	108.0%

Animals were treated with either saline (control) or fluoxetine (3 mg/kg/day, see Methods) for varying periods. Optical density (OD) of 5-HT_{1B} m RNA hybridization signal, measured by in situ hybridization histochemistry as described in Methods and Figure 1, was quantified in each region shown from saline-treated and fluoxetine-treated rats. Because the data related to each duration of fluoxetine treatment were collected in separate assays, it is not possible to make direct comparisons of OD values between different periods of treatment. However, control, and fluoxetine-related values were measured in parallel within each assay, and % of control values are indicated for fluoxetine-treated cases.

[a] $p = .058$.

[b] $p = 0.021$ (Student's two-tailed *t*-test).

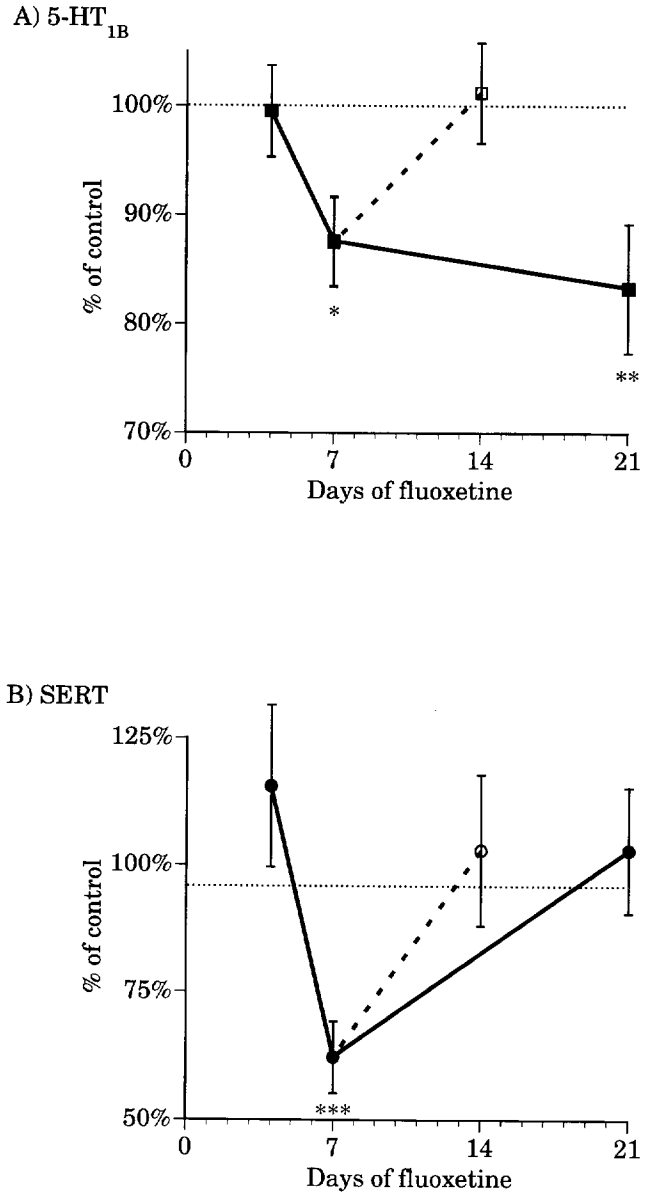

Figure 2. Time-dependent effects of fluoxetine on 5-HT_{1B} and SERT mRNA in the dorsal raphe nucleus. **A**: Chronic treatment with fluoxetine led to a gradual reduction in dorsal raphe 5-HT_{1B} mRNA signal whereas no such trend was observed in other brain regions. *Filled squares*, animals treated with fluoxetine (3 mg/kg/day) for 4, 7, or 21 days (n = 8, 6, 12; respectively) as described in the Methods section. *Open squares*, rats (n = 6) were treated for 7 days with fluoxetine then allowed 7 days of drug washout prior to being killed. **B**: Animals were treated with fluoxetine (3mg/kg/day, *filled circles*) for 4, 7, or 21 days (n = 7, 6, 9; respectively) as described in the Methods section. Compared with saline-treated controls, rats treated with fluoxetine for 7 days had significantly lower SERT mRNA levels in dorsal raphe nucleus but had returned to control levels either after 7 days of drug washout (*open circles*, n = 6) or after 21 days of fluoxetine treatment. SERT mRNA levels were determined in a subset comprising a majority of the brains used for 5-HT_{1B} determinations. Hybridization signal (OD) values are expressed as percentage of saline-treated controls in parallel. *p = .058; **p = .021; ***p = .0167 (Student's two-tailed t-test).

allowed an additional 7 days of drug washout, SERT hybridization signal returned to control levels. After 21 days of treatment, SERT mRNA levels had returned to control levels again (Figure 2 *B*).

DISCUSSION

5-HT_{1B} mRNA and receptors are detected in both serotonergic (presynaptic) and nonserotonergic (postsynaptic) neurons and have been previously described as inhibitory terminal receptors that regulate neurotransmitter release in several neuron types (Boeijinga and Boddeke 1993; Johnson et al. 1992; Maura and Raiteri 1986; Raiteri et al. 1986). SSRI antidepressants appear to affect presynaptic serotonergic function preferentially, perhaps by desensitizing presynaptic terminal autoreceptors (Blier et al. 1987). The major observation in this report is that fluoxetine reduced 5-HT_{1B} mRNA levels in the dorsal raphe nucleus, and this decrease was specific to this area. Nonsignificant increases were detected in several nonserotonergic neuronal types in other brain regions. Reduction of dorsal raphe 5-HT_{1B} mRNA occurs gradually, taking at least one week to become evident (Figure 2 *A*). As in the clinical setting, premature discontinuation of fluoxetine (i.e., washout after 7 days) led to reversal of the antidepressant effect. Although SSRI antidepressants inhibit serotonin reuptake soon after administration (Jordan et al. 1994), the time-course of changes relevant to the relief of depressive symptoms is likely to involve gradual changes, because patients often do not respond to these medications for several weeks.

There have been several reports of the effects of antidepressants on presynaptic serotonergic mechanisms, although these studies have yielded contradictory re-

sults. In electrophysiologic experiments in which afferent fibers from dorsal raphe to the hippocampus were electrically stimulated, chronic administration of SSRI increased serotonin release and reduced the sensitivity to autoreceptor blockade by methiothepin (Blier et al. 1987, 1990; Chaput et al. 1991). These important data suggested that the autoreceptors located on dorsal raphe neuron terminals may be down-regulated by SSRI antidepressants. Using a different technique, Moret and Briley (1990) detected increased [^{3}H]-5-HT release from hypothalamic slices prepared from rats 24 hours after being treated with citalopram for 21 days; the nonselective 5-HT agonist LSD had reduced potency in activating inhibitory autoreceptors in these slices. However, Bosker (1995) found no effect of chronic fluvoxamine on 5-HT$_{1B}$ receptor activity in inhibiting the release of serotonin in hippocampus. Both if these studies examined basal serotonin release rather than stimulated serotonin release, which may not be sensitive enough to detect the full range of autoreceptor activity. Mansari et al. (1995) found that paroxetine and fluoxetine treatment for 21 days increased electrically stimulated serotonin release from tissue slices from guinea pig frontal cortex and provided evidence for regional differences between frontal and orbitofrontal cortex after 8 weeks of treatment. Despite attempts to control for residual SSRI in the tissue at the time of assay, some of the differences between these studies may relate to the extent of SSRI washout prior to assay, because this will influence the measurement of serotonin release. Furthermore, it is difficult to rule out the impact on serotonin release of sudden SSRI withdrawal after chronic treatment.

Radioligand binding studies have yielded conflicting data. Because lesion studies demonstrate that only a small minority of 5-HT$_{1B}$ binding sites in the brain are presynaptic (Frankfurt et al. 1994; Offord et al. 1988) and these are anatomically intermingled with postsynaptic binding sites, it is unlikely that radioligand binding studies could detect even large changes in presynaptic 5-HT$_{1B}$ receptor levels. Thus, Montero (1991) detected no change in [^{125}I]-cyanopindolol binding to rat frontal cortex 72 hours after 15 days of chronic tianeptine, iprindole, or clomipramine exposure. On the other hand, Johanning et al. (1992) found imipramine reduced RU24969-displaceable [^{3}H]-5-HT binding density of rat brain 5-HT$_{1B}$-like binding sites by 19%. Clearly, techniques that can discriminate presynaptic from postsynaptic 5-HT$_{1B}$ receptors offer advantages in addressing these actions of SSRI antidepressants. The technique used in this report, in situ hybridization histochemistry, presents both advantages and problems. The mRNA measurement is not impaired by the presence of antidepressant drug in the tissue. The oligonucleotide probes used identify 5-HT$_{1B}$ mRNA with extremely high specificity in the cell bodies that synthesize the receptor, thus allowing confidence in assessing which population of neurons demonstrate any changes after manipulation. A major caveat of in situ hybridization histochemistry is that posttranslational regulation is not reflected. However, mRNA levels may reflect the cell's capacity to synthesize receptors over time; this is particularly relevant to gradual, adaptive modifications in neurotransmission such as those seen after chronic antidepressant administration. A previous study examined antidepressant effects on whole rat brain 5-HT$_{1B}$ mRNA levels, along with a number of other serotonergic mRNA species (Spurlock et al. 1994) and found no effects in any case. However, the use of whole brain RNA extracts prevented the detection of significant regional differences that may have existed and are demonstrated here. Indeed, in our study regional increases and decreases in 5-HT$_{1B}$ mRNA were detected that may have cancelled out in this previous study.

Several considerations suggest that presynaptic and postsynaptic 5-HT$_{1B}$ receptors may be regulated independently. Chemical axotomy with 5,7-dihydroxytryptamine that spared dorsal raphe serotonergic cell bodies had no detectable effect on 5-HT$_{1B}$ mRNA levels in several forebrain regions, but dorsal raphe 5-HT$_{1B}$ mRNA hybridization was reduced by 62% (Neumaier et al. 1996b). This may reflect the interruption of synaptic interactions that are important in maintaining normal levels of 5-HT$_{1B}$ autoreceptors. Learned helpless rats had a 25% increase in 5-HT$_{1B}$ hybridization signal in dorsal raphe without any significant changes in forebrain areas (Neumaier et al. 1996a). In the present study, several postsynaptic brain areas showed small trends toward increased 5-HT$_{1B}$ mRNA levels after chronic antidepressant treatment, whereas dorsal raphe (presynaptic) 5-HT$_{1B}$ mRNA levels were significantly reduced. It is possible that the anatomy of the serotonin system may help explain these differences. Because rat dorsal raphe neurons make few classic synaptic contacts (Jacobs and Azmitia 1992), the serotonin released from these terminals may diffuse some distance and become diluted prior to reaching postsynaptic receptor sites. Consequently, presynaptic 5-HT$_{1B}$ autoreceptors on serotonergic terminals may be exposed to higher concentrations of serotonin than postsynaptic 5-HT$_{1B}$ receptors when SSRI antidepressants block reuptake of 5-HT. This may be the basis for specific down-regulation of presynaptic 5-HT$_{1B}$ autoreceptors.

In this study, dorsal raphe SERT mRNA was reduced substantially after 7 days of fluoxetine administration, but this effect was reversed by drug washout and was not maintained after 21 days of treatment (Figure 2 B). Previous reports have differed on this issue. In rat dorsal raphe nucleus, chronic antidepressants for 2 weeks (Kuroda et al. 1994) or 3 weeks (Lesch et al. 1993) reduced SERT mRNA, increased SERT after 3 weeks) (Lopez et al. 1994), or produced no change after 8 weeks

of treatment (Burnet et al. 1994). Perhaps these discrepant results may be partially explained by considering the different durations of chronic antidepressant treatment. The present study suggests that there is a temporary reduction in SERT mRNA during chronic fluoxetine treatment that is not sustained over longer periods. This proposal would tend to explain most of the previously published results and further emphasizes that antidepressant effects on SERT are only an early step in inducing clinical benefits.

In summary, fluoxetine reduces 5-HT_{1B} mRNA levels in the rat dorsal raphe nucleus in a time-dependent manner, whereas 5-HT_{1B} mRNA levels in several nonserotonergic brain regions increased nonsignificantly. These results add to evidence that reducing presynaptic serotonin autoreceptor activity is an important aspect of antidepressant action in augmenting serotonergic neurotransmission.

ACKNOWLEDGMENTS

John Neumaier was supported by a young investigator award from the National Alliance for Research in Schizophrenia and Depression. Daniel Root was supported by a summer research fellowship from the Stanley Foundation. Mark Hamblin was supported by the Department of Veterans Affairs Merit Review Program.

REFERENCES

Blakely RD, Berson HE, Fremeau RT Jr, Caron MG, Peek MM, Prince HK, Bradley CC (1991): Cloning and expression of a functional serotonin transporter from rat brain. Nature 354:66–70

Blier P, De Montigny C, Chaput Y (1987): Modifications of the serotonin system by antidepressant treatments: Implications for the therapeutic response in major depression. J Clin Psychopharmacol 7:24S–35S

Blier P, De Montigny C, Chaput Y (1990): A role for the serotonin system in the mechanism of action of antidepressant treatments: Preclinical evidence. J Clin Psychiatry 51:4(Suppl)14–21

Boeijinga PH, Boddeke HW (1993): Serotonergic modulation of neurotransmission in the rat subicular cortex in vitro: A role for 5-HT_{1B} receptors. Naunyn Schmiedebergs Arch Pharmacol 348:553–557

Bolanos Jim'enez F, Manhaes de Castro R, Fillion G (1994): Effect of chronic antidepressant treatment on 5-HT_{1B} presynaptic heteroreceptors inhibiting acetylcholine release. Neuropharmacology 33:77–81

Boschert M, Amara DA, Segu L, Hen R (1994): The mouse 5-hydroxytryptamine$_{1B}$ receptor is localized predominantly on axon terminals. Neuroscience 58:167–182

Bosker FJ (1995): Chronic treatment with fluvoxamine by osmotic minipumps fails to induce persistent functional changes in central 5-HT (1A) and 5-HT (1B) receptors, as measured by in vivo microdialysis in dorsal hippocampus of conscious rats.

Briley M, Moret C (1993): Neurobiological mechanisms involved in antidepressant therapies. Clin Neuropharmacol 16:387–400

Bruinvels AT, Landwehrmeyer B, Gustafson EL, Durkin MM, Mengod G, Branchek TA, Hoyer D, Palacios JM (1994): Localization of 5-HT_{1B}, 5-$HT_{1Dalpha}$, 5-HT_{1E} and 5-HT_{1F}, receptor messenger RNA in rodent and primate brain. Neuropharmacology 33:367–386

Burnet PW, Michelson D, Smith MA, Gold PW, Sternberg EM (1994): The effect of chronic imipramine administration on the densities of 5-HT1A and 5-HT2 receptors and the abundances of 5-HT receptor and transporter mRNA in the cortex, hippocampus and dorsal raphe of three strains of rat. Brain Res 638:311–324

Chaput Y, de Montigny C, Blier P (1991): Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments: An in vivo electrophysiologic study in the rat. Neuropsychopharmacology 5:219–229

Davidson C, Stamford JA (1995): Evidence that 5-hydroxytryptamine release in rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. Br J Pharmacol 114:1107–1109

Edwards E, Harkins K, Wright G, Henn FA (1991): 5-HT_{1B} receptors in an animal model of depression. Neuropharmacology 30:101–105

Edwards E, Kornrich W, Houtten PV, Henn FA (1992): Presynaptic serotonin mechanisms in rats subjected to inescapable shock. Neuropharmacology 31:323–330

Frankfurt M, McKittrick CR, Mendelson SD, McEwen BS (1994): Effect of 5,7-dihydroxytryptamine, ovariectomy and gonadal steroids on serotonin receptor binding in rat brain. Neuroendocrinology 59:245–250

Fujita M, Shimada S, Maeno H, Nishimura T, Tohyama M (1993): Cellular localization of serotonin transporter mRNA in the rat brain. Neurosci Lett 162:59–62

Fuller RW, Wong DT (1987): Serotonin reuptake blockers in vitro and in vivo. J Clin Psychopharmacol 7:36S–43S

Hamblin MW, McGuffin RW, Metcalf MA, Dorsa DM, Merchant KA (1992): Distinct 5-HT_{1B} and 5-HT_{1D} serotonin receptors in rat: Structural and pharmacological comparison of the two cloned receptors. Mol Cell Neurosci 3:578–587

Hjorth S, Tao R (1991): The putative 5-HT_{1B} receptor agonist CP-93,129 suppresses rat hippocampal 5-HT release in vivo: Comparison with RU 24969. Eur J Pharmacol 209:249–252

Hjorth S, Suchowski CS, Galloway MP (1995): Evidence for 5-HT autoreceptor-mediated, nerve impulse-independent, control of 5-HT synthesis in the rat brain. Synapse 19:170–176

Hoyer D, Middlemiss DN (1989): Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. Trends Pharmacol Sci 10:130–132

Jacobs BL, Azmitia EC (1992): Structure and function of the brain serotonin system. Physiol Rev 72:165–229

Johanning H, Plenge P, Mellerup E (1992): Serotonin receptors in the brain of rats treated chronically with imi-

pramine or RU24969: Support for the 5-HT_{1B} receptor being a 5-HT autoreceptor. Pharmacol toxicol 70:131–134

Johnson SW, Mercuri NB, North RA (1992): 5-Hydroxytryptamine$_{1B}$ receptors block the $GABA_B$ synaptic potential in rat dopamine neurons. J Neurosci 12:2000–2006

Jordan S, Kramer GL, Zukas PK, Moeller M, Petty F (1994): In vivo biogenic amine efflux in medial prefrontal cortex with imipramine, fluoxetine, and fluvoxamine. Synapse 18:294–297

Kuroda Y, Watanabe Y, McEwen BS (1994): Tianeptine decreases both serotonin transporter mRNA and binding sites in rat brain. Eur J Pharmacol 268:R3–5

Lesch KP, Aulakh CS, Wolozin BL, Tolliver TJ, Hill JL, Murphy DL (1993): Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. Mol Brain Res 17:31–35

Lopez JF, Chalmers DT, V'Azquez DM, Watson SJ, Akil H (1994): Serotonin transporter mRNA in rat brain is regulated by classical antidepressants. Biol Psychiatry 35:287–290

Maes M, Meltzer HY (1995): In Bloom FE, Kupfer DJ (eds), The Serotonin Hypothesis of Major Depression. Psychopharmacology: The Fourth Generation of Progress. New York, Raven Press, pp 933–944

Mansari ME, Bouchar C, Blier P (1995): Alteration of serotonin release in the guinea pig orbito-frontal cortex by selective serotonin reuptake inhibitors: Relevance to treatment of obsessive-compulsive disorder. Neuropsychopharmacology 13:117–127

Martin P, Puech AJ (1991): Is there a relationship between 5-HT_{1B} receptors and the mechanisms of action of antidepressant drugs in the learned helplessness paradigm in rats? Eur J Pharmacol 1992:193–196

Martin KF, Hannon S, Phillips I, Heal DJ (1992): Opposing roles for 5-HT_{1B} and 5-HT_3 receptors in the control of 5-HT release in rat hippocampus *in vivo*. Br J Pharmacol 106:139–142

Maura G, Raiteri M (1986): Cholinergic terminals in rat hippocampus possess 5-HT_{1B} receptors mediating inhibition of acetylcholine release. Eur J Pharmacol 129:333–337

Montero D (1991): Acute or chronic antidepressants do not modify [125I] cyanopindolol binding to 5-HT_{1B} receptors in rat brain. Eur J Pharmacol 196 (3):327–9

Moret C, Briley M (1990): Modulation by drugs of the release of total tritium and ^{3}H-5-HT from rat hypothalamic slices. Naunyn Schmiedebergs Arch Pharmacol 341(5): 398–403

Neumaier JF, Petty F, Kramer GL, Szot P, Hamblin MW (1996a): Learned helplessness increases 5-HT_{1B} receptor mRNA levels in the rat dorsal raphe nucleus. Biol Psychiatry (in press)

Neumaier JF, Szot P, Peskind E, Dorsa DM, Hamblin MW (1996b): Serotonergic lesioning differentially affects presynaptic and postsynaptic 5-HT_{1B} Receptor mRNA levels in rat brain. Brain Res (in press)

O'Connor JJ, Cruk ZL (1992): Pharmacological characteristics of 5-hydroxytryptamine autoreceptors in rat brain slices incorporating the dorsal raphe or the suprachiasmatic nucleus. Br Pharmacol 106:524–532

O'Connor JJ, Kruk ZL (1994): Effects of 21 days treatment with fluoxetine on stimulated endogenous 5-hydroxytryptamine overflow in the rat dorsal raphe and surachiasmatic nucleus studied using fast cyclic voltammetry in vitro. Brain Res 640:328–335

Offord SJ, Ordway GA, Frazer A (1988): Application of [^{125}I]iodocyanopindolol to measure 5-hydroxytryptamine$_{1B}$ receptors in the brain of the rat. J Pharmacol Exp Ther 244:144–153

Paxinos G, Watson C (1986): The Rat Brain in Stereotaxic Coordinates. Sydney, Academic Press Australia

Petty F, Kramer G, Wilson L (1992): Prevention of learned helplessness: In vivo correlation with cortical serotonin. Pharmacol Biochem Behav 43:361–367

Raiteri M, Maura G, Bonanno G, Pittaluga A (1986): Differential pharmacology and function of two 5-HT_1 receptors modulating transmitter release in rat cerebellum. J Pharmacol Exp Ther 237:644–648

Spurlock G, Buckland P, O'Donovan M, McGuffin P (1994): Lack of effect of antidepressant drugs on the levels of mRNAs encoding serotonergic receptors, synthetic enzymes and 5HT transporter. Neuropharmacology 33:433–440