High Levels of Serotonin Transporter Occupancy With Low-Dose Clomipramine in Comparative Occupancy Study With Fluvoxamine Using Positron Emission Tomography

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Context: Serotonin transporters (5-HTT) are regarded as one of the major therapeutic targets of antidepressants. However, there have only been a few studies about 5-HTT occupancy, and in particular, data concerning classical antidepressants are still limited.

Objective: To investigate the relationship between 5-HTT occupancy and a wide range of antidepressant dosing protocols.

Design, Setting, and Participants: Antidepressant occupancies of 5-HTT were measured using positron emission tomography (PET) with [11C](+)McN5652. Twenty-seven healthy volunteers were measured with and without pretreatment with single low doses of antidepressants, and long-term doses were evaluated in 10 patients. Scan data were collected between December 12, 1995, and August 7, 2002, and data were analyzed during the 2001-2002 period at the National Institute of Radiological Sciences (Chiba, Japan).

Intervention: Four different doses of clomipramine hydrochloride (5-50 mg) and 3 different doses of fluvoxamine maleate (12.5-50 mg) were used for single administration. Long-term doses were 20 to 250 mg per day for clomipramine hydrochloride, and 25 to 200 mg per day for fluvoxamine maleate.

Main Outcome Measure: Occupancies in the thalamus were calculated using the individual baseline of [11C](+)McN5652 for single-dose studies and 2 long-term–dose studies, and the mean value of healthy volunteers as the baseline for 8 long-term–dose studies. The average data from inactive enantiomers [11C](−) McN5652 were used for the estimation of nonspecific binding.

Results: Occupancy of 5-HTT increased in a curvilinear manner. Even 10 mg of clomipramine hydrochloride showed approximately 80% occupancy, which was comparable with that of 50 mg of fluvoxamine maleate. Estimated median effective dose (ED50) of clomipramine hydrochloride was 2.67 mg for oral dose and 1.42 ng/mL for plasma concentration; those of fluvoxamine maleate were 18.6 mg and 4.19 ng/mL, respectively.

Conclusions: Clinical doses of clomipramine and fluvoxamine occupied approximately 80% of 5-HTT, and dose escalation would have minimal effect on 5-HTT blockade. Ten milligrams of clomipramine hydrochloride was enough to occupy 80% of 5-HTT in vivo.

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occupancy by less selective ligands of 5-HTT, such as $[^{125}]$-labeled CIT, requires some circumpection, and the baseline of the calculation of occupancy also needs to be addressed with some caution because of the possible difference in nonspecific binding. A recent study using $[^{11}]$-iodo-DASB [3-$[^{11}]$-amino-4-(2-dimethylaminomethylphenylsulphonyl)benzonitrile] reported approximately 85% 5-HTT occupancy during treatment with 10 to 20 mg/d of paroxetine hydrochloride and 20 mg/d of citalopram hydrobromide. However, the relationship with low doses is not yet clear, and no data are as yet available on 5-HTT occupancy with classic tricyclic antidepressants like clomipramine.

McN5652 (trans-1,2,3,5,6,10-hexahydro-6-[4-(methylthio)phenyl]pyrrolo[2,1-a]isoquinoline) is a selective serotonin reuptake inhibitor that has nanomolar affinity for serotonin 5-HT transporters. Its pharmacologically active enantiomer, (+)McN5652, has at least 2 orders of magnitude higher affinity than its pharmacologically inactive enantiomer, (–)McN5652. (+)McN5652 is currently being used as a PET tracer for 5-HTT with good test-retest reproducibility. How- ever, a relatively high fraction of nonspecific binding and occupancies accurately. Nevertheless, by the use of (+)McN5652, we have been able to calculate 5-HTT occupancy despite the regional differences of nonspecific binding.

As for pharmacological evidence regarding the clinical dose of antidepressants, there have been only limited in vivo data, especially in relationship to 5-HTT occupancy. The purpose of this study was to investigate the precise relationship between 5-HTT occupancy and the dose of the classic tricyclic antidepressant clomipramine, one of the SSRIs, fluvoxamine maleate, with a wide range of dosing protocols using $[^{11}]$-labeled McN5652.

### Methods

#### Subjects

Twenty-seven healthy male volunteers (mean±SD age, 22.0±2.3 years; range, 20-29 years) participated in single low-dose studies, and 10 patients (mean±SD age, 35.7±12.1 years; range, 21-55 years) with psychiatric disorders who were receiving long-term treatment with antidepressants were included in this study (Table 1 and Table 2). All healthy volunteers were recruited from among university students and hospital employees. They filled out a questionnaire about their medical history and medications, and were then interviewed by a medical staff member. They had no history of present or past psychiatric, neurological, or somatic disorders, and no alcohol- or drug-related problems. They had not taken any kind of medication for at least 2 weeks prior to the start of the study. Six patients (age range, 21-50 years) had been taking clomipramine, 4 patients (age range, 24-55 years) had been taking fluvoxamine, and 6 of the 10 patients were also taking 1 or 2 benzodiazepines as anxiolytic or hypnotic treatments. The doses, duration of treatment, and diagnoses are presented in Table 2.

This study was approved by the ethics and radiation safety committees of the National Institute of Radiological Sciences (Chiba, Japan). Written informed consent was obtained from each subject.

### PET Measurement

$[^{11}]$-labeled McN5652 and $[^{11}]$-labeled McN5652 were synthesized by S-methylation of the corresponding desmethyl precursor, which was stabilized by adding a protecting agent for mercapto groups, dithiothreitol, into the reaction medium immediately after the demethylation of McN5652 by an automated procedure. The radiochemical purity was higher than 95%.

For 34 of the 37 subjects, PET scans were performed using a Siemens ECAT47 system (CTI-Siemens, Knoxville, Tenn), which provides 47 slices with 3.375-mm (center-to-center) thickness. Radioactivity was measured in 2-dimensional mode, and the data were reconstructed with a ramp filter with a cut-off frequency of 0.5 (full-width half-maximum [FWHM], 6.3 mm). The other 3 subjects were measured using an EXACT HR+ scanner (CTI-Siemens, Knoxville, Tenn), which pro-

### Table 1. Summary of Single-Dose Studies

<table>
<thead>
<tr>
<th>Drug Dose, mg</th>
<th>No. of Doses</th>
<th>Mean (SD) Occupancy, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clomipramine hydrochloride</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>Fluvoxamine maleate</td>
<td>12.5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4</td>
</tr>
</tbody>
</table>

### Table 2. Summary of Long-term Dose Studies With Patients

<table>
<thead>
<tr>
<th>Drug Dose, mg/d</th>
<th>Duration of Treatment</th>
<th>Concentration, ng/mL</th>
<th>Diagnosis</th>
<th>Occupancy, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clomipramine hydrochloride</td>
<td>250 (qid)</td>
<td>3 mo</td>
<td>660</td>
<td>OCD</td>
</tr>
<tr>
<td></td>
<td>100 (qid)</td>
<td>8 mo</td>
<td>84</td>
<td>MDD</td>
</tr>
<tr>
<td></td>
<td>70 (tid)</td>
<td>3 mo</td>
<td>53</td>
<td>OCD</td>
</tr>
<tr>
<td></td>
<td>55 (qid)</td>
<td>2 mo</td>
<td>NA</td>
<td>MDD</td>
</tr>
<tr>
<td></td>
<td>20 (bid)</td>
<td>2 wk</td>
<td>17</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>20 (qd)</td>
<td>1 wk</td>
<td>11</td>
<td>AD</td>
</tr>
<tr>
<td>Fluvoxamine maleate</td>
<td>200 (bid)</td>
<td>5 mo</td>
<td>143</td>
<td>MDD</td>
</tr>
<tr>
<td></td>
<td>100 (bid)</td>
<td>3 wk</td>
<td>NA</td>
<td>MDD</td>
</tr>
<tr>
<td></td>
<td>75 (tid)</td>
<td>6 mo</td>
<td>54</td>
<td>BPD</td>
</tr>
<tr>
<td></td>
<td>25 (qd)</td>
<td>3 wk</td>
<td>NA</td>
<td>AD</td>
</tr>
</tbody>
</table>

Abbreviations: AD, anxiety disorder; bid, twice daily; BPD, bipolar disorder; MDD, major depressive disorder; NA, not applicable; OCD, obsessive-compulsive disorder; qd, daily; qid, 4 times daily; tid, 3 times daily.
vides 63 planes and a 15.5-cm field-of-view, and the data were reconstructed with a Hanning filter cut-off frequency 0.4 (FWHM, 7.5 mm). The subjects were placed in the supine position with eyes closed and ears unplugged. To minimize head movement during each scan, head fixation devices (Fissler Instruments, Stockholm, Sweden) and thermoplastic attachments made to fit the individuals were used. A transmission scan of 10 minutes with a germanium 68–gallium 68 source was followed by a dynamic scan for 90 minutes with a bolus injection of 581 to 20.51 mCi (215–759 MBq) of [11C](+) McN5652. The specific radioactivities ranged from 0.31 to 5.25 Ci/µmol (11.6–194.3 GBq/µmol) (mean ± SD radioactivity, 2.61 ± 1.19 Ci/µmol [96.4 ± 44.1 GBq/µmol]) at the time of injection. A dynamic scan for 90 minutes with a bolus injection of 12.30 to 19.86 mCi (455–735 MBq) (mean ± SD injection, 18.11 ± 2.68 mCi [670–99 MBq]) of [11C](−)McN5652 was performed on 14 healthy volunteers. The specific radioactivities were 0.69 to 3.66 Ci/µmol (25.6–135.6 GBq/µmol) (mean, 2.11 ± 0.95 [77.9 ± 33.2]) at the time of injection.

To measure the occupancy by a single administration of antidepressants, 13 healthy volunteers took part in the clomipramine study. Initial PET scans were carried out to establish the baseline data. Second PET scans were performed 5 hours after orally taking 5 to 50 mg of clomipramine hydrochloride (2 volunteers for 5 mg; 2 for 10 mg; 3 for 25 mg; and 6 for 50 mg) (Table 1). Blood samples (10 mL) were taken to measure the plasma concentration of clomipramine just before tracer injection. Fourteen healthy volunteers took part in the fluvoxamine study. Initial PET scans were carried out to attain the baseline data. Second PET scans were performed 5 hours after oral administration of different doses of fluvoxamine maleate (3 volunteers at 12.5 mg; 7 at 25 mg; 4 at 50 mg) (Table 1). Three subjects were scanned with the EXACT HR+ scanner for first and second scans having been given 25 mg of fluvoxamine maleate. Blood samples (10 mL) were taken to measure the plasma concentration of fluvoxamine just before tracer injection.

The plasma concentration of clomipramine was measured using high-performance liquid chromatography (HPLC) with ultraviolet detection, and that of fluvoxamine was measured using high-performance liquid chromatography (HPLC) and a mass spectrometer.

To measure 5-HTT occupancy by long-term doses of antidepressants, the 10 patients were measured for 3-HHT binding during long-term treatment with clomipramine or fluvoxamine (Table 2). The PET scans were performed 7 hours after intake of fluvoxamine for 2 patients; 4 hours for 1 patient; 1 hour for 1 patient. Scans were performed 10 hours after intake of clomipramine for 1 patient; 4 hours for 3 patients; and 1 hour for 2 patients. Blood samples (10 mL) were taken to measure their plasma concentrations just before tracer injection. One of the patients taking clomipramine and 2 of the patients taking fluvoxamine refused the blood sampling.

DATA ANALYSIS

Positron emission tomographic images summed for 90 minutes were coregistered to magnetic resonance imaging (MRI) data with SPM99 (Wellcome Department of Cognitive Neurology, London, England), and regions of interest were defined over the cerebellum and thalamus based on these coregistered MRI and PET images. Because the specific binding of [11C](+)McN5652 in the cerebral cortex is too low for quantitative analysis,10,11 we focused on the thalamus to quantify its specific binding. Binding potential (BP) was used for the quantification that is expressed as k3 /k4 in compartment models. In the 3-compartment model, K1 was used to describe the uptake of the tracer across the blood-brain barrier; k5 represented back diffusion from tissue to the vascular space; and k3 and k4 described the binding and dissociation of the radioligand at the specific binding site. The ratio k3/k4 is expressed by the equation

\[ k_3/k_4 = \text{DVR} - 1, \]

where DVR is the distribution volume ratio based on the assumption that K1/k2 in the target tissue is equal to that in the reference tissue. However, the amount of nonspecific binding in the target tissue (the thalamus) was different from that in the reference tissue (cerebellum) with [11C](+)McN5652.11 The DVR of the target tissue to the reference tissue is given by the following equations for [11C](+)McN5652 and the pharmacologically inactive enantiomer [11C](−)McN5652:

\[ \text{DVR}(+) = \frac{K_{1r+}}{K_{2r+}}, \]

\[ \text{DVR}(−) = \frac{K_{1r−}}{K_{2r−}}, \]

respectively, where K1r+, K1r−, K2r+, and K2r− are the estimated parameters of [11C](+)-McN5652 for the target tissue; K1r+ and K2r+ are the estimated parameters of [11C](+)-McN5652 for the reference tissue without a specific binding site, and K1r−, K2r−, K1r+, and K2r− are those of [11C](−)-McN5652. Then k3+/k4− is expressed as

\[ \frac{k_{3+}}{k_{4−}} = \frac{\text{DVR}(+) - 1}{\text{DVR}(−)}. \]

If it is assumed that (K1r+/k2r−)/(K1r−/k2r+) is equal to DVR(−), then BP can be derived from the equation

\[ \text{BP} = \frac{\text{DVR}(+) - 1}{\text{DVR}(−)} = 1, \]

where the DVR of the thalamus to the cerebellum was calculated without arterial input function by the graphical method.11 This assumption is based on the result that the value of DVR(+) in the saturation study of [11C](+)-McN5652 was almost equal to DVR(−).11

CALCULATION OF 5-HT OCCUPANCY

Serotonin transporter occupancy (Occu [%]) using [11C](+)-McN5652 is expressed as

\[ \text{Occu} (%) = 100 \cdot \left( \frac{\text{BP}_{\text{baseline}} - \text{BP}_{\text{drug}}}{\text{BP}_{\text{baseline}}} \right) = 100 \cdot \left( \frac{[1-(\text{DVR}(+)_{\text{drug}} - \text{DVR}(−))]/(\text{DVR}(+)_{\text{baseline}} - \text{DVR}(−))} \right), \]

where DVR(+) and DVR(−) are the distribution volume ratios of [11C](+)-McN5652 and [11C](−)-McN5652 as estimated by graphical method with the reference tissue17, the test-retest variability and reliability (intraclass correlation coefficient) of [11C](+)-McN5652 for this graphical method were 10.8% and 0.83, respectively, based on
the 10-subject data (unpublished data). In this study, the mean ± SD value of DVR(−) was estimated as 1.16 ± 0.06 based on the data of 14 healthy volunteers (mean ± SD age, 23.4 ± 4.1 years), and it was used as a fixed value for the calculation of occupancy since not all subjects underwent the PET scans with [11C](−)McN5652.

In 8 of the 10 patients studied, the mean ± SD value of DVR(+)_baseline for the 27 healthy volunteers (1.89 ± 0.21 [range, 1.59-2.40]) was used for the calculation because the individual DVR(+)_baseline values were not available for these 8 patients.

The relationship between 5-HTT occupancy and the dose or plasma concentration of antidepressants was modeled by the equation

$\text{5-HTT}_{\text{occ}} = \frac{100 \cdot D}{(ED_{50} + D)}$,  

where 5-HTT$_{\text{occ}}$ is 5-HTT occupancy, ED$_{50}$ (the in vivo median effective dose) is a constant, and D is the concentration of the drug near the 5-HTT. Dose and plasma concentration were used as functional surrogates of D.\(^{18}\)

### RESULTS

The occupancy of 5-HTT in the thalamus was dose-dependently increased both by clomipramine and fluvoxamine (Table 1, Figure). In the single-dose studies, clomipramine occupied more than 60% of 5-HTT even with the lowest dose of 5 mg (Table 1 and Figure). The ranges of occupancy were from 61.3% to 100% between 5 mg and 50 mg of clomipramine hydrochloride and from 7.7% to 87.7% between 12.5 mg and 50 mg of fluvoxamine maleate. There were hyperbolic relationships between 5-HTT occupancy and oral dose ($r=0.780$) and plasma concentration of clomipramine ($r=0.752$) (Figure) (2 of the plasma samples were under the detection limit and are not shown in the Figure). There were also hyperbolic relationships between 5-HTT occupancy and oral dose ($r=0.686$) and plasma concentration of fluvoxamine ($r=0.808$) (Figure).

The calculated ED$_{50}$ was 2.67 mg for oral dose and 4.20 ng/mL for plasma concentration of clomipramine, and that of fluvoxamine was 18.6 mg for oral dose and 4.19 ng/mL for plasma concentration.

In this study, we used the patient data with long-term dose to estimate 5-HTT occupancy. The occupancies from long-term dose ranged from 83.9% to 100% for clomipramine and 76.6% to 93.6% for fluvoxamine (Table 2). Including the patient data, the saturation curves did not significantly deviate against the dose or plasma concentration (clomipramine: $r=0.724$; fluvoxamine: $r=0.722$) or plasma concentration (clomipramine: $r=0.726$; fluvoxamine: $r=0.835$). The calculated ED$_{50}$ including the patient data was 2.62 mg for oral dose and 1.40 ng/mL for plasma concentration of clomipramine. The calculated ED$_{50}$ of fluvoxamine was 17.4 mg for oral dose and 4.20 ng/mL for plasma concentration including the patient data.

### COMMENT

The present results demonstrated that 5-HTT was occupied by clomipramine or fluvoxamine in dose-dependent manners (Table 1), and curvilinear functions against drug concentration were clearly demonstrated (Figure). The present results showed marked high 5-HTT occupancy even with a small dose of clomipramine (Table 1 and Figure). Approximately 80% of 5-HTT was occupied with 10 mg of clomipramine hydrochloride (Table 1 and Figure). On the other hand, the 5-HTT occupancy of fluvoxamine increased slowly compared with that of clomipramine. The ED$_{50}$ values of fluvoxamine were several times larger than those of clomipramine. Although several different values using different methods have been reported, this order of ED$_{50}$ was generally consistent with the binding parameters in vitro.\(^{19,20}\) However, the daily clinical doses of those antidepressants were not so different between clomipramine hydrochloride (at 75-300 mg/d [50-100 mg/d in Japan]) and fluvoxamine maleate (at 100-300 mg/d [50-100 mg/d in Japan]).\(^4\)

The present results indicated that the variations in 5-HTT occupancy with low doses (12.5 mg and 23 mg) of fluvoxamine maleate were relatively large, but 50 mg of fluvoxamine maleate consistently occupied approximately 80% of 5-HTT (Table 1 and Figure). This suggested that at least 50 mg of fluvoxamine maleate was necessary to obtain certainly high 5-HTT occupancy (Table 2). These results of 5-HTT occupancy were concordant with those reporting that a dose of 50 to 150 mg of fluvoxamine maleate per day was therapeutically effective and that the minimally effective dose was 50 mg/d.\(^{21}\)
A recent study indicated that the clinical doses of paroxetine and citalopram occupy 70% to 80% of 5-HTT in living human brain. It seems that close to 80% of 5-HTT occupancy is necessary to obtain therapeutic effects. That is a value of occupancy similar to that reported for the dopamine D2 receptor with a clinical dose of antipsychotics. Furthermore, saturated 5-HTT with high doses of antidepressants (Table 2) can explain the fact that there was no relationship between the plasma concentration of fluvoxamine and clinical response during the treatment with a relatively high oral dose (200-300 mg/d or 150-300 mg/d).

On the other hand, ED50 of clomipramine was much smaller compared with the usual clinical dose. The present results showed that 10 mg of clomipramine hydrochloride would be enough for nearly 80% of 5-HTT occupancy. It has been reported that there was no relationship between clinical effects and plasma concentration of clomipramine at a fixed dose of 150 mg/d23,28 or different doses between 30 and 75 mg/d.27-29 These reports support the present results that clomipramine occupied close to 80% of 5-HTT with an oral dose of 10 mg and that only a minimal increase in 5-HTT occupancy could be expected with a high dose. However, some reports have suggested a relationship between clinical response and dose (25-200 mg20) or plasma concentration of clomipramine and its metabolite desmethylclomipramine.30,31 Other studies showed a relationship between clinical response and the plasma concentration of desmethylclomipramine only.25,32 Since desmethylclomipramine is a potent noradrenaline reuptake inhibitor and clomipramine has relatively high affinities to several receptors, mechanisms other than 5-HTT blockade can be supposed after sufficient saturation of 5-HTT.

Although the clinical merits of therapeutic drug monitoring of SSRIs are controversial,34 5-HTT occupancy correlates well with the plasma concentration as compared with the dose especially in the case of fluvoxamine (Figure). This suggested the possible use of therapeutic drug monitoring of SSRIs. If a patient does not respond well to an SSRI despite a high plasma concentration, this would be a good reason to switch antidepressants since there are many SSRI nonresponders.35,36 There would be no merit in a dose escalation in a highly saturated dose range.36 The beneficial alternative would be switching to a non-SSRI with different pharmacological properties.35,37,38 Clomipramine has been reported to show a better clinical outcome compared with citalopram or paroxetine in severely depressed hospitalized patients.39,40 A high dose of clomipramine can work on multiple neurotransmitter systems.

There are several limitations to the present study, especially with regard to the 5-HTT occupancy of the 8 patients being treated with antidepressants long-term. We used the mean DVR(+) baseline of the 27 healthy volunteers as the baseline. The variance of the calculated occupancy of 83.9% would be 73.3% to 90.7% using the extreme values of DVR(+) (1.59 and 2.40). This variance was similar to that of dopamine receptor occupancy using the average value as a baseline.22 However, since BPs of [11C] (+)McNS652 in the thalamus were higher in patients with mood disorders13 and no data are available for the 5-HTT of obsessive-compulsive disorders in vivo, some of the occupancy values calculated in this study might be underestimated or overestimated. In addition, the age effect of BPs was not considered in this study, since the effect of age in these different diseases was not clear. In this study we used the mean value of DVR(−) (1.16±0.06) from 14 healthy volunteers as an estimate of nonspecific binding. The estimation of nonspecific binding can affect the absolute value of occupancy.41 For example, the calculated occupancy of 82.6% can vary between 78.9% and 86.7% based on the deviation of DVR(−) value. Furthermore, if the nonspecific binding of the patients was different from that of the healthy volunteers, the estimated value of occupancy might be inaccurate.

In this study we have demonstrated that clinical doses of clomipramine and fluvoxamine occupied about 80% of 5-HTT, and that a small dose (such as 10 mg) of clomipramine can occupy about 80% of 5-HTT in vivo. However, further studies are needed to investigate the relationship between clinical effects and occupancy especially for the use of a long-term small dose of clomipramine.

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