Platelet Serotonin (5-HT)\textsubscript{2A} Receptor Binding Sites in Affective Disorders: A Quantitative Receptor Autoradiographic Study with [\textsuperscript{125}I]Lysergic Acid Diethylamide

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We used the quantitative receptor autoradiographic method with a radioligand of [\textsuperscript{125}I]lysergic acid diethylamide ([\textsuperscript{125}I]LSD) to quantitate platelet serotonin (5-HT)\textsubscript{2A} receptors in affective disorders. Specific binding of [\textsuperscript{125}I]LSD to human platelet pellet sections was saturable, and of high affinity and single. Both ketanserin and spiperone, 5-HT\textsubscript{2A} selective ligands, inhibited [\textsuperscript{125}I]LSD binding to human platelet pellets with high potency (IC\textsubscript{50} values of 0.15 and 0.19 nM, respectively), whereas 5-HT and paroxetine, selective 5-HT re-uptake inhibitors, inhibited binding with a very low potency. These data confirmed that binding sites of human platelet pellets specifically labelled by [\textsuperscript{125}I]LSD were 5-HT\textsubscript{2A} receptors.

The number of 5-HT\textsubscript{2A} receptors (B\textsubscript{max} of [\textsuperscript{125}I]LSD binding) of human platelets obtained from drug-free depressed patients was significantly higher than those of healthy volunteers. There were no statistical differences in the number of 5-HT\textsubscript{2A} receptors between depressed patients with and without suicidal behaviors. The increased number in platelet 5-HT\textsubscript{2A} receptors may indicate a hyperfunction of the central 5-HT\textsubscript{2A} receptors. The method with human platelets pellet sections we used is simple and sensitive for investigating platelet 5-HT\textsubscript{2A} receptors, a diagnostic and therapeutic marker in depressive disorders, in the clinical research.

**Key words**: 5-HT\textsubscript{2A} receptors, platelet, affective disorders, quantitative receptor autoradiography, [\textsuperscript{125}I]LSD

**Introduction**

Affective disorders are thought to be associated with dysfunctions in the central serotonergic neurons. Concentrations of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) was reduced in the cerebrospinal fluid of drug-free depressive patients, and in the postmortem brains of depressive and suicidal patients. A decreased number of 5-HT transporter binding sites was also noted in the postmortem brains of depressive and suicidal patients. Interestingly, Arora and Meltzer found an increase in the density of 5-HT\textsubscript{2A} receptor binding sites in the postmortem brains of depressive and suicidal patients.

As a similarity in binding characteristics with 5-HT\textsubscript{2A} ligands between human blood platelets and frontal cortex tissues was reported and a nucleotide sequence of human platelet 5-HT\textsubscript{2A} cDNA was found to be identical to that of human frontal cortex, human platelets are attracting attention as a model for the central 5-HT neurons. Changes in the density of 5-HT\textsubscript{2A} receptors and 5-HT transpoter binding sites were detected in membrane preparations of blood platelets obtained from patients with affective disorders. The 5-HT\textsubscript{2A} ligand-binding method with membrane preparations seems adequate to detect 5-HT\textsubscript{2A} receptor binding sites in human platelets of patients with affective disorders, since it needs a large volume of blood (i.e., 30 ml). In fact, the binding characteristic in human platelets may vary from human blood-sampling processes. Himeno and Saavedra found that the quantitative receptor autoradiographic method with platelet-pellet sections can detect 5-HT receptor binding sites in platelets obtained from a small volume of human blood (1.0 ml). Therefore, taking advantage of the high sensitivity, to characterize platelet 5-HT\textsubscript{2A} receptors of patients with affective disorders, we used the quantitative receptor autoradiographic method with a radioligand of [\textsuperscript{125}I]lysergic acid diethylamide ([\textsuperscript{125}I]LSD) in the present study.
Materials and Methods

Preliminary experiments for characterizing human platelet 5-HT2A receptors

Basic experiments to quantitate human platelet 5-HT2A receptors were performed with five healthy volunteers with no histories of psychiatric and neurologic disorders. All subjects were in good physical health, and were free from psychotropic medications. Informed consent of healthy volunteers was obtained after the study procedures had been fully explained.

Depressive patients and normal controls

Outpatients with depressive disorder were recruited in our University-affiliated clinics. All patients enrolled in the protocol met ICD-10 classification of mental and behavioral disorders: diagnostic criteria for research ICD-10/DCR for mood (affective) disorders as determined by interviews with plural psychiatrists. All depressed patients were determined to be physically healthy on the basis of their medical histories, physical examination, and routine laboratory tests. Clinical ratings were performed in close juxtaposition to the venipuncture by staff who were blind to the binding results. In addition, binding experiments were also performed by other staff who were blind to the clinical ratings. Patients diagnosed with depressive disorder according to ICD-10/DCR criteria were studied. Eight depressive patients (male: 6, female: 2) with no history of neurologic disorders ranged in age from 26 to 69 years. The mean age of the subjects was 45.1±5.2 (mean±SEM) years. Depressive patients were in good physical health, and were free for at least 6 months from psychotropic medication. Subjects for normal controls (male: 4, female: 1) with no history of psychiatric or neurologic disorders ranged in age from 25 to 45 years. The mean age of the subjects was 36.8±3.4 years. Control subjects were in good physical health and were free from psychotropic medication. There was no statistical differences in age and sex between the two groups. Informed consent of depressive patients and normal controls was obtained after the study procedures had been fully explained.

The categories of ICD-10 system for these patients were F31.5 bipolar affective disorder, current episode severe with psychotic symptoms, F32.1 moderate depressive episode, F33.1 recurrent depressive disorder, current episode moderate, F33.2 recurrent depressive disorder, current episode moderate without psychotic symptoms, and F33.3 recurrent depressive disorder, current episode severe with psychotic symptoms.

Clinical assessment of the severity of patient's current depressive symptoms

The severity of the patient's current depressive symptoms was assessed using the Hamilton Depression Scale (HAMD).

Quantitative receptor autoradiographic method with [125I]LSD

We drew whole blood from normal subjects via a 21-gauge needle into 5 ml vacuum glass tubes containing EDTA-2Na. Platelet-rich plasma (PRP) was obtained by centrifugation of blood at 180×g for 15 min at room temperature. Following centrifugation, we removed PRP to conical polypropylene tubes containing 20 μl of M-1 embedding matrix. We centrifuged the tubes at 1200×g for 8 min at room temperature. After discarding the supernatant, we slowly added 250 μl of M-1 embedding matrix on top of the pellet, together with a thin wooden stick. The tubes were frozen in isopentane on dry ice. We separated the pellets by lightly warming the tubes and pulling the stick. The frozen platelet pellet was mounted on a cryostat chuck (-20°C) with mounting medium and was sectioned at 20 μm thickness.

Triplicate sections were preincubated for 15 min at room temperature in buffer solution (50 mM Tris-HCl, 120 mM NaCl, 5 mM, 1 mM MgCl2, 0.05% ascorbate, pH 7.4). After preincubation, sections were incubated with [125I]LSD (0.08 nM–3.64 nM) in the presence or absence of competing ligands for 15, 30, 60 min or 180 min at 4 °C, 23°C or 37°C in buffer solution (50 mM Tris-HCl, 120 mM NaCl, 5 mM, 1 mM MgCl2, 0.05% ascorbate, pH 7.4). Slices were then washed at room temperature, dipped three times for 1 min each in fresh ice-cold buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM, 1 mM MgCl2, 0.05% ascorbate, pH 7.4) or three times for 3 min each in fresh ice-cold buffer and for 1 sec in ice-cold distilled water. Slide sections were then dried under a stream of cold air, and the radioactivities of these sections were analyzed using the imaging plate system. Autoradiographic [125I]micro-scales (Amersham) were used for standards. A Scatchard analysis was performed by the method of least squares.

In the inhibition study we used a single 0.35 nM [125I]LSD concentration, and six concentrations (10⁻⁸ to 10⁻⁵ M) of unlabeled ketanserin, spiroerone, 5-HT, and...
paroxetine. Ketanserin and spiperone are the 5-HT₂₅ selective ligands, and paroxetine is a selective 5-HT reuptake inhibitor.

Data analysis

The two-tailed Student's t-test was used to contrast values for the 5-HT₂₅ receptor binding parameters between patients and normal controls or patient subgroups.

Results

Experimental conditions for in vitro quantitation of [³⁵S] LSD binding in human platelets

The incubation temperatures for the [³⁵S]LSD binding were set at either 4°C, 23°C, or 37°C (Fig.1). Specific binding of [³⁵S]LSD at an incubation temperature of 37°C was two to three times higher than that at 4°C or 23°C. The ratio of specific/total binding of [³⁵S]LSD at 37°C was 50~60%.

The incubation times for the [³⁵S]LSD binding were at 15 min, 30 min, 60 min, or 180 min (Fig.2). Specific binding of [³⁵S]LSD at an incubation time of 60 min was much higher than that at 15 min, 30 min, or 180 min. The ratio of specific/total binding of [³⁵S]LSD at an incubation time of 60 min was also highest.

Two different washing times were used. A washing time of 3 min in fresh ice-cold buffer showed a much higher specific binding of [³⁵S]LSD than a washing time of 9 min (Fig.3).

Fig.1. Incubation temperature of in vitro quantitation of [³⁵S]LSD binding in human platelet sections.

Sections were incubated with [³⁵S]LSD (0.22 nM) in the presence or absence of competing ligands for 60 min, at 4°C, 23°C or 37°C in buffer solution (50 mM Tris-HCl, 120 mM NaCl, 5 mM, 1 mM MgCl₂, 0.05% ascorbate, pH 7.4). Specific binding of [³⁵S]LSD at an incubation temperature of 37°C was two to three times higher than that at 4°C or 23°C. The ratio of specific/total binding of [³⁵S]LSD at 37°C was 50~60%.

Fig.2. Incubation time of in vitro quantitation of [³⁵S]LSD binding in human platelet pellet sections.

Sections were incubated with [³⁵S]LSD (0.22 nM) in the presence or absence of competing ligands for 15 min, 30 min, 60 min or 180 min, at 37°C in buffer solution. Each point represents the specific binding. Specific binding of [³⁵S]LSD at an incubation time of 60 min was much higher than that at 15 min, 30 min, or 180 min. The ratio of specific/total binding of [³⁵S]LSD at an incubation time of 60 min was also highest.

Fig.3. Washing time of in vitro quantitation of [³⁵S]LSD binding in human platelet sections.

Slices were then washed at room temperature, dipped three times for 1 min each in fresh ice-cold buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM, 1 mM MgCl₂, 0.05% ascorbate, pH 7.4) or three times for 3 min each in fresh ice-cold buffer and for 1 sec in ice-cold distilled water. A washing time of 3 min in fresh ice-cold buffer showed a much higher specific binding of [³⁵S]LSD than a washing time of 9 min.
Fig. 4. Autoradiographic images of [³²P]LSD binding in human platelet pellet sections
 Autoradiographic images of sections incubated with 0.35 nM [³²P]LSD in the absence(A) or presence(B) of 1 μM unlabeled ketanserin.

Based on these results, we chose a [³²P]LSD binding condition of incubation temperature of 37°C and incubation time of 60 min, with three washes of 1 min each in fresh ice-cold buffer and then 1 sec in ice-cold distilled water. Under the binding condition, we observed considerable amounts of specific [³²P]LSD binding to pellet sections of human platelets (Fig. 4).

Saturation study and Scatchard analysis of [¹²⁵]I-LSD binding to human platelet pellet sections

After preincubation, sections were incubated with [¹²⁵]I-LSD (0.08 nM–3.64 nM) in the presence or absence of 1 μM ketanserin for 60 min at 37°C in buffer solution. Specific binding of [¹²⁵]I-LSD to human platelet pellet sections was saturable and of high affinity.

The Scatchard analysis of these data demonstrated a correlation coefficient close to unity, indicating a single binding site (Fig. 5).

Inhibition of specific binding of [¹²⁵]I-LSD to human platelet pellet sections (Table 1)

Both ketanserin and spiperone, the 5-HT₂a selective ligands, inhibited [¹²⁵]I-LSD binding to human platelet pellets with a high potency, with IC₅₀ values of 0.15 and 0.19 nM, respectively, whereas 5-HT and paroxetine (selective 5-HT reuptake inhibitor) inhibited binding with a very low potency (IC₅₀ values >1000 nM). This data confirms that the binding sites of human platelet pellets labelled by [¹²⁵]I-LSD are 5-HT₂a receptors.

Table 1. Inhibition of specific binding of [¹²⁵]I-LSD to human platelet pellet sections.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC₅₀ (nM)</th>
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</thead>
<tbody>
<tr>
<td>ketanserin</td>
<td>0.15</td>
</tr>
<tr>
<td>spiperone</td>
<td>0.19</td>
</tr>
<tr>
<td>serotonin</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>paroxetine</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

In the inhibition study we used a single 0.35 nM [¹²⁵]I-LSD concentration, and six concentrations (10⁻¹⁰ to 10⁻⁵ M) of unlabeled ketanserin, spiperone, 5-HT, and paroxetine. Ketanserin and spiperone are the 5-HT₂a selective ligands, and paroxetine is a selective 5-HT reuptake inhibitor.

Clinical assessment of the severity of patient's current depressive symptoms (Table 2)

The severity of the depressive state of these patients at the time of blood sampling before treatment was just over moderate severity, with patients receiving a HAMD score (17 items) of 17-37 points (25.4±2.9, mean±SEM).

Binding parameters (Kd and Bmax) of platelet [¹²⁵]I-LSD binding in the depressive patient group and the control group were calculated by Scatchard analysis

The binding parameters of platelet [¹²⁵]I-LSD binding in these two groups at the time of blood sampling before treatment were calculated by Scatchard analysis. The mean (±SEM) Kd of [¹²⁵]I-LSD binding for the control group was 0.87±0.06 nM (n=5), which was not significantly different from that of depressive
Table 2. Clinical Characteristics of Depressive Patients and Normal Controls

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, sex</th>
<th>Diagnosis (ICD-10)</th>
<th>HAMD (17 items)</th>
<th>Histories of suicide attempts</th>
<th>Suicidal ideas in this episode</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Depressive Patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32, m</td>
<td>F33.1</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>45, m</td>
<td>F33.1</td>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>26, f</td>
<td>F33.1</td>
<td>19</td>
<td>+</td>
<td>-</td>
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<tr>
<td>4</td>
<td>63, m</td>
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<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>44, m</td>
<td>F33.3</td>
<td>22</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>47, m</td>
<td>F31.5</td>
<td>37</td>
<td>+</td>
<td>+</td>
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<tr>
<td>7</td>
<td>35, m</td>
<td>F33.2</td>
<td>37</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>69, f</td>
<td>F31.5</td>
<td>31</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Mean±SEM</strong></td>
<td></td>
<td></td>
<td>45.1±5.2</td>
<td>25.4±2.9</td>
<td></td>
</tr>
<tr>
<td><strong>Normal Control</strong></td>
<td></td>
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<td>1</td>
<td>37, m</td>
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<td></td>
<td></td>
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<tr>
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<td>25, f</td>
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<tr>
<td>3</td>
<td>42, m</td>
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<td></td>
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<tr>
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<td>35, m</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>45, m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean±SEM</strong></td>
<td></td>
<td></td>
<td>36.8±3.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Bmax of [3H]LSD binding and the HAMD score for eight depressive patients

A comparison of the Bmax of [3H]LSD binding for the eight depressive patients free from psychotropic medication for at least 6 months with the HAMD scores at the time of blood sampling before treatment showed no significant correlation (r=0.16, p=0.56, Fig.7).
Table 3. Binding parameters (Kd and Bmax) of platelet [125I]LSD binding in the depressive patients, free from psychotropic medication at least six months and the normal controls

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Depressive Patients</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kd (nM)</td>
<td>Bmax (f mol/mg)</td>
</tr>
<tr>
<td>1</td>
<td>0.89</td>
<td>5.08</td>
</tr>
<tr>
<td>2</td>
<td>1.16</td>
<td>3.85</td>
</tr>
<tr>
<td>3</td>
<td>1.09</td>
<td>7.50</td>
</tr>
<tr>
<td>4</td>
<td>0.68</td>
<td>4.94</td>
</tr>
<tr>
<td>5</td>
<td>1.22</td>
<td>6.40</td>
</tr>
<tr>
<td>6</td>
<td>0.94</td>
<td>4.70</td>
</tr>
<tr>
<td>7</td>
<td>0.96</td>
<td>7.10</td>
</tr>
<tr>
<td>8</td>
<td>1.39</td>
<td>4.60</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>1.04±0.08</td>
<td>5.52±0.46</td>
</tr>
</tbody>
</table>

* ** p (0.01, two-tailed Student’s t-test, as compared to normal controls

Bmax of [125I]LSD binding for patients with suicidal behavior and without suicidal behavior (Fig.8)

In the eight depressive patients, four had past histories of suicidal attempts (Table 2). In addition, four of the patients had suicidal ideas, show over position 2 in the item “suicide” of HAMD, at the time of blood sampling (Table 2).

The Bmax for the patient subgroup with histories of suicidal attempts was 5.80±0.70 f mol/mg (mean ± SEM, n=4), which was not significantly different from the patient subgroup without histories of suicidal attempts 5.24±0.68 f mol/mg (mean ± SEM, n=4). The Bmax for the patient subgroup with suicidal ideas at the time of blood sampling was 5.70±0.62 f mol/mg (mean ± SEM, n=4), which was also not significantly different from the patient subgroup without suicidal ideas at the time of blood sampling 5.34±0.77 f mol/mg (mean ± SEM, n=4). The Bmax for every patient subgroup was significantly increased compared to the control group (p <0.05).

The Kd for the patient subgroup with histories of suicidal attempts was 1.16±0.10 nM (mean ± SEM, n=4), which was not significantly different from the patient subgroup without histories of suicidal attempts 0.92±0.10 nM (mean ± SEM, n=4). The Kd for the patient subgroup with suicidal ideas at the time of blood sampling was 1.13±0.11 nM (mean ± SEM, n=4), which was also not significantly different from the patient subgroup without suicidal idea at the time of blood sampling 0.96±0.11 nM (mean ± SEM, n=4).

Platelet 5-HT2A receptor binding and scores on Hamilton’s rating scale for depression (HAMD) after treatment in two cases

Case No.5 44-year-old man, head of the office
Diagnosis: F33.3 recurrent depressive disorder, current episode severe with psychotic symptoms

The patient had his first depressive episode at age 25, and at age 27, he had his second depressive episode. Since then, he has had some minor depressive episodes without any difficulties in business. He had no history of psychiatric treatment or psychotropic medication. In the present episode, at age 44, after he was very busy on business, he had depressive symptoms including a depressive mood, insomnia, and appetite loss. One month later, his follower caused a traffic
accident. He blamed himself for that accident and felt severe guilt. He also felt that he was subject to a policeman's superintendence. One month later he made a suicidal attempt (hanging), and he visited our psychiatric clinic and was admitted to our psychiatric ward the same day. After admission, his HAMD score was evaluated and a platelet $[^{125}\text{I}]$LSD binding assay was performed every week. At the first week of admission, flunitrazepam was prescribed. In addition, at the second week, an antidepressant treatment (maprotiline maximum dose 60 mg/day) was started. His depressive symptoms subsided in response to the antidepressant treatment. The Bmax of platelet $[^{125}\text{I}]$LSD binding showed a gradually decrease, corresponding to the decreased HAMD number, which was also in response to the antidepressant treatment (maprotiline maximum dose 60 mg/day). A comparison of the Bmax of $[^{125}\text{I}]$LSD binding and the HAMD score demonstrated a significant linear correlation ($r=0.99$, $p=0.00002$).

Case No.9 35-year-old man, cook
Diagnosis: F31.3 bipolar affective disorder, current episode mild or moderate depression

The patient had his first depressive episode at age 23, and at age 24, he had first hypomanic episode. At age 25, he had his second depressive episode, and at age 27, he had his third depressive episode. In the present episode, at age 34, he had depressive symptoms that included a depressive mood and low self confidence, because he was worried about his prearranged marriage. His depressive state gradually deteriorated. Four month later he broke off his engagement. At that time he also had suicidal ideas and tried to commit suicide. One month later he visited our psychiatric clinic and was admitted to our psychiatric ward the same day. After admission, his HAMD score was evaluated and a platelet $[^{125}\text{I}]$LSD binding assay was performed every week. After the start of treatment with an experimental new drug (double blind study), his depressive symptoms improved initially, but from the sixth week of admission they became worse. Correlatively, the Bmax of platelet $[^{125}\text{I}]$LSD binding decreased at first and then increased. A comparison of the Bmax of $[^{125}\text{I}]$LSD binding and the HAMD scores also demonstrated a significant linear correlation ($r=0.71$, $p=0.02$).
initially, but from the sixth week of admission they became worse. Correlatively, the Bmax of platelet \(^{[125I]}\) LSD binding decreased at first and then increased. A comparison of the Bmax of \(^{[125I]}\) LSD binding and the HAMD scores also demonstrated a significant linear correlation (r=0.71, p=0.02, Fig.10).

The significant linear correlation between the Bmax of \(^{[125I]}\) LSD binding and the HAMD scores in these cases suggests that there is a possible relationship between increased platelet 5-HT\(_{2A}\) receptor concentrations and the severity of depressive symptoms in the clinical course.

**Discussion**

In recent years, serotonin receptors were divided into 14 different subtypes\(^{[18,25]}\), including 5-HT\(_{1A}\) family of receptors, 5-HT\(_{3A}\), 5-HT\(_{3B}\) and 5-HT\(_{3C}\). There appears to be a striking similarity in the pharmacological characteristics of platelet and brain 5-HT\(_{3A}\) receptors\(^{[21,22]}\). In addition, 5-HT\(_{3A}\) receptors are linked to the phosphoinositide second messenger system in both platelets\(^{[20]}\) and brain\(^{[18]}\). And the nucleotide sequence of human platelet 5-HT\(_{3A}\) cDNA is identical to that reported for the human frontal cortex 5-HT\(_{3A}\) receptor\(^{[23]}\). These results suggest that the regulation of 5-HT\(_{3A}\) receptors at the gene level may be the same both platelets and brain. In the present study, we applied in vitro receptor autoradiography to characterize \(^{[125I]}\) LSD binding to human platelet pellet sections. The present method revealed a single class of high affinity binding sites for \(^{[125I]}\) LSD in human platelets. Both ketanserin and spiperone, the 5-HT\(_{3A}\) selective ligands, inhibited \(^{[125I]}\) LSD binding to human platelet pellets with high potency (IC\(_{50}\) values of 0.15 and 0.19 nM, respectively), whereas 5-HT and paroxetine (selective 5-HT reuptake inhibitor) inhibited binding with a very low potency. These data confirmed that the binding sites of human platelet pellets labelled by \(^{[125I]}\) LSD using this method revealed 5-HT\(_{3A}\) receptors. This method required a much smaller volume of blood (5ml) than a classical membrane binding assay (30ml).

In a comparison of \(^{[125I]}\) LSD binding to human platelet pellets in patients with depressive disorder before treatment in this episode, in patients who had been drug free for at least six months, and in normal controls, Bmax was significantly higher in depressive patients before treatment than in the controls. Patients were considered to show over moderate severity by which showed 17-37 points (25±4±2.9, mean±SEM) in the HAMD (17 items). In the depressive group, the comparison of the Bmax of \(^{[125I]}\) LSD binding for eight depressive patients who had been free from psychotropic medication at least 6 months and the HAMD at the time of blood sampling before treatment demonstrated no significant correlation. The small number of depressive patients may have influenced this result.

We recorded the platelet 5-HT\(_{3A}\) measurements and the scores on the HAMD repeatedly after treatment in two cases. The comparison of the Bmax of \(^{[125I]}\) LSD binding and the HAMD demonstrated significant linear correlation in these cases (Fig.9, Fig.10). There is a possible relationship between the increased platelet 5-HT\(_{3A}\) receptor concentrations and the symptoms of depression. In the first case (No.5), the Bmax of \(^{[125I]}\) LSD binding showed gradual decreases, corresponding to the decreased HAMD scores in response to antidepressants treatment. It was considered that the decreased numbers of platelet 5-HT\(_{3A}\) receptors in response to the antidepressants treatment might result in a down-regulation. In the second case (No.9), soon after the start of treatment with an experimental new drug (double blind study), the depressive symptoms began to improve, but after the sixth week, symptoms began to worsen again. Correlatively, the Bmax decreased at first and then increased again. This could not be fully explained by the antidepressant treatment alone.

It is very interesting that the density of platelet 5-HT\(_{3A}\) receptors in depressive patients who had been free from psychotropic medication for at least 6 months was higher than that in normal controls. This finding showing increased values for the density of platelet 5-HT\(_{3A}\) receptors in depressive patients compared to normal controls is in agreement with the study of Biegon et al.\(^{[28]}\), but in disagreement with those of Cowen et al.\(^{[20]}\) and McBride et al.\(^{[20]}\), all of whom found similar mean values for the density of platelet 5-HT\(_{3A}\) receptors in depressive patients and normal controls. On the other hand, Pandey et al.\(^{[20,22]}\) have showed increased platelet 5-HT\(_{3A}\) receptors in suicidal patients independent of psychiatric diagnosis. In our experiments there was no statistical difference between the depressive patients with suicidal behavior and the depressive patients without suicidal behavior. Discrepancies in the results of the platelet 5-HT\(_{3A}\) receptor binding studies in depressive disorders may be a reflection of number of factors, including the effects of the length of the psychotropic medication free interval on platelet 5-HT\(_{3A}\) receptor binding\(^{[20]}\). In our experiments, the drug-free interval of at least 6 months was longer than that in other studies. The effects of exposure to psychotropic medication on platelet 5-HT\(_{3A}\) receptor binding in our experiments, therefore...
may be smaller than the effects observed in other studies. Other factors including patients sample size and depressive subtype (psychotic or nonpsychotic, with or without melancholia) may be considered. Platelet 5-HT$_2A$ receptors may be regulated by blood 5-HT concentration, monoamine oxidase activities in platelets and plasma cortisol levels throughout the hypothalamic-pituitary-adrenal axis. It is suggested that a depressive state may be reflected by increased value for the density of platelet 5-HT$_2A$ receptors in depressive patients through regulation by these factors.

The increased density of platelet 5-HT$_2A$ receptors in depressive disorder may reflect a hyperfunction of the central 5-HT$_2A$ receptors. We would suggest that the increased density of platelet 5-HT$_2A$ receptors may be a possible state marker in depressive disorder. This method could be very useful in clinical research for investigating the platelet 5-HT$_2A$ receptors as diagnostic and therapeutic markers in depressive disorders.

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