

Decrease in Brain Serotonin 2 Receptor Binding in Patients With Major Depression Following Desipramine Treatment

A Positron Emission Tomography Study With Fluorine-18–Labeled Setoperone

Lakshmi N. Yatham, MBBS, FRCPC; Peter F. Liddle, PhD, MBBS; Joelle Dennie, MSc; I-Shin Shiah, MD; Michael J. Adam, PhD; Carol J. Lane, MSc; Raymond W. Lam, MD; Thomas J. Ruth, PhD

Background: The neuroreceptor changes involved in therapeutic efficacy of various antidepressants remain unclear. Preclinical studies have shown that long-term administration of various antidepressants causes down-regulation of brain serotonin 2 (5-HT₂) receptors in rodents, but it is unknown if similar changes occur following antidepressant treatment in depressed patients. Our purpose, therefore, was to assess the effects of treatment with desipramine hydrochloride on brain 5-HT₂ receptors in depressed patients using positron emission tomography (PET) and fluorine-18 (¹⁸F)–labeled setoperone.

Methods: Eleven patients who met *DSM-IV* criteria for major depression as determined by a structured clinical interview for *DSM-III-R* diagnosis and suitable for treatment with desipramine were recruited. Ten patients underwent a PET scan before and another after 3 to 4 weeks of treatment with desipramine.

Results: Eight of the 10 patients responded to desipramine treatment as indicated by more than 50% decrease in Hamilton Depression Rating Scale scores. Depressed patients showed a significant decrease in 5-HT₂ receptor binding as measured by setoperone binding in frontal, temporal, parietal, and occipital cortical regions following desipramine treatment. The decrease in 5-HT₂ receptor binding was observed bilaterally and was particularly prominent in frontal cortex.

Conclusions: Depressed patients showed a significant reduction in available 5-HT₂ receptors in the brain following desipramine treatment, but it is unknown if this change in 5-HT₂ receptors is due to clinical improvement or an effect of desipramine that is unrelated to clinical status.

Arch Gen Psychiatry. 1999;56:705-711

From the Divisions of Mood Disorders (Drs Yatham, Shiah, and Lam and Ms Dennie) and Schizophrenia (Dr Liddle and Ms Lane), Department of Psychiatry, and the TRIUMF Positron Emission Tomography Program (Drs Adam and Ruth), The University of British Columbia, Vancouver; and the Department of Psychiatry, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan (Dr Shiah).

THE MECHANISMS by which antidepressants exert their therapeutic effects in major depression have remained elusive. Since there is a time lag of about 2 to 4 weeks between initiating antidepressant treatment and the clinical response, it has been suggested that alterations in neurotransmitter receptors occurring within this time frame might be relevant to therapeutic effects of these drugs.¹ Because 5-hydroxytryptamine (serotonin [5-HT]) has been implicated in depression,^{2,3} a number of animal studies⁴⁻¹⁵ have assessed the changes in 5-HT receptors after 2 to 4 weeks of the administration of antidepressants. Serotonin 2 receptors are the most widely studied,^{4,9,11-13,15} and these studies have shown that long-term administration of tricyclic antidepressants,^{4-6,8,9,11-13} monoamine oxidase inhibitors,^{4,6,8,12} atypical antidepressants such as iprindole^{4,6,12} and mianserin hydrochloride,^{7,8,13} and most but not all serotonin reuptake inhibitors^{4,9,12,16-19} decreases the density of cortical 5-HT₂ re-

ceptors in rats. It is, however, unknown if antidepressant treatment would lead to similar changes in 5-HT₂ receptor density in depressed patients.

The purpose of our study was to ascertain the effects of 3- to 4-week treatment with an antidepressant on brain 5-HT₂ receptors using positron emission tomography (PET) in patients with major depression. Ligands such as carbon 11 (¹¹C) (R)-(+)-4-(1-hydroxy-1-[2,3-dimethoxyphenyl]methyl)-N-2-(4-fluorophenylethyl)piperidine (MDL 100 907),²⁰ ¹¹C-N-methylspiperone,²¹ ¹⁸F-setoperone,²² and ¹⁸F-altanserin²³ are available for imaging 5-HT₂ receptors in human brain.²⁴ We chose ¹⁸F-setoperone because (1) it has a total-nonspecific binding ratio of greater than 2; (2) it was recommended by the European Task Force as a suitable ligand for imaging 5-HT₂ receptors²⁴; and (3) it has been used successfully by other groups to explore 5-HT function in psychiatric disorders.²⁵⁻²⁸ We chose to test the effects of the antidepressant desipramine hydrochloride on 5-HT₂ receptors because this medi-

SUBJECTS AND METHODS

SUBJECTS

Eleven patients who met *DSM-IV* criteria for major depressive disorder²⁹ and who were suitable for treatment with desipramine were recruited from the University of British Columbia Hospital, Vancouver. The study was approved by the University Ethics Committee, and subjects gave written informed consent. The diagnosis of major depression was made using the Structured Clinical Interview for *DSM-III-R*.³⁰ None had other Axis I diagnoses or current or past alcohol or substance abuse. All subjects were physically healthy as determined by history and results of physical examination. The severity of depression was quantified using the 21-item Hamilton Depression Rating Scale (HAM-D).³¹ All study subjects were free of psychotropic drugs for at least 2 weeks (5 weeks in the case of fluoxetine hydrochloride) before baseline PET scan, and none had ever received electroconvulsive therapy.

PET PROCEDURE

Radiosynthesis of ¹⁸F-setoperone was accomplished by a modified method of Crouzel and colleagues,²² as described by Adam et al.³² Radioactivity in brain tissue was measured with a PET system (ECAT 953B/31; CTI/Siemens, Knoxville, Tenn). The spatial resolution of images is 6 mm (full width at half maximum). Subjects were supine with the head slightly tilted to obtain brain slices parallel to the canthomeatal line. Each subject had a 10-minute transmission scan for correcting PET images for attenuation. Following this, subjects were given 148 to 259 MBq of ¹⁸F-setoperone intravenously, and a 15-frame scan was performed. The numbers and durations of frames were as follows: 5 frames for 2 minutes each, 4 frames for 5 minutes each, 4 frames for 10 minutes each, and 2 frames for 20 minutes each (total duration, 110 minutes).

TREATMENT

Each patient started desipramine hydrochloride therapy, 50 mg, and the dose was increased to 100 mg on day 4. Patients underwent assessment weekly with a clinical interview and a 21-item HAM-D. If there was no improvement in symptoms by the second week, the dose of desipramine hydrochloride was increased to 150 mg; a further increase in dose was made if no improvement was seen by the third week. No other psychotropic medication was allowed except lorazepam or oxazepam as required for night sedation. Subjects had a second PET scan 3 to 4 weeks after the commencement of desipramine therapy, 12 to 20 hours after the last dose.

DATA ANALYSIS

Using the multipurpose imaging (MPI) tool³³ to define regions in frontal, temporal, and parietal cortex and cerebellum, we confirmed previous observations³⁴ that cortical-to-cerebellar ratio is constant, indicating pseudoequilibrium, between 70 to 110 minutes after injection of ¹⁸F-setoperone in depressed patients.

Statistical parametric mapping (SPM96) software^{35,36} was used to align PET images in the 70- to 110-minute period, to coregister them to magnetic resonance images, and to transform the magnetic resonance images (and PET images) into the standard coordinate frame used for templates in SPM96. Images were smoothed by applying a 12-mm (full width at half maximum) isotropic Gaussian filter to improve the signal-to-noise ratio.

For trace doses of setoperone at equilibrium, the amount of setoperone bound to receptors is proportional to the binding potential, B_{max}/K_d , where B_{max} is the total number of receptors and K_d is the equilibrium-binding constant. To determine the binding potential from the measured concentration of setoperone in cerebral tissue, it is necessary to allow for variation in the amount of setoperone in plasma. This can be performed by comparing the setoperone concentration in the region of interest with that in a reference region. Provided the capacity for

cation consistently has been shown to decrease the density of 5-HT₂ receptors in rat studies.

RESULTS

One patient could not tolerate the side effects of desipramine and dropped out on day 4. The other 10 patients completed the study, and the data analysis is presented for these 10.

The subjects had a mean age of 40.6 ± 9.2 years. Three patients had no previous depressive episodes, and 2 had 1 previous episode. The other 5 patients had from 3 to 12 previous episodes of depression (**Table 1**). The duration of current depressive episode ranged from 20 to 120 weeks. Four patients had been free of psychotropic medication for more than 6 months, and 1 patient was drug naive. The other 5 patients were drug free from 2 to 5 weeks. Three subjects received nighttime sedation with lorazepam and/or oxazepam during the study. The mean dose of desipramine hydrochloride was 160.0 ± 21.0 mg (range, 150-200 mg). The patients had a mean 21-item HAM-D

score of 27.0 ± 5.6 , and the mean HAM-D score decreased to 7.0 ± 7.8 following treatment. Eight of 10 patients improved (defined as $\geq 50\%$ reduction in 21-item HAM-D score) with treatment by second PET scan.

Depressed patients showed a significant reduction in setoperone binding in several cortical areas, including frontal, temporal, parietal, and occipital regions, following desipramine treatment. Analysis using SPM96 demonstrated an extensive cluster of voxels in which there was a significant decrease in setoperone binding following treatment with desipramine (**Table 2**, **Figure 1**, and **Figure 2**). This cluster was highly significant after correcting for multiple comparisons ($P < .001$). It included 26 695 voxels, and this corresponds to approximately 46% of the volume of gray matter that was in the field of view. The entire cluster embraced medial frontal cortex, insula, and lateral frontal cortex and extended back to temporoparietal and occipital cortices bilaterally. Within this cluster, 383 voxels satisfied the criteria for significance irrespective of their membership in the cluster. Table 2 gives the location and z value for change in setoperone binding

nonspecific binding is identical in the region of interest and the reference region, the ratio of setoperone concentration in the region of interest (C_{local}) to that in the reference region ($C_{\text{reference}}$) is given using the following formula:

$$C_{\text{local}}/C_{\text{reference}} = f \cdot \text{BP}_{\text{local}} + 1,$$

where BP_{local} is the local binding potential, and f is a quantity that reflects the total binding capacity (specific + nonspecific) in the reference region. Although cerebellum has been used as a reference region, Petit-Taboue et al³⁷ have demonstrated that in humans, nonspecific binding of setoperone in cerebellum is different from that in cortex. Hence this equation is not accurate, and its use leads to errors of approximately 20% in the estimation of $f \cdot \text{BP}_{\text{local}}$ if cerebellum is used as a reference region. However, Petit-Taboue et al³⁷ demonstrated that nonspecific binding does not vary substantially between different cortical regions. Therefore, when the object is to measure regional changes in setoperone binding, an alternative approach is to use the whole cortex as the reference region.

The following equation is used when the whole cortex is treated as the reference region:

$$f = 1/(\text{BP}_{\text{global}} + k5/k6),$$

where $\text{BP}_{\text{global}}$ is mean global binding potential, and $k5$ and $k6$ are the transfer coefficients for attachment to and detachment from nonspecific binding sites. If $\text{BP}_{\text{global}} + k5/k6$ is not substantially affected by treatment, the change in $C_{\text{local}}/C_{\text{global}}$ is proportional to the change in binding potential during treatment. If $\text{BP}_{\text{global}} + k5/k6$ changes during treatment, change in $C_{\text{local}}/C_{\text{global}}$ is proportional to change in ratio of local binding potential to mean global binding capacity (specific + nonspecific), but there is reduced power to detect local changes in binding potential in regions where local change is in the same direction as the mean global change. Provided there is no substantial change in global binding, it is preferable to use the whole cortex as the reference region for the determination of local changes, as this avoids errors due to differences between cortical and cerebellar nonspecific binding.

We evaluated C_{global} using the middle 10 slices of the image and $C_{\text{cerebellum}}$ in a cerebellar region drawn using the MPI tool. The pretreatment value of the ratio $C_{\text{global}}/C_{\text{cerebellum}}$ was 1.173, whereas the posttreatment ratio was 1.148 (paired t , 0.514; $P = .62$). The minimal observed change in the ratio $C_{\text{global}}/C_{\text{cerebellum}}$ justifies the use of whole cortex as the reference region for determining local changes in setoperone binding.

Using SPM96, we then determined the change in ratio of local setoperone concentration to mean global setoperone concentration during treatment with proportional scaling. The threshold for including voxels in the analysis was set at 1.3 times mean global cerebral image intensity to exclude non-gray matter voxels. For each voxel, the general linear model was used to estimate the mean change in $C_{\text{local}}/C_{\text{global}}$. The significance of the change for each voxel was determined applying the method developed by Worsley³⁸ based on the theory of Gaussian fields, as implemented in SPM96.^{35,36} Although there are approximately 58 000 voxels in the image, the image intensity in adjacent voxels is strongly correlated, so that there are only about 90 independent measurements. In effect, Worsley's method determines the number of independent measurements and applies the appropriate Bonferroni-type correction.³⁸ Voxels in which the significance of z satisfies this correction can be accepted as significant irrespective of whether they are a part of a contiguous set of voxels exhibiting significant change.

The theory of Gaussian fields was also applied to determine the statistical significance of clusters of contiguous voxels in which change during treatment exceeded a specified threshold. For this calculation, we set the threshold of $z = 1.96$ corresponding to $P < .025$ one-tailed (or $P < .05$ two-tailed). Worsley's method³⁸ calculates the significance of clusters, taking account of the extent and peak z value within the cluster, and then applies a correction allowing for the multiple comparisons performed. Cluster significance level was set at $P < .05$.

Pearson correlations between changes in relative setoperone binding in the significant clusters and changes in 21-item HAM-D score and HAM-D suicide item score between pretreatment and posttreatment conditions were computed. Unless otherwise indicated, data are given as mean \pm SD.

at those individual voxels where z exceeded 4.00 ($P < .05$ after stringent correction for multiple comparisons). The mean decrease in setoperone binding for the entire cluster was 8.1%, and it ranged from 8.0% to 15.7% for individual voxels that were significant (Table 2). The sites where most significant decreases in setoperone binding were observed included left medial and orbitomedial frontal gyri, left inferior frontal gyrus, left middle and inferior temporal gyri, right inferior frontal gyrus, right lingual gyrus, and right middle occipital gyrus (Table 2 and Figures 1 and 2). At all these sites, decreases in setoperone binding occurred in all 10 subjects, including the 2 nonresponders. The magnitude of decrease in binding at these sites was similar in responders and nonresponders (**Figure 3**; data shown only for left medial frontal gyrus) and drug-naive and drug-free patients.

There were, however, no significant correlations between change in setoperone binding and change in 21-item HAM-D or suicide item scores (data not shown). As predicted, depressed patients did not show a significant increase in setoperone binding in any of the brain areas.

COMMENT

This is the first study to assess the effects of desipramine treatment on brain 5-HT₂ receptors in living, depressed patients. The results indicated that depressed patients had a significant decrease in brain setoperone binding in various cortical areas, including frontal, temporal, parietal, and occipital regions, following desipramine treatment. The decrease in setoperone binding was observed bilaterally, with decreases highly significant in left medial and orbitomedial frontal gyri, left inferior frontal gyrus, left middle and inferior temporal gyri, right inferior frontal gyrus, right lingual gyrus, and right middle occipital gyrus.

Several interpretations must be considered for a decrease in setoperone binding in our study subjects. First, the methods used in our study would not permit an independent determination of B_{max} and K_d . Hence, a decrease in setoperone binding observed following desipramine treatment in our study could be due to a decrease in B_{max} or an alteration in K_d . However, postmortem stud-

Table 1. Sociodemographic and Illness Characteristics for Depressed Patients Receiving Desipramine Treatment*

Subject No./Sex	Age, y	No. of Previous Depressive Episodes	Duration of Current Episode, wk	Duration of Drug-Free Period	Pretreatment HAM-D Score	Posttreatment HAM-D Score
1/F	49	4	78	Drug naïve	24	11
2/M	46	12	50	5 wk	24	21
3/M	41	1	32	30 wk	25	7
4/M	34	0	120	4 wk	23	20
5/M	30	0	100	5 wk	20	2
6/F	47	1	20	>1 y	24	0
7/M	47	10	70	2 wk	27	3
8/F	50	0	52	3 wk	38	0
9/F	40	7	26	26 wk	34	5
10/F	22	3	26	26 wk	31	1
All, mean ± SD	40.6 ± 9.2	3.8 ± 4.4	57.4 ± 34.0	...	27.0 ± 5.6	7.0 ± 7.8

*HAM-D indicates 21-item Hamilton Depression Rating Scale; ellipses, cannot be calculated. Desipramine was given as desipramine hydrochloride.

Table 2. Changes in Setoperone Binding During Desipramine Treatment in Depressed Patients*

Cluster			Voxels									
Size	z Score	Corrected P	z Score	Corrected P	¹⁸ F-Setoperone Binding			Coordinates†			Brain Regions	
					Pretreatment	Posttreatment	Change, %	x	y	z		
26 695	4.53	<.001	4.53	.007	1.5626 ± 0.0514	1.3526 ± 0.0514	-13.3	-4	58	-2	Left medial frontal gyrus	
			4.51	.008	1.4617 ± 0.0590	1.3407 ± 0.0572	-8.2	-32	26	-14	Left inferior frontal gyrus	
			4.50	.008	1.5003 ± 0.0941	1.3281 ± 0.0938	-11.4	-10	46	18	Left medial frontal gyrus	
			4.18	.03	1.6116 ± 0.0403	1.3966 ± 0.0402	-13.2	16	-64	4	Right lingual gyrus	
			4.17	.03	1.6981 ± 0.0538	1.4281 ± 0.0536	-15.7	-4	46	-16	Left orbitomedial frontal gyrus	
			4.10	.04	1.3829 ± 0.0971	1.2440 ± 0.0971	-9.9	26	26	-14	Right inferior frontal gyrus	
			4.10	.04	1.3316 ± 0.0313	1.2237 ± 0.0310	-8.0	28	-74	12	Right middle occipital gyrus	
			4.08	.04	1.3451 ± 0.0723	1.2192 ± 0.0721	-9.2	-46	-44	-6	Left middle temporal gyrus	
			4.04	.05	1.6257 ± 0.0338	1.4290 ± 0.0338	-11.9	-60	-16	-16	Left inferior temporal gyrus	

*¹⁸F-setoperone indicates fluorine 18-labeled setoperone. Desipramine was given as desipramine hydrochloride.

†Coordinates are in millimeters from the origin at the midpoint of anterior commissure, in the coordinate frame employed in statistical parametric mapping.

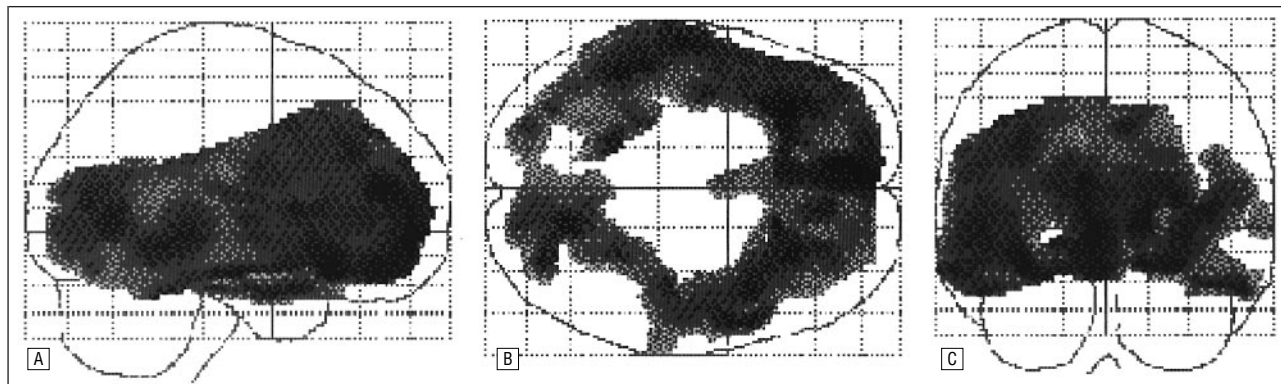


Figure 1. Statistical parametric maps of t values displayed as projections on the sagittal (A), transverse (B), and coronal (C) renderings of the brain. These projections illustrate regions of decreased binding of fluorine 18-labeled setoperone following desipramine hydrochloride treatment.

ies of brain 5-HT₂ receptors in depressed patients have shown a decrease in B_{max} without any alteration in K_d for 5-HT₂ receptors.³⁹⁻⁴³ Similarly, studies of the effects of antidepressant treatments on brain 5-HT₂ receptors in rodents also have shown a decrease in B_{max} without a change in K_d for 5-HT₂ receptors.^{4,7,8,13,17,18} Therefore, the decrease in setoperone binding observed in depressed patients following desipramine treatment in this study is

likely to be due to a decrease in B_{max}, indicating a decrease in 5-HT₂ receptor density.

Second, although desipramine does not inhibit 5-HT reuptake, long-term treatment with desipramine might lead to an increase in brain 5-HT levels through some other mechanism. An increase in brain 5-HT levels could occupy 5-HT₂ receptors, leaving a decreased number of receptors available for setoperone binding, thus account-

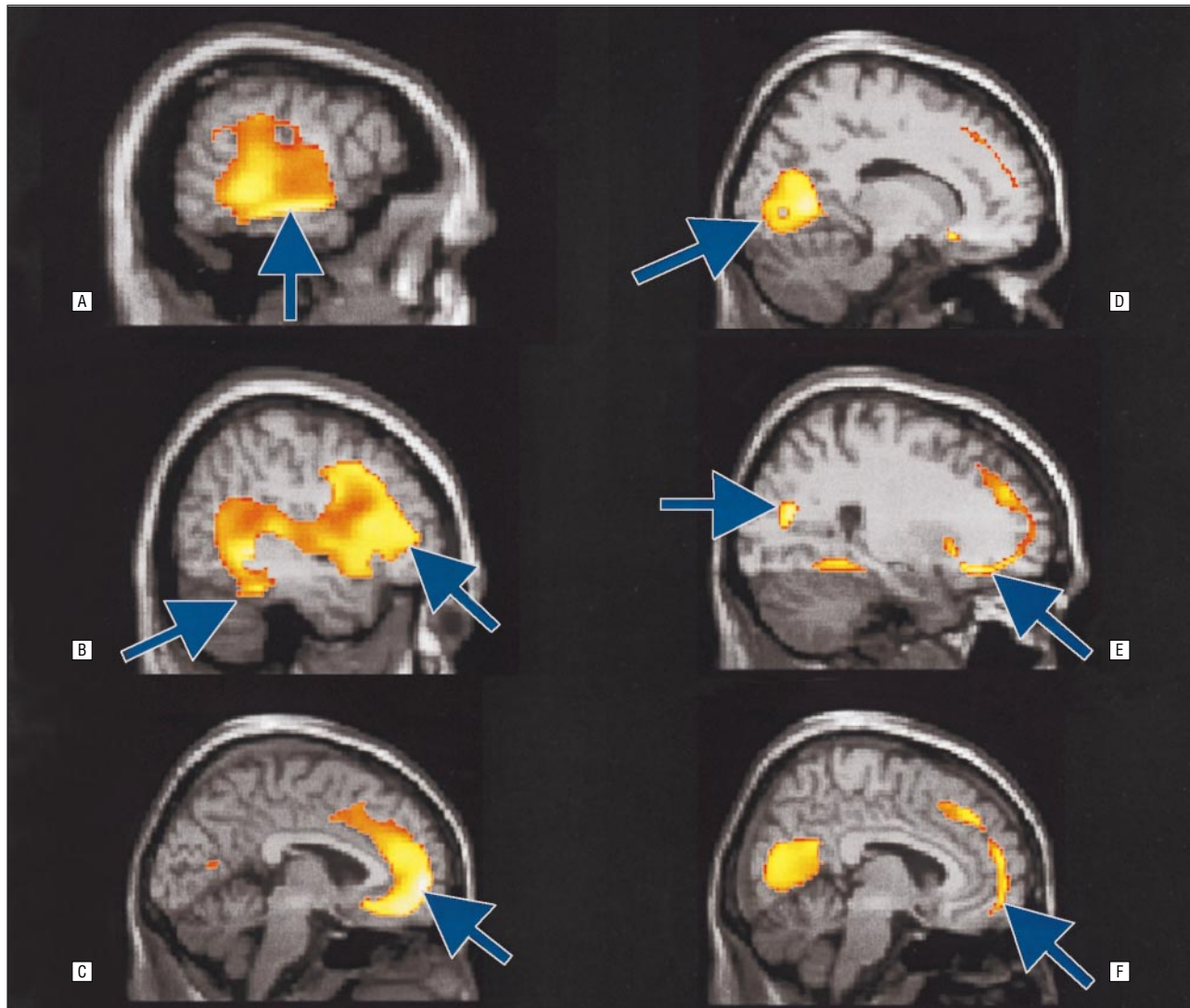


Figure 2. Areas of significant decreases in fluorine 18-labeled setoperone binding on the sagittal renderings of the brain indicated by arrows (A, left inferior temporal gyrus; B, left middle temporal gyrus and left inferior frontal gyrus; C, left medial frontal gyrus; D, right lingual gyrus; E, right middle occipital gyrus and right inferior frontal gyrus; and F, right inferior frontal gyrus) in depressed patients following desipramine hydrochloride treatment.

ing for a decreased binding we observed. However, a recent PET study⁴⁴ using ¹⁸F-setoperone reported no significant change in 5-HT₂ receptor binding following an acute challenge dose of paroxetine, the most potent 5-HT reuptake inhibitor in healthy subjects. This would suggest that elevation in 5-HT levels has no measurable impact on 5-HT₂ receptor binding as assessed with PET, thus suggesting that decreased setoperone binding observed in the study subjects is not due to a confounding effect of elevation in endogenous brain 5-HT levels.

Third, since patients underwent scanning while receiving treatment with desipramine, we cannot exclude the possibility that the decrease in setoperone binding is due to desipramine binding to 5-HT₂ receptors and not due to a decrease in 5-HT₂ receptor density. However, this is unlikely, because desipramine binding would be expected to occur wherever there are 5HT₂ receptors, but we observed no substantial global change in setoperone binding.

Our study has some limitations. First, although setoperone binds with higher affinity to 5-HT_{2A} receptors

($K_i = 0.37$ nmol/L),³⁴ it also has some affinity ($K_i = 50$ nmol/L) to 5-HT_{2C} receptors,⁴⁵ and hence we cannot tell whether a reduction in binding observed is due to a down-regulation of 5-HT_{2A} or 5-HT_{2C} or both receptor populations. Second, most patients in our study improved by the time the second PET scan was performed. Hence, we cannot tell if the decrease in 5-HT₂ receptor binding is due to clinical improvement that may or may not have been induced by desipramine or due to an effect of desipramine that was unrelated to clinical improvement. The fact that all 10 patients showed a reduction in 5-HT₂ receptor binding but only 8 improved substantially supports the latter possibility. Conversely, in a preliminary PET study,⁴⁶ we reported that electroconvulsive therapy, another effective treatment for depression, induced a significant decrease in 5-HT₂ receptor binding in all 6 depressed patients who improved, supporting the former possibility.

Based on our finding of a decrease in 5-HT₂ receptor binding following desipramine treatment, one would expect an increase in 5-HT₂ receptor binding to be associated with depression. In this regard, several^{39-42,47} al-

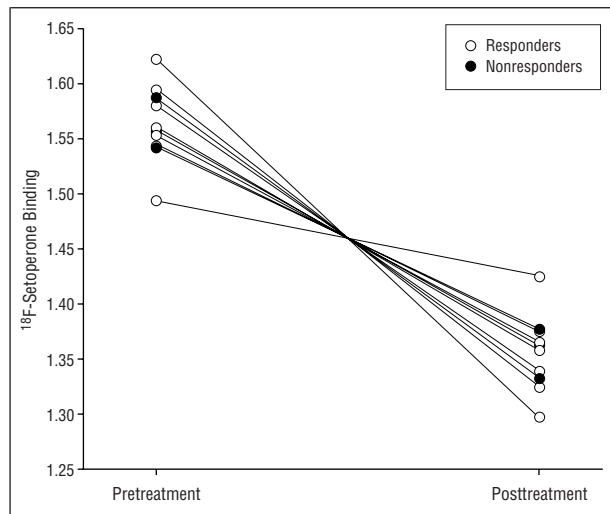


Figure 3. Binding of fluorine 18-labeled setoperone (^{18}F -setoperone) in left medial frontal gyrus ($x = -4, y = 58, z = -2$) for 10 depressed patients (8 responders and 2 nonresponders) before and after treatment with desipramine hydrochloride.

though not all⁴⁸⁻⁵¹ postmortem studies that examined 5-HT₂ receptor binding in brain samples from suicides reported increased B_{max} with no changes in K_d in various brain regions, particularly in frontal cortex, compared with control subjects. Similarly, 5-HT₂ receptor binding was also reported to be increased in postmortem brain samples of depressed patients who died of natural causes, compared with controls,^{43,52} although not in all studies.⁴⁸ Of the 2 studies that assessed brain 5-HT₂ receptor density in living, depressed patients, the single-photon emission tomography scan study with 2-iodine 123-ketanserin reported an increase in B_{max} within parietal cortex and right inferior frontal region,⁵³ whereas the PET study by Biver et al⁵⁴ using [^{18}F]altanserin reported a decrease in 5-HT₂ receptor binding in the right posterolateral orbitofrontal cortex and anterior insular cortex in depressed patients compared with controls. The discrepancy in findings between the latter study and previous single-photon emission tomography and postmortem studies that showed an increased 5-HT₂ receptor binding in depression might be related to differences in duration of drug-free periods before ascertaining 5-HT₂ receptor binding.

In conclusion, to our knowledge, we demonstrated for the first time in humans that depressed patients show a decrease in brain 5-HT₂ ligand binding following desipramine treatment. Our results, however, cannot tell us whether the decrease in 5-HT₂ receptor binding was related to clinical improvement or to an effect of desipramine that was unrelated to clinical status. Further studies assessing the effects of antidepressant treatment on brain 5-HT₂ receptors in depressed patients are needed to verify the hypothesis that 5-HT₂ receptors are an important target for antidepressant therapies.

Accepted for publication April 13, 1999.

This study was supported by a National Alliance for Research on Schizophrenia and Depression Young Investigator Award (Dr Yatham).

Preliminary results of this study were presented at the 21st Collegium Internationale Neuro-Psychopharmacologic Congress, Glasgow, Scotland, July 13, 1998.

Reprints: Lakshmi N. Yatham, MBBS, FRCPC, Mood Disorders Clinical Research Unit, The University of British Columbia, 2255, Wesbrook Mall, Vancouver, British Columbia, Canada V6T 2A1 (e-mail: yatham@unixg.ubc.ca).

REFERENCES

1. Stahl SM. Antidepressants and mood stabilizers. In: Stahl SM, ed. *Essential Psychopharmacology: Neuroscientific Basis and Practical Applications*. New York, NY: Cambridge University Press; 1996:131-166.
2. Maes M, Meltzer HY. The serotonin hypothesis of major depression. In: Bloom F, Kupfer D, eds. *Psychopharmacology: The Fourth Generation of Progress*. New York, NY: Raven Press; 1995:933-944.
3. Smith KA, Cowen PJ. Serotonin and depression. In: Honig A, Van Praag HM, eds. *Depression: Neurobiological, Psychopathological and Therapeutic Advances*. New York, NY: John Wiley & Sons Inc; 1997:129-146.
4. Peroutka SJ, Snyder SH. Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. *Science*. 1980;210:88-90.
5. Peroutka SJ, Snyder SH. Regulation of serotonin-2 (5-HT₂) receptors labeled with [^3H]spiroperidol by chronic treatment with the antidepressant amitriptyline. *J Pharmacol Exp Ther*. 1980;215:582-587.
6. Kellar KJ, Casio CS, Butler JA, Kurtzke RN. Differential effects of electroconvulsive shock and antidepressant drugs on serotonin-2 receptors in rat brain. *Eur J Pharmacol*. 1981;69:515-518.
7. Blackshear MA, Sanders-Bush E. Serotonin receptor sensitivity after acute and chronic treatment with mianserin. *J Pharmacol Exp Ther*. 1982;221:303-308.
8. Goodwin GM, Green AR, Johnson P. 5-HT₂ receptor characteristics in frontal cortex and 5-HT₂ receptor-mediated head twitch behavior following antidepressant treatment to mice. *Br J Pharmacol*. 1984;83:235-242.
9. Sanders-Bush E, Breeding M, Knoth K, Tsutsumi M. Sertraline-induced desensitization of the serotonin 5HT-2 receptor transmembrane signaling system. *Psychopharmacology*. 1989;99:64-69.
10. Welner SA, De Montigny C, Desroches J, Desjardins P, Suranyi-Cadotte BE. Autoradiographic quantification of serotonin1A receptors in rat brain following antidepressant drug treatment. *Synapse*. 1989;4:347-352.
11. Newman ME, Drummer D, Lerer B. Single and combined effects of desipramine and lithium on serotonergic receptor number and second messenger function in rat brain. *J Pharmacol Exp Ther*. 1990;252:826-831.
12. Eison AS, Yocca FD, Gianutsos G. Effect of chronic administration of antidepressant drugs on 5-HT₂-mediated behavior in the rat following noradrenergic or serotonergic denervation. *J Neural Transm Gen Sect*. 1991;84:19-32.
13. Klimek V, Zak-Knapik J, Mackowiak M. Effect of repeated treatment with fluoxetine and citalopram, 5-HT uptake inhibitors, on 5-HT_{1A} and 5-HT₂ receptors in the rat brain. *J Psychiatr Neurosci*. 1994;19:63-67.
14. Jolas T, Haj-Dahmane S, Kidd EJ, Langlois X, Lanfumey L, Fattaccini CM, Vantalon V, Laporte AM, Adrien J, Gozlan H. Central pre- and postsynaptic 5-HT_{1A} receptors in rats treated chronically with a novel antidepressant, cericlamine. *J Pharmacol Exp Ther*. 1994;268:1432-1443.
15. Le Poul E, Lima L, Laporte AM, Even C, Doucet E, Fattaccini CM, Laaris N, Hamon M, Lanfumey L. Central serotonin receptors and chronic treatment with selective serotonin reuptake inhibitors in the rat: comparative effects of fluoxetine and paroxetine. *Encephale*. 1995;21:123-132.
16. Stolz JF, Marsden CA, Middlemiss DN. Effects of chronic antidepressant treatment and subsequent withdrawal on [^3H]-5-hydroxytryptamine and [^3H]-spiperone binding in rat frontal cortex and serotonin receptor mediated behavior. *Psychopharmacology*. 1983;80:150-155.
17. Wamsley JK, Byerley WF, McCabe RT, McConnell EJ, Dawson TM, Grosser BJ. Receptor alterations with serotonergic agents: an autoradiographic analysis. *J Clin Psychiatry*. 1987;48:19-25.
18. Paul IA, Duncan GE, Powell KR, Mueller RA, Hong JS, Breese GR. Regionally specific neural adaptation of beta adrenergic and 5-hydroxytryptamine₂ receptors after antidepressant administration in the forced swim test and after chronic antidepressant drug treatment. *J Pharmacol Exp Ther*. 1988;246:956-962.
19. Hrdina PD, Vu TB. Chronic fluoxetine treatment upregulates 5-HT uptake sites and 5-HT₂ receptors in rat brain: an autoradiographic study. *Synapse*. 1993;14:324-331.
20. Lundkvist C, Halldin C, Ginovart N, Nyberg S, Swahn CG, Carr AA, Brunner F, Farde L. [^{11}C]MDL 100907, a radioligand for selective imaging of 5-HT_{2A} receptors with positron emission tomography. *Life Sci*. 1996;58:187-192.

21. Dannals RF, Ravert HT, Wilson AA, Wagner HNJ. An improved synthesis of (3-N-[¹¹C]methyl)piperone. *Appl Radiat Isot.* 1986;37:433-434.
22. Crouzel C, Venet M, Irie T, Sanz G, Goullais C. Labelling of a serotonergic ligand with ¹⁸F-¹⁸F-setoperone. *J Labeled Compounds Radiopharmaceuticals.* 1988;25:403-414.
23. Biver S, Goldman S, Luxen A, Monclus M, Forestini M, Mendlewicz J, Lotstra F. Multicompartmental study of fluorine-18 altanserine binding to brain 5HT₂ receptors in humans using positron emission tomography. *Eur J Nucl Med.* 1994;21:937-946.
24. Crouzel C, Guillaume M, Barre L, Lemaire C, Pike VW. Ligands and tracers for PET studies of the 5-HT system: current status. *Nucl Med Biol.* 1992;19:857-870.
25. Kapur S, Zipursky R, Remington G, Jones C, McKay G, Houle S. PET evidence that loxapine is an equipotent blocker of 5-HT₂ and D₂ receptors: implications for the therapeutics of schizophrenia. *Am J Psychiatry.* 1997;154:1525-1529.
26. Kapur S, Zipursky RB, Remington G, Jones C, DaSilva J, Wilson AA, Houle S. 5-HT₂ and D₂ receptor occupancy of olanzapine in schizophrenia: a PET investigation. *Am J Psychiatry.* 1998;155:921-928.
27. Trichard C, Paillere-Martinot ML, Attar-Levy D, Recassens C, Monnet F, Martinot JL. Binding of antipsychotic drugs to cortical 5-HT_{2A} receptors: a PET study of chlorpromazine, clozapine, and amisulpride in schizophrenic patients. *Am J Psychiatry.* 1998;155:505-508.
28. Trichard C, Paillere-Martinot ML, Attar-Levy D, Blin J, Feline A, Martinot JL. No serotonin 5-HT_{2A} receptor density abnormality in the cortex of schizophrenic patients studied with PET. *Schizophr Res.* 1998;31:13-17.
29. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.* Washington, DC: American Psychiatric Association; 1994.
30. Spitzer RL, Williams JBW, Gibbon M, First MB. *Structured Clinical Interview for DSM-III-R, Patient Edition.* Washington, DC: American Psychiatric Association; 1990.
31. Hamilton M. A rating scale of depression. *J Neurol Neurosurg Psychiatry.* 1960;23:56-62.
32. Adam MJ, Lu S, Jivan S, Huser J. Simplified purification and synthesis of F-18 setoperone. *J Labeled Compounds Radiopharmaceuticals.* 1997;40:258.
33. Pietrzyk U, Herholz K, Fink G, Jacobs A, Mielke R, Slansky I, Würker M, Heiss WD. An interactive technique for three-dimensional image registration: validation for PET, SPECT, MRI, and CT brain studies. *J Nucl Med.* 1994;35:2011-2018.
34. Blin J, Sette G, Fiorelli M, Bletry O, Elghozi JL, Crouzel C, Baron JC. A method for the in vivo investigation of the serotonergic 5-HT₂ receptors in the human cerebral cortex using positron emission tomography and ¹⁸F-labeled setoperone. *J Neurochem.* 1990;54:1744-1754.
35. Friston KJ, Frith CD, Liddle PF, Frackowiak RSJ. Comparing function (PET) images: the assessment of significant change. *J Cereb Blood Flow Metab.* 1991;11:690-699.
36. Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. *Hum Brain Mapp.* 1995;2:189-210.
37. Petit-Taboue M-C, Landeau B, Barre L, Onfroy M-C, Noel M-C, Baron J-C. Parametric PET imaging of 5HT_{2A} receptor distribution with ¹⁸F-setoperone in the normal human neocortex. *J Nucl Med.* 1999;40:25-32.
38. Worsley KJ. Local maxima and the expected Euler characteristic of excursion sets of χ^2 , F and t fields. *Adv Appl Probability.* 1994;26:13-42.
39. Stanley M, Mann JJ. Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet.* 1983;1:214-216.
40. Mann JJ, Stanley M, McBride A, McEwan BS. Increased serotonin₂ and β -adrenergic receptor binding in the frontal cortices of suicide victims. *Arch Gen Psychiatry.* 1986;43:954-959.
41. Arora RC, Meltzer HY. Serotonergic measures in the brains of suicide victims: 5-HT₂ binding sites in the frontal cortex of suicide victims and control subjects. *Am J Psychiatry.* 1989;146:730-736.
42. Hrdina PD, Demeter E, Vu TB, Sotonyi P, Palkovits M. 5-HT uptake sites and 5-HT₂ receptors in brain of antidepressant-free suicide victims/depressives: increase in 5-HT₂ sites in cortex and amygdala. *Brain Res.* 1993;614:37-44.
43. Yates M, Leake A, Candy JM, Fairbairn AF, McKeith JG, Ferrier IN. 5HT₂ receptor changes in major depression. *Biol Psychiatry.* 1990;27:489-496.
44. Cho R, Kapur S, Jones C, Kennedy S, Zipursky RB. PET comparison of 5-HT₂ receptor blockade by SSRIs: nefazodone vs paroxetine. *Biol Psychiatry.* 1998;43(suppl):18S-19S.
45. Hoyer D. Functional correlates of serotonin 5-HT₁ recognition sites. *J Recept Res.* 1988;8:59-81.
46. Yatham LN, Dennie J, Lane C, Shiah IS, Liddle PF. A PET study of effects of desipramine or ECT on 5-HT₂ receptors in depression. Paper presented at: the 21st Collegium Internationale Neuro-Psychopharmacologicum (CINP) Congress; Glasgow, Scotland; July 13, 1998:278.
47. Arango V, Ernsberger P, Marzuk PM, Chen JS, Tierney H, Stanley M, Reis DJ, Mann JJ. Autoradiographic demonstration of increased serotonin 5-HT₂ and β -adrenergic receptor binding sites in the brain of suicide victims. *Arch Gen Psychiatry.* 1990;47:1038-1047.
48. Crow TJ, Cross AJ, Cooper SJ, Deakin JFW, Poulter M, Lofthouse R, Corsellis JAN, Chambers DR, Blessed G, Perry EK, Perry RH, Tomlinson BE. Neurotransmitter receptors and monoamine metabolites in the brains of patients with Alzheimer-type dementia and depression, and suicides. *Neuropharmacology.* 1984;23:1561-1569.
49. Cheetham SC, Crompton MR, Katona CLE, Horton RW. Brain 5HT₂ receptor binding sites in depressed suicide victims. *Brain Res.* 1988;443:272-280.
50. Lowther S, De Paermentier F, Crompton MR, Katona CLE, Horton RW. Brain 5-HT₂ receptors in suicide victims: violence of death, depression and effects of antidepressant treatment. *Brain Res.* 1994;642:281-289.
51. Stockmeier CA, Dilley GE, Shapiro LA, Overholser JC, Thompson PA, Meltzer HY. Serotonin receptors in suicide victims with major depression. *Neuropsychopharmacology.* 1997;16:162-173.
52. McKeith IG, Marshall EF, Ferrier IN, Armstrong MM, Kennedy WN, Perry RH, Perry EK, Eccleston D. 5-HT receptor binding in post-mortem brain from patients with affective disorder. *J Affect Disord.* 1987;13:67-74.
53. D'haenen H, Bossuyt A, Mertens J, Bossuyt-Piron C, Gijsemans M, Kaufman L. SPECT imaging of serotonin₂ receptors in depression. *Psychiatr Res Neuroimag.* 1992;45:227-237.
54. Biver F, Wikler D, Lotstra F, Damhaut P, Goldman S, Mendlewicz J. Serotonin 5-HT₂ receptor imaging in major depression: focal changes in orbito-insular cortex. *Br J Psychiatry.* 1997;171:444-448.