Extrastriatal dopamine D2 receptor density and affinity in the human brain measured by 3D PET

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Abstract

The aim of the present study was to quantify the density and affinity of human extrastriatal dopamine D2 receptors using positron emission tomography (PET). [11C]FLB-457, a high-affinity dopamine D2 receptor antagonist with various specific radioactivities (SA) was used. Eight healthy male subjects, age 20–35 yr, participated twice or three times at different SAs (1–279 GBq/µmol), and serial dynamic scans were performed in the 3D data acquisition mode. The peak of the specific binding was not well defined with high SA due to the flatness of the curves after 60 min but was observed within the PET measurement. In the experiment with low SA, the peak came earlier than that with high SA. Scatchard analysis was performed using the maximal specific binding value (transient equilibrium) and the radioactivity in the cerebellum as free ligand concentration. The highest density was observed in the thalamus (2.3 ± 0.6 pmol/ml), followed by the temporal cortex (1.5 ± 0.5 pmol/ml), hippocampus (1.4 ± 0.5 pmol/ml), parietal cortex (0.9 ± 0.4 pmol/ml), frontal cortex (0.8 ± 0.2 pmol/ml) and occipital cortex (0.7 ± 0.3 pmol/ml). There was no significant difference in Kd values in these six regions. The present results demonstrate that dopamine D2 receptor densities in the extrastriatal regions were only 2–8% of that in the striatum. Although the density of extrastriatal dopamine D2 receptor was low, significant regional differences were observed in the present study, as reported in postmortem studies.

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Key words: Extrastriatal dopamine D2 receptor, Bmax, Kd, human brain, PET, [11C]FLB-457.

Introduction

Research on dopamine D2 receptors in schizophrenia using positron emission tomography (PET) or single photon emission tomography (SPECT) has almost exclusively focused on the striatum. Although the results are still controversial (Farde et al., 1990; Wong et al., 1986), most in vivo imaging studies measuring striatal dopamine D2 receptors in schizophrenia reported no significant difference between controls and neuroleptic-free schizophrenic patients (Hietala et al., 1994; Martinot et al., 1994; Nordström et al., 1995; Okubo et al., 1997).

The mesocortico-prefrontal dopaminergic neurons are of major interest in schizophrenia since they are important in emotional responses and cognitive processes (Glowinski et al., 1984). In a recent PET study we reported decreased dopamine D1 receptor binding in the prefrontal cortex of drug-naive and drug-free schizophrenic patients (Okubo et al., 1997). The reduction in the dopamine D1 receptor binding in the prefrontal cortex correlated with negative symptoms and cognitive deficits (Okubo et al., 1997). However, not only the extrastriatal D1 receptor but also the D2 receptor may be related to the pathophysiology of schizophrenia. Pharmacological studies indicated that the extrastriatal dopamine D2 receptor may be a common site of action of antipsychotics (Lidow and Goldman-Rakic, 1997).

In vivo imaging studies of extrastriatal dopamine D2 receptors have been limited by a lack of suitable radioligands for detection of the low density of receptors in the extrastriatal regions. The substituted benzamide such as FLB-457 [(S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-bromo-2,3-dimethoxybenzamide] and epidepride have very high affinity for dopamine D2 receptors in vitro (Halldin et al., 1995). [128I]-labelled epidepride has been introduced as an effective SPECT ligand for extrastriatal dopamine D2 receptors (Kessler et al., 1992; Kornhuber et al., 1995; Kuikka et al., 1997), and [11C]FLB-457 has been...
shown to provide a good signal in extrastriatal regions by PET (Farde et al., 1997). The shorter half-life of $^{13}$C (20 min) compared to $^{123}$I (13 h) can be an advantage for multiple experiments within a short period. The purpose of the present study was to measure the density and affinity of extrastriatal dopamine D2 receptors in the living human brain using PET.

Materials and methods

Subjects

Eight healthy male subjects, age 20–35 yr, with no history of drug- or alcohol-related problems, were recruited from university students and hospital employees. They had no medical history of somatic or psychiatric disorders. None of them showed brain abnormalities when examined by magnetic resonance imaging (MRI).

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.

Chemistry

The precursor for synthesis of $^{13}$CFLB-457 was kindly supplied by Astra Arcus (Södertälje, Sweden). FLB-457 was labelled with $^{13}$C by N-methylation of FLB-604 (2-hydroxy precursor) and purified automatically using specially designed equipment (Halldin et al., 1995; Suzuki et al., 1990). The radiochemical purity was higher than 99%. The specific radioactivities (SA), calculated by dividing the concentration of radioactivity (GBq/ml) by the concentration of FLB-457 ($\mu$mol/ml), were from $1$ to $279$ GBq/$\mu$mol at the time of injection (Table 1).

The concentration of FLB-457 was determined by injecting $300 \mu$l of the saline solution of $^{13}$CFLB-457 into a Diasil 5C18 column (4 mm i.d. x 150 mm, Chromatotec, Tokyo, Japan) under the following conditions: eluent, CH$_2$CN/0.1 M H$_2$PO$_4$ = 22/78; flow rate, 1.5 ml/min; UV, 210 nm ($R_t = 8.7$ min); and by comparing the peak area with the calibration curve obtained at the concentration ranges of 12.5, 125 and 1000 ng/ml.

Metabolite study

Rats were intravenously injected with $^{13}$CFLB-457 (about 100 MBq) and sacrificed at 30 and 70 min after tracer injection. The striatum was homogenized in 1 ml solution of H$_2$O/20% TCA in CH$_2$CN = 50/50 (v/v). After centrifugation at 15000 g for 1 min, the supernatant was examined using the HPLC system coupled to a radiodetector under the following conditions: column, $\mu$Bondapak C18 (300 x 7.5 mm i.d.); eluent, 0.01 M

Table 1. Description of $^{13}$CFLB-457 at time of i.v. injection

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Injection dose (MBq)</th>
<th>Scan duration (min)</th>
<th>Specific radioactivity (GBq/$\mu$mol)</th>
<th>Injected mass (nmol/body)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>141</td>
<td>90</td>
<td>184</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>325</td>
<td>90</td>
<td>15</td>
<td>21.0</td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td>108</td>
<td>90</td>
<td>135</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>373</td>
<td>90</td>
<td>11</td>
<td>33.5</td>
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<tr>
<td>C</td>
<td>23</td>
<td>131</td>
<td>60</td>
<td>279</td>
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<tr>
<td></td>
<td></td>
<td>288</td>
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<td>22</td>
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<td></td>
<td></td>
<td>328</td>
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<td>6</td>
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</tr>
<tr>
<td>D</td>
<td>26</td>
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<td>90</td>
<td>184</td>
<td>0.9</td>
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<td></td>
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<td>322</td>
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<td>5</td>
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<td>90</td>
<td>151</td>
<td>1.3</td>
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<td>217</td>
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<td>54.0</td>
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<td>F</td>
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<td>109</td>
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<td>83</td>
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<td></td>
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<td>183</td>
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<td>3</td>
<td>64.1</td>
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<td>70</td>
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<td>1</td>
<td>65.7</td>
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<tr>
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<td>32</td>
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<td>90</td>
<td>71</td>
<td>2.4</td>
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<tr>
<td></td>
<td></td>
<td>290</td>
<td>60</td>
<td>4</td>
<td>71.2</td>
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<tr>
<td>H</td>
<td>35</td>
<td>194</td>
<td>90</td>
<td>197</td>
<td>1.0</td>
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<tr>
<td></td>
<td></td>
<td>174</td>
<td>60</td>
<td>6</td>
<td>31.5</td>
</tr>
</tbody>
</table>
PET scan

Tomography was performed with an ECAT HR + scanner (CTI-Siemens, Knoxville, TN, USA) which provides 63 transaxial slices with an axial field of view of 15.5 cm (Brix et al., 1997). To ensure accuracy on re-positioning, the head of each subject was immobilized during measurement using a head fixation device with thermoplastic attachments for individual fit (Fixter Instruments, Stockholm, Sweden). A transmission scan for attenuation correction was performed using a $^{68}$Ge–$^{68}$Ga source before the first emission scan. Acquisitions were done in 3D mode with the interplane septa retracted (Brix et al., 1997).

Two ($n = 4$) or three ($n = 4$) PET scans were performed on each subject, using $[^{11}C]$FLB-457 with high SA (71–279 GBq/$\mu$mol) and middle or low SA (1–22 GBq/$\mu$mol) (Table 1). Two measurements were performed on the first experimental day. The first (with highest SA) and second (low SA) were performed at least 2 h apart. $[^{11}C]$FLB-457 was rapidly administered intravenously into the antecubital vein with a 10 ml saline flush at a radioactivity of 70–373 MBq (Table 1). Serial scanning was started together with the start of infusion. An initial set of ten 2-min scans was followed by ten 4-min scans for 60 min and an additional six 5-min scans for 90 min (Table 1).
Data analysis

All emission scans were reconstructed using PROMIS reconstruction (Brix et al., 1997) with a Hanning filter cut-off frequency of 0.4 (FWHM = 7.5 mm). After the reconstruction, the tissue concentration of radioactivity was obtained from seven regions of interest (ROIs) defined on the PET images. The regions were the thalamus, temporal cortex, hippocampus, parietal cortex, frontal cortex, occipital cortex and cerebellar cortex. Circular ROIs were set at 8 mm diameter covering 10–15 slices for cortical regions, 5–7 slices for the cerebellum and 5 slices for the thalamus, with guidance from MRI and a brain atlas (Matui and Hirano, 1977). All samples taken were averaged to provide the mean count density for a given region. The average values of left and right ROIs were used to increase the signal-to-noise ratio for the calculations.

The ROI values were expressed in kBq/ml, normalized to the injected radioactivity of 185 MBq (5 mCi), and plotted against time.

Reference region

The cerebellum has commonly been used as a reference region for quantitation of dopamine D2 receptor binding with PET and SPECT (Farde et al., 1986; Kornhuber et al., 1995; Pilowsky et al., 1997). The method assumes that there is negligible density of dopamine D2 receptors in the cerebellum. However, it needs to be confirmed whether the specific binding of [11C]FLB-457 in the cerebellum is negligible. SAs were categorized into high SA (71–279 GBq/µmol) and low SA (1–15 GBq/µmol) as an independent variable (Table 1). The eight cerebellar uptake curves of high SA were normalized to the injected radioactivity and averaged to compare them with those of low SA. Repeated two-factor ANOVA (SA × time) was performed. Since injected mass and the effect on specific binding vary within subjects, cerebellar uptake curves were compared again among subjects whose specific binding reductions in the thalamus were more than 50%. The differences of SA at each time-point were determined using a simple main effect test.

Transient equilibrium analysis

Transient equilibrium analysis was introduced for obtaining values of density and affinity of dopamine D2 receptors in the striatum with substituted benzamide [11C]raclopride (Farde et al., 1986). The data were obtained at systematically varied ligand concentrations and analysed by means of saturation curves and Scatchard plots (Farde et al., 1986, 1989, 1995). The saturation curve is given by the following equation:

\[ B = \frac{B_{\text{max}} \cdot F}{K_d + F} \]  

(1)

where \( B \) is the concentration of specific radioligand binding (pmol/ml), \( F \) the concentration of free radioligand (pmol/ml), \( B_{\text{max}} \) the receptor density (pmol/ml), and \( K_d \) the dissociation constant (nM). For Scatchard analysis, eqn (1) can be rearranged into this linear expression:

\[ B = \frac{B_{\text{max}} - B}{K_d} \frac{F}{K_d}. \]  

(2)

A basic condition for this analysis is that the transient equilibrium state of ligand–receptor interactions occurs in vivo within the time of a PET experiment. Following a bolus injection of radioligand, the transient equilibrium is established when the specific binding radioactivity is maximal (Farde et al., 1989, 1995; Ito et al., 1998). Specific binding was defined as the difference between radioactivity in the cortical regions or thalamus and that in the cerebellum. The curve of specific binding data was fitted and the peak point was defined automatically by in-house software. Specifically bound \( B \) and free \( F \) radioligand concentrations were obtained by dividing the measured specific and free radioactivities by SA (GBq/µmol).
After intravenous injection of \(^{[11]}\text{C}\)FLB-457, the accumulation of radioactivity was highest in the striatum, lower in the thalamus and temporal cortex, and lowest in the cerebellum (Figure 1). The average regional time–activity curves of the extrastriatal regions obtained after the injection of \(^{[11]}\text{C}\)FLB-457 with high SA are shown in Figure 2. The uptake in the thalamus reached a peak around 60 min and began to decrease. Uptake in the cerebellum peaked at 5 min while uptake in the cortical regions was between the values for thalamus and cerebellum uptakes.

The metabolite study using rats showed that there were no detectable metabolites in the brain either at 30 or 70 min after \(^{[11]}\text{C}\)FLB-457 injection.

The average cerebellar uptake curves of high and low SA are shown in Figure 3. Repeated two-factor ANOVA showed a significant interaction between SA and time \([F(19, 266) = 2.73, p < 0.01]\). But the results of the simple main effect test concerning time revealed no significant differences between high and low SA at any time-point. SA × time interaction indicated the time-related change of the radioactivity in each SA. Comparison among the six subjects with more than 50% reduction in thalamic uptake did not show significant reduction in cerebellar uptake either. Therefore radioactivity in the cerebellum was regarded as a valid estimate for free and non-specific binding in this study.

The radioactivity in the regions with specific binding sites was markedly reduced when the mass of injected ligand was increased (low SA) (Figure 4, Table 1). The
peak of the specific binding was not well defined with high SA due to the flatness of the curves after 60 min but was observed within the PET measurement. In the experiment with low SA, the peak came earlier than that with high SA (Figure 5, Table 2). The in vivo Scatchard plots in the thalamus, temporal cortex and frontal cortex based on 2 (subject E) or 3 (subject D) experiments are shown in Figure 6. The average $B_{\text{max}}$ values and $K_d$ values in six brain regions are given in Table 3. One-way ANOVA revealed a significant regional main effect [$F(5,41) = 15.29, p < 0.001$], and multiple comparisons (Fisher’s PLSD) indicated significant regional differences. The highest $B_{\text{max}}$, 2.3 ± 0.6 pmol/ml (mean ± s.d.), was obtained in the thalamus where the value was significantly higher than in any other extrastriatal regions ($p < 0.01$ vs. all of other regions). $B_{\text{max}}$ in the temporal cortex (1.5 ± 0.5 pmol/ml) and hippocampus (1.4 ± 0.5 pmol/ml) were significantly higher than those in the parietal (0.9 ± 0.4 pmol/ml), frontal (0.8 ± 0.2 pmol/ml) and occipital cortex (0.7 ± 0.3 pmol/ml) ($p < 0.01$, temporal

Table 2. Peak time of specific binding of extrastriatal regions

<table>
<thead>
<tr>
<th>Region</th>
<th>Low specific radioactivity (min ± s.d.)</th>
<th>High specific radioactivity (min ± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporal</td>
<td>42 ± 9</td>
<td>74 ± 14</td>
</tr>
<tr>
<td>Frontal</td>
<td>41 ± 11</td>
<td>58 ± 15</td>
</tr>
<tr>
<td>Parietal</td>
<td>38 ± 11</td>
<td>64 ± 16</td>
</tr>
<tr>
<td>Occipital</td>
<td>42 ± 15</td>
<td>62 ± 20</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>39 ± 8</td>
<td>79 ± 13</td>
</tr>
<tr>
<td>Thalamus</td>
<td>45 ± 0</td>
<td>79 ± 13</td>
</tr>
</tbody>
</table>

Figure 5. The time-course for the specific binding of [$^{11}$C]FLB-457 with high SA and low SA in the thalamus (○, ▲) and temporal cortex (△, ▲) of subject E. The difference between radioactivity in the thalamus, temporal cortex and that in the cerebellum was defined as specific binding and fitted to a set of four exponentials. Transient equilibrium was defined as the peak point.

Figure 6. Scatchard plots for determinations of $B_{\text{max}}$ and $K_d$ in the thalamus (○, ●), temporal cortex (▲, △) and frontal cortex (●, ○). Two points for subject E (——) and three points for subject D (———).
The binding of \( \left[{ }^{11} \text{C}\right] \)PD-128907 also indicated high density in living human brain regions of the human brain (Landwehrmeyer et al., 1993). An autoradiographic study using the selective dopamine D3 receptor ligand \( \left[{ }^{11} \text{C}\right] \)raclopride (Farde et al., 1997). In the previous data, uptake in the thalamus peaked around 15 min after injection and gradually decreased. On the other hand, the present results demonstrated that the uptake in the thalamus reached a peak at around 60 min. Uptake in the cortical regions also showed a more rapid decrease in the previous study than in the present one. The differences between these data were due to the injected mass of the ligand. In the previous study the injected mass was 7–13 nmol based on the injected radioactivity of about 300 MBq in the 2D data acquisition mode and the specific radioactivities of 22–44 GBq/µmol (Farde et al., 1997). The injected mass was several times larger than that used in the present study with high SA (0.5–2.4 nmol) (Table 3). In the previous study more than 10% of the receptors were assumed to be occupied by the injected mass. This explains the different appearance of the time–activity curves in the two studies.

Dopamine receptor densities in vivo have been calculated using the cerebellum as the reference region for non-specific binding and free-ligand concentration in the brain (Farde et al., 1986; 1989). However, a very low but detectable dopamine D2 receptors has been reported in the cerebellum (Bouthenet et al., 1991; Hall et al., 1996a). Although there is a tendency for a decrease in the cerebellar uptake curve after the injection of \( \left[{ }^{11} \text{C}\right] \)FLB-457 with low SA, the present results did not show a significant decrease.

**Table 3. Extrastriatal dopamine D2 receptor density and affinity in living human brain**

<table>
<thead>
<tr>
<th>Region</th>
<th>( B_{\text{max}} ) (pmol/ml)</th>
<th>( K_d ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td>2.3 ± 0.6</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Temporal</td>
<td>1.5 ± 0.5*</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.4 ± 0.5*</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td>Parietal</td>
<td>0.9 ± 0.4*†‡†</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Frontal</td>
<td>0.8 ± 0.2*§‖</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.7 ± 0.3*§‖</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± s.d.; *p < 0.01 vs. thalamus; †p < 0.05 vs. temporal cortex; §p < 0.01 and ‖p < 0.05 vs. hippocampus.

vs. frontal and occipital cortex, hippocampus vs. occipital cortex; \( p < 0.05 \), temporal vs. parietal cortex, hippocampus vs. frontal and parietal cortex) (Table 3). On the other hand, the \( K_d \) values were not significantly different among any of the regions \( F(5,41) = 1.18 \).

**Discussion**

The present quantitative analysis was based on \( \left[{ }^{11} \text{C}\right] \)FLB-457, a radioligand which has high affinity not only for dopamine D2 but also for dopamine D3 receptors in vitro (Hallldin et al., 1995). Dopamine D3 receptor mRNA has been identified in human nucleus accumbens and the islands of Calleja, but not in significant signals in other brain regions (Landwehrmeyer et al., 1993). An autoradiographic study using the selective dopamine D3 receptor ligand \( \left[{ }^{11} \text{C}\right] \)PD-128907 also indicated high density in nucleus accumbens but no binding in the neocortex and thalamus (Hall et al., 1996b). However, mRNA of dopamine D3 receptor has been identified in human nucleus accumbens and the thalamus (Hall et al., 1996b). However, mRNA of dopamine D3 receptor has been identified in the human medial temporal lobe (Meador-Woodruff et al., 1994), and a recent report suggests low but more widely expressed D3 receptor mRNA in the human brain (Suzuki et al., 1998). The binding of \( \left[{ }^{11} \text{C}\right] \)FLB-457 might represent both dopamine D2 and D3 receptors in some extrastriatal regions of the human brain.

The affinity of FLB-457 for dopamine D4 receptors was not thoroughly investigated. However, substituted benzamides including epidepride, eticlopride and raclopride, which have affinity for the dopamine D2 receptor, have 2 or 3 orders of magnitude lower affinity for the dopamine D4 receptor (Hall et al., 1996a; Van Tol et al., 1991). In addition, our preliminary displacement experiment indicated that there was no detectable effect on the \( \left[{ }^{11} \text{C}\right] \)FLB-457 time-course of the monkey cerebral cortex by intravenous injection of 0.1 or 0.4 mg/kg of high-affinity D4 ligand \( N\)-(2-[4-(4-chlorophenyl)piperazin-1-yl](ethyI)-3-methoxybenzamide (IC\(_{50} 0.057 \text{ nm} \)) (Perrone et al., 1998) (data not shown). Thus the role of the dopamine D4 receptor can not be important in \( \left[{ }^{11} \text{C}\right] \)FLB-457 binding.

\( \left[{ }^{11} \text{C}\right] \)FLB-457 accumulated to a high degree in the striatum (Figure 1). However, data from high-density receptor regions with very high-affinity radioligands do not fit the standard three-compartment model (Gifford et al., 1998; Votaw et al., 1993). The sensitivity of receptor occupancy by antipsychotic drugs is much lower in \( \left[{ }^{11} \text{C}\right] \)FLB-457 compared to the relatively low-affinity substituted benzamide \( \left[{ }^{11} \text{C}\right] \)raclopride (Farde et al., 1997).

High-affinity ligands show very slow clearance from the high-density receptor region where the radioligand delivery can be rate limiting. The slow clearance of the ligand can be explained by rebinding to receptors within a dense receptor region (Frost and Wagner, 1984; Gifford et al., 1998; Votaw et al., 1993). In this study we did not evaluate the striatal data since \( \left[{ }^{11} \text{C}\right] \)FLB-457 is not a suitable ligand for the quantitative analysis of the striatum (Farde et al., 1997).

There was a conspicuous concentration of radioactivity in the extrastriatal regions, but the time–activity curves in those regions were not exactly the same between the present results (Figure 2) and previous human data with \( \left[{ }^{11} \text{C}\right] \)FLB-457 (Farde et al., 1997). In the previous data, uptake in the thalamus peaked around 15 min after injection and gradually decreased. On the other hand, the present results demonstrated that the uptake in the thalamus reached a peak at around 60 min. Uptake in the cortical regions also showed a more rapid decrease in the previous study than in the present one. The differences between these data were due to the injected mass of the ligand. In the previous study the injected mass was 7–13 nmol based on the injected radioactivity of about 300 MBq in the 2D data acquisition mode and the specific radioactivities of 22–44 GBq/µmol (Farde et al., 1997). The injected mass was several times larger than that used in the present study with high SA (0.5–2.4 nmol) (Table 1). In the previous study more than 10% of the receptors were assumed to be occupied by the injected mass. This explains the different appearance of the time–activity curves in the two studies.
reduction. Further, no reduction of $[^{11}]$CFLB-457 binding in the cerebellum was reported after haloperidol administration despite 70–80% of striatal D2 receptor occupancy (Farde et al., 1997). These observations support the use of the cerebellum as allowing a valid estimate for free and non-specific binding in this study. However, since the amount of total injected ligand was limited in this study, it can not be ruled out that there is a significant specific binding of $[^{11}]$CFLB-457 in the cerebellum.

The present study demonstrated that specific binding of FLB-457, presumably to dopamine D2 receptor densities in the extrastriatal regions was 0.7–2.3 pmol/ml. This represented approx. 2–8% of the density in the putamen, which is 30 pmol/ml as measured by PET using $[^{11}]$Craclopride (Farde et al., 1995). These results are consistent with previous in vitro data of the human brain which indicated that the extrastriatal density is approx. 1–6% of that in the putamen (Joyce et al., 1991; Kessler et al., 1993).

The $K_i$ values were not significantly different among extrastriatal regions, but the absolute $K_i$ values were 30- to 40-fold higher than the previously reported in vitro data using $[^{12}]$Epidepride (Joyce et al., 1991; Kessler et al., 1993). The in vitro affinity of epidepride ($K_i = 0.024$ nM) for the dopamine D2 receptor is nearly the same as that of FLB-457 ($K_i = 0.018$ nM) (Halldin et al., 1995). This discrepancy of $K_i$ values between in vitro and in vivo has been reported with several ligands. The $K_i$ values in vivo have been reported to be 1 or 2 orders of magnitude greater than that obtained in vitro (Cunningham et al., 1991; Leslie and Bennett, 1987a, b; Votaw et al., 1993). Several factors may contribute to this discrepancy. It has been assumed that only a minor fraction of the radioligand in the brain is free to associate with the receptors (Farde et al., 1989). Furthermore, in the microscopic receptor region, local diffusional gradients have been suggested to exist in the synapse (Cunningham et al., 1991; Zeeberg et al., 1988). This means that the true free-ligand concentration around the microscopic receptor region in the synapse might be lower than that calculated from the reference region. These factors may partly account for the difference between in vivo and in vitro $K_i$ values (Farde et al., 1989; Gifford et al., 1998; Leslie and Bennett, 1987a).

A number of reports have pointed out a significant role of the extrastriatal dopamine system in the pathophysiology of schizophrenia. However, the functional role of the extrastriatal dopamine D2 receptors in the brain has not been fully understood. The present results indicate that there are significant regional differences in receptor distribution, which has been reported in post-mortem studies (Joyce et al., 1991; Kessler et al., 1993). Among the cortical regions, the D2 receptor densities in the temporal cortex and hippocampus were significantly higher than in other cortical regions (Figure 1, Table 3). In the temporal cortex of schizophrenic patients, significant, regionally specific disrupted patterns of dopamine D2 receptors are reported; this phenomenon has been discussed in relation to auditory hallucinations (Goldsmith et al., 1997). Dopamine D2 receptors in the extrastriatal regions have some functions in generating psychiatric symptoms. The temporal cortex is connected with the prefrontal cortex via the mediodorsal nucleus of the thalamus. The dopamine D2 receptor density in the thalamus was 2–3 times higher than in the cortical regions (Figure 1, Table 3). In addition, the thalamus has been suggested to act as an information filter for cortical input, and an elevation of the dopamine content in the thalamus of schizophrenic patients has been reported (Oke and Adams, 1987).

The extrastriatal dopamine D2 receptor has been considered an important target of antipsychotic drugs, especially atypical ones like clozapine (Lidow and Goldman-Rakic, 1997). Increased neural activity indicated by the induction of Fos protein is observed in the thalamus in response to the antipsychotic drugs haloperidol and clozapine (Cohen and Wan, 1995). This supports the hypothesis that a thalamic site may play an important role in mediating the clinical effects of antipsychotic drugs. Considerable reduction of $[^{11}]$CFLB-457 binding is observed in the thalamus and neocortex after oral administration of haloperidol (4 mg), and to a lesser degree with clozapine (Farde et al., 1997). There is a report that clozapine has a selective effect on the temporal cortex (Pilowsky et al., 1997).

The present study demonstrates the pharmacokinetic characteristics of $[^{11}]$CFLB-457 and its saturability of specific binding. The results show the feasibility of $[^{11}]$CFLB-457 for PET determination of the density and affinity of extrastriatal dopamine D2 receptor in the living human brain. The function of dopamine D2 receptors in the thalamus and the temporal cortex, where the densities are relatively high in the extrastriatal regions, remains to be investigated in relation to the pathophysiology of schizophrenia and the site of action of antipsychotics.

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