

# Tritiated Imipramine Binding

## A Peripheral Marker for Serotonin in Parkinson's Disease

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• Tritiated imipramine binding in platelets has been used to evaluate serotonin activity in depression in previous studies. This article examined this marker as a possible measure of central nervous system serotonergic activity for depression in patients with Parkinson's disease (PD). The number of binding sites was significantly lower in depressed patients with PD than in a healthy control group. Patients with PD who were not depressed had lower values than the comparison group, but this difference was not significant. We also found a significant correlation between the receptor site values in platelets and cerebrospinal fluid levels of the serotonin metabolite, 5-hydroxyindoleacetic acid ( $r = .59$ ), but this was independent of a diagnosis of depression. Receptor site values were examined to identify appropriate cutoff scores to predict depression in the group of patients with PD. A maximum sensitivity of 50% was achieved with a specificity of 64%. Our results strongly support a generalized alteration in serotonin metabolism in depressed patients with PD, but tritiated imipramine binding in platelets is not a useful diagnostic tool for depression. (*Arch Neurol.* 1991;48:1052-1054)

The "serotonin hypothesis" of depression in Parkinson's disease (PD) is based on the evidence of a generalized deficiency of serotonin in patients with PD and depression.<sup>1-5</sup> The cerebrospinal fluid (CSF) concentration of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), is reduced in patients with PD compared with age-matched controls<sup>4</sup>; levels are even lower in patients with depression.<sup>3</sup> Serotonin also plays a role in depression among individuals who do not have PD, since CSF 5-HIAA levels are lower in depressed patients and in suicide victims.<sup>6,7</sup>

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An alternative measure of serotonergic function is tritiated imipramine (<sup>3</sup>H-imipramine) binding, which measures presynaptic serotonergic receptor sites and has been examined in platelets and brain.<sup>8</sup> Tritiated imipramine binding is lower in the platelets of patients without PD but with depression and is reduced in the brains of depressed patients dying of natural causes or suicide when compared with the brains from those who did not commit suicide.<sup>9</sup> However, others have found no difference in brain or platelet binding between depressed and nondepressed individuals.<sup>10,11</sup>

Correlations between platelet and brain <sup>3</sup>H-imipramine binding capacity in animal studies<sup>12</sup> suggest that platelet binding may reflect central nervous system activity. Although Raisman et al<sup>13</sup> found <sup>3</sup>H-imipramine binding decreased in the brains of patients with PD (in whom depression was not assessed), earlier reports indicated no difference between individuals with PD and controls.<sup>14</sup> Schneider et al<sup>15</sup> found platelet binding reduced in depressed patients with PD. These conflicting studies used small groups and the diagnostic information concerning depression was not systematically obtained.

This study sought to examine the relationship among depression, CSF 5-HIAA, and <sup>3</sup>H-imipramine binding in platelets in patients with PD. Second, it sought to determine whether <sup>3</sup>H-imipramine binding in platelets could be a sensitive and specific marker for depression in these patients.

### SUBJECTS AND METHODS

#### Patient Group

Seventy patients with idiopathic PD (mean age, 66.9 ± 9.1 years) participated in this study. The diagnosis of idiopathic PD was defined by the presence of at least two of the following four features: tremor, gait disorder, bradykinesia, and rigidity. Clinical history and other physical symptoms had to be consistent with the idiopathic form of the disease. Patients were excluded if there was evidence implicating another cause for the parkinsonism (eg, phenothiazine exposure or

history of encephalitis) and if they had taken any type of antidepressant medication or other psychoactive drug in the last 60 days. However, patients who were taking dopamine agonists or dopamine precursors or anticholinergics for PD were included. The type and dose of medication were recorded.

#### Control Group

This group consisted of 53 age-matched, healthy elderly individuals (mean age, 66.5 ± 4.7 years) who were accompanying patients to the medical center. This included spouses, companions, and significant others who were not blood relatives of the patient group. These individuals were screened to eliminate central nervous system disease, a history of major depression or other psychiatric illness, or other life-threatening medical illnesses. Individuals were excluded if they had taken antidepressant medication within the past 60 days.

#### Procedures

After giving informed consent all subjects received the following assessments.

**Standardized Medical History.**—This included a record of all major medical and neurologic problems, psychiatric treatment history, and current medications. For the patients with PD, a detailed history of the onset of the disease was taken and all treatment regimens were recorded.

**PD Severity Ratings.**—The Unified Parkinson's Disease Rating Scale<sup>16</sup> was used to assess the presence and severity of motor manifestations. Those items that assess mentation, behavior, and mood (items 1 through 4) were excluded because they were measured with alternate instruments described below. The maximum score possible on the remaining items is 172, with higher scores indicating more severe motor disturbance. The Schwab and England<sup>17</sup> inventory of Activities of Daily Living<sup>17</sup> was used. This inventory rates by percentage the degree of independence in Activities of Daily Living, with 100% referring to total independence and 0% indicating the patient is bedridden with impairment of vegetative functions. The Hoehn and Yahr Scale<sup>18</sup> was also completed. This six-stage index ranges from 0 ("no sign of disease") to 5 ("bedridden or wheelchair bound").

**Mental Status.**—The modified Mini-Mental State Examination was administered to assess intellectual function. This modifica-

Table 1.—Comparison of Depressed and Nondepressed Patients With Parkinson's Disease (PD) and Healthy Elderly Volunteers\*

Group	n	HDRS	Kd	B <sub>max</sub>
Patients with PD				
Depressed	14	17.4 ± 4.5†	1.6 ± 1.2	740.9 ± 198‡
Nondepressed	56	4.2 ± 3.4	1.6 ± 1.4	862.0 ± 352
Controls	53	4.0 ± 4.2†	2.1 ± 0.8	950.3 ± 255‡

\*HDRS indicates Hamilton Depression Rating Scale score; Kd, affinity constant; and B<sub>max</sub>, number of receptor sites. The total group (patients with PD) was classified as described: depressed, Hamilton Score 12 or more; nondepressed, Hamilton Score less than 12.

†Groups were significantly different ( $P < .01$ ).

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tion of the procedure described by Folstein et al<sup>19</sup> includes additional items that measure naming, language, and attention and has a maximum score of 57. The mean modified Mini-Mental State score for healthy elderly individuals is 52.5 (± 4.3).<sup>20</sup>

**Psychiatric Evaluation.**—A standardized semistructured interview for the Hamilton Depression Rating Scale (HDRS)<sup>21</sup> was administered. Based on previous findings,<sup>1,2</sup> those patients with HDRS scores greater than 12 were classified as clinically depressed.

**<sup>3</sup>H-Imipramine Binding.**—Binding of <sup>3</sup>H-imipramine in platelets was determined using the method described by Briley et al<sup>22</sup> and modified by Asarch et al.<sup>23</sup>

**CSF Studies.**—Nineteen of the 70 patients with PD participated in an extended study of biogenic amine metabolites in CSF. These patients were selected because they had cognitive or affective complaints and were admitted to the General Clinical Research Center to complete all assessments. In addition to the HDRS, psychiatric diagnoses for depression were made according to the criteria of the *Diagnostic and Statistical Manual, Revised Third Edition*,<sup>24</sup> using the Structured Clinical Interview<sup>25</sup> completed by a trained interviewer with the patient (and an informant when available).

Lumbar puncture for CSF studies was performed after 18 hours of bed rest. Twenty milliliters of CSF was obtained for biochemical analysis. High-pressure liquid chromatography with amperometric detection was used to measure 5-HIAA and homovanillic acid by a method previously described.<sup>1</sup>

### Data Analysis

For <sup>3</sup>H-imipramine-binding studies, Scatchard analysis was used to determine the number of receptor sites (B<sub>max</sub>) and the affinity constant (Kd)<sup>26</sup> for each subject. Tritiated imipramine-binding results were correlated with demographic and disease severity variables. Group mean comparisons were used to examine differences in binding measures between controls and patients. Pearson correlations were used to examine the association between CSF 5-HIAA measures and platelet binding measures. Nonparametric techniques were used to examine the sensitivity and specificity<sup>27</sup> of platelet binding for assessment of depression in PD.

### RESULTS

Tritiated imipramine-binding measures for the PD group and the control

group were initially compared using a *t* test. This comparison yielded a significant difference between the two groups ( $P \leq .05$ ). Patients with PD were then classified as depressed, using an HDRS score of 12 or greater for depression, or not depressed. The <sup>3</sup>H-imipramine-binding measures for the two PD groups and the control group are given in Table 1. An analysis of variance of the B<sub>max</sub> values yielded a significant F test ( $P < .05$ ) for the comparison of depressed patients with PD, nondepressed patients with PD, and the control group. Post hoc analysis demonstrated significantly lower B<sub>max</sub> values in the depressed patients with PD than in the controls. The group mean value for the nondepressed patients with PD fell between the other two groups, but was not significantly different from either. Based on effect size reported by Schneider et al,<sup>15</sup> this study had sufficient group size to achieve 65% power for detecting such a difference at the .05 level of significance.

### <sup>3</sup>H-Imipramine-Binding and Disease Severity Measures

The mean duration of illness in the PD group was 8.6 (± 2.2) years and the mean Unified Parkinson's Disease Rating Scale score was 41.8 (± 26.1). The mean Hoehn and Yahr rating in the PD group was 2.7 (± .79), with 39.1% receiving a score of 1 or 2 (mild disease), 48.4% receiving a score of 3 (moderate severity), and 12.5% receiving a score of 4 or 5 (marked severity). The mean Schwab and England rating was 68.3% (± 24.1%) with 23.8% of the patients experiencing serious disability, scoring below 50%. None of these measures were significantly correlated with B<sub>max</sub> or Kd values.

The mean modified Mini-Mental State score in the PD group was 44.7 (± 11.5). This is significantly lower ( $P < .05$ ) than the mean for the control group (52.3 ± 3.4). However, no significant correlation was noted between this measure and B<sub>max</sub> or Kd values.

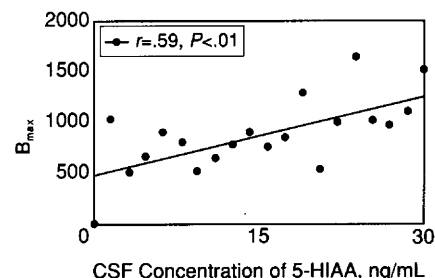
Fifty-five (78.6%) patients were tak-

Table 2.—Comparison of Cerebrospinal Fluid (CSF) Metabolites and Binding Measures in Depressed and Nondepressed Patients With Parkinson's Disease\*

	Patients With Parkinson's Disease	
	Depressed (n = 8)	Nondepressed (n = 11)
HDRS	16.1 (7.1)†	5.8 (3.6)†
CSF 5-HIAA	14.2 (6.4)	18.8 (6.8)
CSF HVA	52.7 (34.3)	30.5 (12.1)
Kd	1.2 (0.72)	1.5 (0.72)
B <sub>max</sub>	826.9 (253)	904.2 (384)

\*HDRS indicates Hamilton Depression Rating Scale; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; Kd, affinity constant; and B<sub>max</sub>, number of receptor sites. Numbers in parentheses are SDs.

†Groups are significantly different ( $P < .01$ ).



Relationship between the amount of 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) and the number of tritiated imipramine binding sites in platelets of patients with Parkinson's disease. B<sub>max</sub> indicates number of receptor sites.

ing parkinsonian medication. The correlation between the dose of medication and the B<sub>max</sub> was not significant ( $r = -.2$ ).

### <sup>3</sup>H-Imipramine-Binding and CSF Measures

Eight (42%) of the 19 patients who had lumbar punctures for the CSF studies were diagnosed as having major depression or dysthymia. They had a mean HDRS score of 16.1 ± 7.1, which represents depression of mild to moderate severity, compared with a mean HDRS score of 5.8 ± 3.6 in the nondepressed patients ( $P < .05$ ). The CSF 5-HIAA and the <sup>3</sup>H-imipramine binding values for these patients are given in Table 2. A significant correlation was noted between B<sub>max</sub> values and CSF 5-HIAA ( $r = .59$ ;  $P < .01$ ) (Figure) but not CSF homovanillic acid ( $r = .39$ ;  $P > .1$ ). The Kd values did not correlate with either CSF metabolite measure.

The mean CSF 5-HIAA level in the depressed group was lower than in the nondepressed group (14.2 ± 6.4 vs 18.8 ± 6.8 ng/mL). This difference was

not statistically significant, perhaps due to our small sample size. Based on our previous work,<sup>1</sup> it was estimated that this study yielded low power for this comparison (0.25).

#### Sensitivity and Specificity of <sup>3</sup>H-Imipramine Binding for Depression in PD

The sensitivity and specificity of <sup>3</sup>H-imipramine binding to detect depression in PD was examined. A B<sub>max</sub> cutoff value of 800 correctly classified the maximum number of patients; 37 (53%) of the 70 patients with PD were correctly identified as depressed or not depressed. This value yielded a specificity of 64% and a sensitivity of 50% for depression among the PD group.

#### COMMENT

This study further supports an alteration in serotonin in PD. First, <sup>3</sup>H-imipramine binding in platelets is reduced in patients with PD. A closer examination of these patients demonstrates that patients with depression and PD have significantly fewer binding sites than controls. Patients with PD who were not depressed had B<sub>max</sub> values that fell between the controls and the depressed group, although they were not significantly different. Second, platelet binding values significantly correlate with CSF 5-HIAA levels, suggesting that this peripheral measure may reflect central serotonergic activity. Together, these results provide additional evidence for a generalized serotonergic abnormality that may be associated with depression in PD.

The binding values in this study were similar to those reported by Suranyi-Cadotte et al,<sup>14</sup> who found no difference between patients with PD and a comparison group. However, the sample size was much smaller in their study. Schneider and coworkers<sup>15</sup> found a 22% decrease in <sup>3</sup>H-imipramine platelet binding in subjects with PD compared with controls. Four of the nine patients with PD had either major depression or dysthymic disorder that may have contributed to the reported difference, although this was not systematically examined. This study demonstrated a 10.9% difference in B<sub>max</sub> values with a large group size.

This work represents the first report of a correlation between CSF and platelet measures of serotonergic activity. It is usually assumed that CSF markers reflect only nonspecific changes in brain chemistry, but in suicide victims there is a correlation between CSF 5-HIAA and <sup>3</sup>H-imipramine-binding sites in the frontal cortex.<sup>28</sup> In depressed patients with PD, Mayberg et al<sup>29</sup> found lowered

metabolism in the caudate and in the inferior orbital-frontal cortex, where a serotonergic mechanism for this frontal hypometabolism has been postulated previously.<sup>30</sup> Significant loss of large neurons in the ascending serotonergic pathway of the dorsal raphe nucleus and decreased serotonin concentrations in the projection areas in the frontal cortex have also been reported in patients with PD.<sup>31</sup>

Although pervasive, the serotonergic deficit may not parallel deterioration of the dopamine system. Our previous work indicates no correlation between CSF 5-HIAA and variables associated with disease severity such as age, motor manifestation, or the use of dopamine precursors or agonists.<sup>3</sup> Similarly, this study found no association between these variables and <sup>3</sup>H-imipramine-binding measures.

A peripheral marker for depression in PD would be useful because symptoms of depression and PD may overlap. When a B<sub>max</sub> value was chosen to provide maximum correct classification of depression among patients with PD, the specificity and sensitivity of the measure were too low to be useful as a screen. Despite this, the decrease in B<sub>max</sub> in depressed patients with PD and the correlation between CSF and platelet measures support a major alteration in serotonergic metabolism in PD that may explain some of the behavioral manifestations of PD.

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