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# Serotonin 5-HT2 receptor binding on blood platelets as a state dependent marker in major affective disorder

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**Abstract.** Serotonin receptors of the 5-HT2 type were studied on platelet membranes from 15 patients suffering from major depression. Receptor binding and clinical state (assessed by the Hamilton and Beck rating scales) were examined in a drug free state upon admission and after 1 and 3 weeks of treatment with the antidepressant maprotiline (MPT). 5-HT2 receptor binding changed in correlation with changes in the clinical state of the patients as judged by the rating scales. Since most patients showed a clinical improvement, the patients as a group exhibited a significant decrease in binding concomitant with a drop in depression scores. However, in those patients in whom there was no clinical change or an increase in depression scores, 5-HT2 receptor binding did not change or increased, respectively, thus resulting in a significant correlation between clinical changes and changes in binding. These results support the use of 5-HT2 receptors on platelets in evaluating depression and its treatment.

**Key words:** Serotonin – Platelets – Depression – 5-HT2 receptors – Maprotiline

Serotonin is a neurotransmitter often implicated in the pathophysiology of affective disorders (Van Praag and Korf 1971; Asberg et al. 1976). In the brain, two major receptor subtypes for serotonin have been identified: the 5-HT1 receptor, with a high affinity to the transmitter, and 5-HT2, with an affinity to serotonin in the μM range (Peroutka et al. 1981). These two receptor types have been characterized and localized in rat (Biegon et al. 1982; Pazos et al. 1985) as well as in human (Biegon et al. 1986) brain. It appears that of the two subtypes, the 5-HT2 receptors are more involved in the biochemistry of depression and its treatment. Thus, there are reports on changes in 5-HT2 receptor levels in the brains of suicide victims and patients with affective disorder studied post mortem (Stanley and Mann 1983; Owen et al. 1983; McKeith et al. 1987). In the rat brain, 5-HT2 receptors were shown to be reduced following chronic treatment with antidepressant drugs (Bergstrom and Kellar 1979), while electroconvulsive treatment induces an increase in receptor density (Bergstrom and Kellar 1983).

We and others have recently identified binding sites with characteristics of 5-HT2 receptors on human whole platelets (MacBride et al. 1983; Geaney et al. 1984) and on frozen platelet membranes (Biegon et al. 1987). In a study of patients with unipolar affective disorder, we have found an increased concentration of 5-HT2 receptors on platelet membranes, which decreased to control levels following successful treatment with the antidepressants amitriptyline and trazodon (Biegon et al. 1987). However, it remained unclear whether the changes in receptor binding precede the clinical changes, coincide with it or follow it. Thus the present study was designed to examine the temporal relationship between changes in receptor binding and clinical state, using maprotiline (MPT), a drug with an extremely low affinity for the 5-HT2 receptor (Pinder 1977; Peroutka et al. 1981).

## Materials and methods

The study included 15 patients, 11 women and 4 men, 33–78 years old (mean 60.5 years), who gave informed consent to participate in the study. All patients were diagnosed as suffering from major depression according to DSM-III (1980). Patients had no other neurological or psychiatric illness and were not taking any medication for at least 2 weeks prior to admission and first blood sampling. The women were all past menopause, so stage of menstrual cycle was not monitored.

Following a clinical interview and the first blood sample (phase 1), patients were started on active treatment (MPT, 150 mg/day given either orally or intravenously). A second blood sample was withdrawn following 1 week of treatment (phase 2) and a third after 3 weeks of treatment (phase 3). Blood was always withdrawn after the clinical evaluation but prior to the daily dose of MPT.

Platelet membranes were prepared as previously described (Biegon et al. 1987). Briefly, blood is collected into plastic centrifuge

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tubes containing citrate buffer (final concentration 0.38%) and centrifuged at 200 $g \times 10$ min to yield platelet rich plasma (PRP). PRP preparations are lysed by mixing with a hypotonic medium (5 mM TRIS-HCl, pH 7.5) and homogenized for 15 s with a polytron at a moderate speed. The lysed preparation is centrifuged at 45000 $g \times 20$ min in a refrigerated centrifuge, the supernatant decanted and the membrane pellet washed twice in 50 mM TRIS-HCl pH 7.5. The final pellet is kept frozen at $-70°$ C until used.

For the binding assay, we used [$^3$H]-ketanserin (NEN specific activity 90 ci/mmole). The binding procedure consists of incubating 800 µl samples of the membrane suspension with 100 µl of a near-saturating concentration of the ligand (3.0 nM) and 100 µl incubation buffer (50 mM TRIS-HCl pH 7.5 for total binding) or cold displacing drug (10 µM mianserin, to define non specific binding) for 60 min at room temperature (25° C). The binding is terminated by filtration, under vacuum, through GF/B filter discs [previously soaked in 1% bovine serum albumin (BSA) and dried] followed by $3 \times 5$ ml washes in ice-cold incubation buffer. The binding assays were performed in triplicate. The three samples from each patient (phase 1, 2 and 3) were always run in the same experiment.

The filters were dried and counted in 4 ml scintillant following 2 h in a shaker bath; using a Packard scintillation counter. Final protein concentration was measured by the method of Lowry et al. (1951). Mianserin was the kind gift of Organon, Holland. All other chemicals used in the experiments were obtained from commercial sources.

## Results

Pretreatment values of ketanserine binding in the present group of patients were similar to those observed at the same concentration in depressed or suicidal subjects (Biegon et al. 1987, 1989) and higher than values in control subjects in those studies. Hamilton and Beck scores were high (Table 1, phase 1). Following 1 week of treatment, receptor binding in the group was significantly decreased by about 30%. Both Hamilton and Beck scores were also significantly decreased. Following 2 more weeks of treatment, the depression scores exhibited a further significant decrease, while receptor binding did not changes any further (Table 1).

The significant decrease in depression scores in the group as a whole reflects the fact that most patients improved on MPT. However, not all of them did. While 11 patients showed considerable clinical improvement, two did not improve and two actually got worse, as evidenced by an increase in depression scores. In those individuals receptor binding remained unchanged or increased, respectively (Fig. 1). To test for possible correlation between changes in clinical state and receptor binding, we have calculated the difference between the pretreatment and post-treatment scores on the Hamilton and Beck depression scales for each individual, as well as the difference between pretreatment and post-treatment receptor binding. We found a significant correlation between the change in the Hamilton depression score and change in receptor binding, with an $R$ (correlation coefficient) of 0.62, significant at the $P < 0.025$ level. An even better and more significant correlation was seen between the change in receptor binding and the Beck depression inventory score ($R = 0.72$, $P < 0.006$). The mode of drug administration had no significant effect on the treatment outcome, neither did we find an effect of age or sex on the biochemical and clinical results.

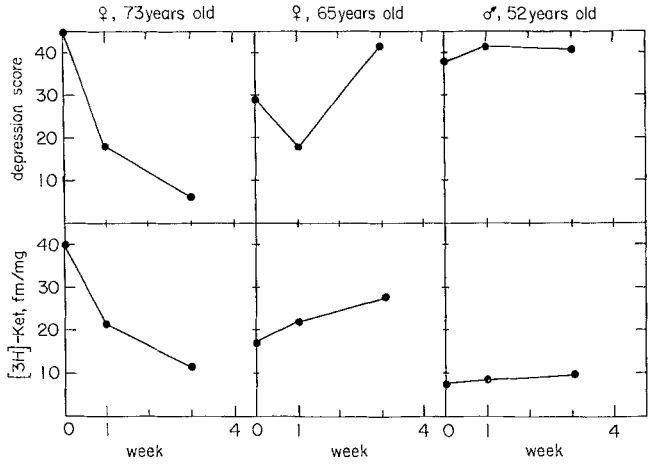


**Fig. 1.** Serotonin 5-HT2 receptors and depression rating in patients before and during antidepressant treatment. The figure shows cases representative of the three possible treatment outcomes: clinical improvement (*left*), increase in depression (*middle*) and no change (*right*). For the illustration, the depression score was calculated as the mean of the Hamilton and Beck scores for each patient

**Table 1.** Effect of antidepressant drug treatment on 5-HT2 receptor binding and depression scores

| Treatment duration (weeks) | 5-HT2 binding (fmole/mg P) | HDS score | BDI score |
|---|---|---|---|
| 0 | 29.5±4.8 | 36.8±1.8 | 38.1±2.9 |
| 1 | 21.8±3.6[a] | 28.6±2.4[b] | 31.1±3.1[c] |
| 3 | 21.7±3.8 | 20.7±2.6 | 22.5±2.7 |

Results are mean±SEM of 15 patients
HDS = Hamilton Depression Scale
BDI = Beck Depression Inventory
[a] Significantly different from pretreatment values, $P < 0.05$, Student's $t$-test for paired values, two tailed
[b] Significantly different from week 0 and week 3, $P < 0.001$ and $P < 0.02$, respectively, Student's $t$-test, two tailed
[c] Significantly different from week 0 and week 3, $P < 0.02$ and $P < 0.05$, respectively, Student's $t$-test, two tailed

## Discussion

Depression is a relatively common disease, and a simple, non-invasive procedure for the determination of a physiological marker for the state of depression could be a major step in aiding diagnosis and assessment of treatment. In the present work, we show that serotonin receptors of the 5-HT2 type do appear to be a promising new condidate for such a role. We (Biegon et al. 1987) and others (Arora and Melzer 1989) have recently demonstrated that depressed individuals have higher platelets 5-HT2 receptor levels than normal controls. Following successful treatment with antidepressant drugs, receptor levels drop down to control levels. Now we show, in a larger group of patients, that the receptor response, seen after only 1 week of treatment, precedes, and may therefore possibly predict, the full clinical response,

which is generally delayed by at least 2–3 weeks. This is true for responders as well as non-responders in the present group, since patients in whom there was no clinical change and those who have even become worse during the treatment period show either no change in receptor binding or an increase, respectively.

It is probably premature to suggest the use of absolute 5-HT2 receptor levels on platelets as a measure for the severity of depression, since the range of normal values is very wide and the possible influence of factors such as age, sex, hormonal status and heredity has not yet been adequately evaluated. However, within an individual, or in comparison with a well matched control group, these receptors appear to faithfully reflect the state of depression. Ongoing studies in our laboratory examine the specificity of the elevation in 5-HT2 receptors to depression by studying other syndromes theoretically linked with the serotonergic system, such as sleep disorders and aggression (e.g. Pucilowski and Kostowski 1980).

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