Effects of fluoxetine treatment of platelet $^3$H-imipramine binding, 5-HT uptake and 5-HT content in major depressive disorder

Anna Wägner 1,3, Dolores Montero 1,3, Björn Mårtensson 1, Bo Siwers 2 and Marie Åberg 1

Department of Psychiatry and Psychology, Karolinska Institute, Karolinska Hospital, S-104 01 Stockholm, Sweden, 2 Department of Psychiatry and 4 Department of Clinical Pharmacology, Karolinska Institute, Huddinge Hospital, S-141 86 Huddinge, Sweden

(Received 9 October 1989)
(Revision received 18 May 1990)
(Accepted 30 May 1990)

Summary

Platelet $^3$H-imipramine binding, serotonin (5-HT) uptake and 5-HT concentrations were studied in 14 hospitalized patients with depressive disorder following 6 weeks of treatment with a selective 5-HT uptake blocker, fluoxetine. After 3 weeks of treatment there was a significant decrease in $B_{max}$ of $^3$H-imipramine binding and a significant increase in $K_d$. A highly significant decrease in $V_{max}$ of 5-HT uptake was seen after 3 weeks of treatment which was accompanied by a slight increase in $K_m$. At the same time the platelet 5-HT content was significantly reduced by about 90% of its original level. The platelet 5-HT content continued to decrease with further treatment while there was a tendency for $V_{max}$ to return to pretreatment levels. The affinity of the 5-HT uptake carrier continued to decrease significantly. There was no further significant change in $B_{max}$ of $^3$H-imipramine binding during further treatment, although there was an increase in $B_{max}$ in the majority of patients. The changes in $B_{max}$ and $V_{max}$ were closely associated throughout the treatment. In some cases the changes in different platelet parameters correlated with the changes in depression rating scores during treatment, but this correlation did not reach statistical significance.

Key words: Fluoxetine treatment; Depressed patients; Platelet 5-HT uptake; Platelet 5-HT content; $^3$H-Imipramine binding

Introduction

During the last decade a series of selective serotonin (5-hydroxytryptamine, 5-HT) uptake inhibitors have been successfully used in the treatment of depression (reviewed in, e.g., Åberg et al., 1986a). Although their main clinical advantage may be their different side effect spectrum, the antidepressant effect of serotonin uptake inhibitors is interesting in view of the many studies linking serotonin to depressive illness (Åberg et al., 1984; reviewed in, e.g., Åberg et al., 1986b,c). A commonly used strategy in these studies is to investigate peripheral human blood platelets which...
have several biochemical and pharmacological similarities to central 5-HT neurons (reviewed in, e.g., Stahl, 1985; Pletcher and Laubscher, 1980) such as active 5-HT transport, 5-HT receptors, \(^{3}H\)-imipramine binding sites and mitochondrial monoamine oxidase.

Although both \(^{3}H\)-imipramine binding and 5-HT uptake in platelets are lower in depressed patients than in healthy controls (reviewed in, e.g., Åberg and Wägner, 1986; Wirz-Justice, 1988) no correlation has hitherto been found between these two parameters, neither in depressed patients nor in healthy controls (Raisman et al., 1982). Antidepressant treatment has been shown to decrease the affinity of the 5-HT uptake transporter (increase in \(K_m\)) (Coppen et al., 1979; Meltzer et al., 1981; Suranyi-Cadotte et al., 1985; Malmgren et al., 1987) as well as to change the number of uptake sites (\(V_{max}\)) (Lingjaerde, 1979a; Kenny et al., 1983; Wood et al., 1983; Beving et al., 1985; Healy et al., 1983; Phillips et al., 1988) and the effects seem to be related to the type of antidepressant used. The changes in platelet \(^{3}H\)-imipramine binding are less clear, since no change, a decrease as well as an increase in the number of binding sites have been reported (reviewed in, e.g., Åberg and Wägner, 1986).

The aim of the present study was to investigate the effects of treatment with a selective 5-HT uptake blocker, fluoxetine (Wong et al., 1975; Horning and Wong, 1976), with known antidepressant effect (reviewed in, e.g., Åberg et al., 1986a; Benfield et al., 1986; Sommi et al., 1987) on some selected parameters in blood platelets from depressed patients.

Fluoxetine is a potent inhibitor of the presynaptic re-uptake of 5-HT with essentially no effect on the re-uptake of other neurotransmitters (Wong et al., 1975, 1983; Horning and Wong, 1976; Stark et al., 1985) and with negligible binding affinity for classical receptor sites (i.e., dopamine, histamine, \(\alpha\) and \(\beta\)-receptors) (Wong et al., 1983). A decrease in 5-HT\(_1\) receptors (Wong et al., 1985) and \(\beta\)-adrenergic receptors (Wansley et al., 1987) has been observed after chronic fluoxetine treatment. The metabolism of fluoxetine (reviewed in Benfield et al., 1986) is not fully known, but about 30% of fluoxetine is metabolized to norfluoxetine which also has selective 5-HT uptake inhibiting properties (Horning and Wong, 1976). In the present study we have determined platelet \(^{3}H\)-imipramine binding, 5-HT uptake and 5-HT concentrations in depressed patients following treatment with fluoxetine for 6 weeks. The effects of fluoxetine on monoamine metabolites in the cerebrospinal fluid (CSF) were also determined, but the results are published elsewhere (Mårtensson et al., 1989). The \(K_m\) values of fluoxetine and norfluoxetine for platelet \(^{3}H\)-imipramine binding and 5-HT uptake were determined in vitro in order to investigate the possibility of using these data including plasma concentrations of fluoxetine and/or the metabolite norfluoxetine to predict the effects on the different platelet parameters in vivo.

Material and methods

Patients

Fourteen inpatients (12 women, two men; ranging in age from 25 to 69 years, mean \(\pm\) SD: 46.2 \(\pm\) 18.8 years) were admitted via the 24-h emergency clinic at the psychiatric clinics of the Karolinska Hospital (\(n = 10\)) and the Huddinge University Hospital (\(n = 4\)). All patients fulfilled DSM-III criteria for major depressive disorder. The decision to hospitalize the patients was always made on clinical grounds and no patient was admitted to the study for research purposes only. Physical illness and signs or symptoms of substance abuse disorder, schizophrenia or organic mental disorder were exclusion criteria.

All patients had been drug-free for at least 1 month before participation in the study, with the exception of low doses of benzodiazepines used for day and night sedation (oxazepam typically not exceeding 45 mg/day for anxiety and nitrazepam 5–10 mg/day for night sedation).

Ratings

Severity of depression was assessed using the Montgomery–Åberg Depression Rating Scale (MADRS) (Montgomery and Åberg, 1979) which is a subscale of the Comprehensive Psychopathological Rating Scale (CPRS; Åberg et al., 1978). Initial rating scores on the MADRS ranged from 15 to 40 (mean \(\pm\) SD: 28.8 \(\pm\) 7.1) reflecting moderate to severe depression. During treatment,
severity of depression was rated on the MADRS once a week for 6 weeks. Patients were classified as responders (n = 8) if their MADRS score had decreased more than 50% after 6 weeks of treatment. The mean MADRS score after 6 weeks was 11.8 ± 9.5 which is a highly statistically significant decrease (t = 4.45, P < 0.01).

**Medication**

The evaluation period preceding active treatment was 4–7 days, during which time placebo capsules were given. Fluoxetine (Eli Lilly and Company) was given in 20-mg capsules in the morning. The first three patients were given 60 mg fluoxetine daily and 11 patients were given 40 mg daily. The reason for reducing the dose was that when the study had already started, new results emerged concerning the optimal therapeutic dose of fluoxetine (Wernicke et al., 1987). The scheduled minimum duration of inpatient treatment was 6 weeks. Patients were to be withdrawn from the study in case of severe deterioration of their mental state or because they did not consent to further participation in the study. Four patients were withdrawn because of deterioration of their mental state or lack of antidepressant effect. Three of them were successfully treated with electroconvulsive therapy and the fourth patient refused further treatment. One patient terminated the treatment after 3 weeks because of an intercurrent viral infection, although her depression ameliorated. Nine patients completed the 6-week study. Data from patients are included in the calculations until the time of withdrawal from the study. During the study moderate doses of oxazepam were allowed to relieve anxiety and/or for night sedation (in one patient nitrazepam).

**Blood sampling**

Blood samples for determination of platelet 5-HT parameters were taken between 8 a.m. and 9 a.m. three times: before treatment, after 3 and 6 weeks of treatment with fluoxetine. All subjects were fasting. Blood samples were taken weekly for determination of plasma concentrations of fluoxetine (FLU) and norfluoxetine (NorFLU).

About 120 ml blood was collected into plastic tubes containing 3% EDTA as anticoagulant (1 ml EDTA to 9 ml blood) and used for determination of 5-HT uptake and $^3$H-imipramine binding (IMI). Another 5 ml blood was collected into a separate cold plastic tube (kept on ice) containing 3% EDTA for determination of endogenous platelet 5-HT concentration.

Lumbar punctures were performed immediately after the blood sampling procedures, i.e., three times during treatment. Cerebrospinal fluid was collected for determination of the monoamine metabolites (5-HIAA, HVA and HMPG) and substance P. The sampling procedure and the results are published elsewhere (Mårtensson et al., 1989).

**Biochemical analysis**

**Platelet 5-HT concentrations**

Platelet 5-HT concentrations were analyzed by high-performance liquid chromatography (HPLC) techniques according to a method described recently (Montero et al., 1989). An aliquot of the sample was taken for determination of protein (Peterson, 1977) and platelet number.

**Determination of $^3$H-imipramine binding**

The procedures for platelet preparation and the $^3$H-imipramine binding assay have been described in detail elsewhere (Wagner et al., 1985). In brief, the total incubation volume was 200 μl (100 μl membranes, 50 μl of $^3$H-imipramine and 50 μl incubation buffer). The $^3$H-imipramine binding was studied using at least seven different concentrations of $^3$H-imipramine (specific activity about 75 Ci/mmol, New England Nuclear) in duplicate between 0.5 and 10 nM. To ensure comparable analysis conditions in the binding assay, all three samples from a patient were analyzed at approximately the same protein concentration (0.3–0.4 mg of protein/ml assay). The protein concentration was determined by the method of Peterson (1977) using bovine serum albumin as a standard.

Maximum binding ($B_{max}$) and the affinity constant ($K_d$) were calculated by linear least squares analysis of individual Scatchard plots for each patient and control. Specific binding was defined as the difference in binding in the absence and presence of desmethylimipramine (final concentration in the assay was 10 μM) and repre-
sented about 80–90% of the total binding at 5 nM 
$^3$H-imipramine.

**Determination of 5-HT uptake**

The method was a modification of the method described by Arora and Meltzer (1981). Aliquots of 250 μl of platelet-rich plasma (PRP) were incubated for 1 min at 37°C with 25μl of various concentrations of $^3$H-5-HT (specific activity 26.2 Ci/mmol, New England Nuclear). The 5-HT uptake was analyzed using seven different concentrations of $^3$H-5-HT (diluted 50 times with unlabeled 5-HT) in duplicate between 0.2 and 1.5 μM. The passive uptake was determined by parallel incubation of temperature-equilibrated PRP (37°C) on ice (4°C). After 1 min incubation, the reaction was stopped by the addition of 1 ml ice-cold phosphate buffer (NaCl 70 mM, KCl 7.5 mM, NaH$_2$PO$_4$ 55 mM, pH = 7.5) containing chlorimipramine (1 μM), after which the tubes were immediately placed on ice. After adding 5 ml ice-cold phosphate buffer (without chlorimipramine), the samples were rapidly filtered through Whatman GF/C filters and washed three times with 5 ml ice-cold phosphate buffer. The filters were placed in scintillation vials and 10 ml of Quickscint 401 (Zinsser Analytic, U.K. Ltd) was added and counted in an Intertechnique SL-4000 scintillation counter (efficiency 35%).

Active uptake was obtained by subtracting the passive uptake (incubation on ice) from the total uptake (incubation at 37°C). The kinetic parameters $V_{\text{max}}$ and $K_m$ were calculated according to the method described by Eadie and Hofstee. The correlation coefficient was > 0.90 in all cases. An aliquot of the PRP was taken for counting the number of platelets.

**Plasma concentrations**

The plasma concentrations of fluoxetine (FLU) and norfluoxetine (NorFLU) were analyzed by gas chromatography with electron capture techniques (Nash et al., 1982). The analysis was performed by Wisconsin Analytical and Research Services, Canada.

**Determination of $K_i$ values for fluoxetine and norfluoxetine**

The $K_i$ values for FLU and NorFLU were determined for $^3$H-imipramine binding and 5-HT uptake. Stock solutions of the drugs (1 mM) were dissolved in 1 mM HCl. About nine duplicate concentrations of the drugs between $10^{-4}$ and $10^{-10}$ nM were incubated with either $^3$H-imipramine or $^3$H-5-HT under the same conditions as described in detail above. The $^3$H-imipramine concentration in the assay was 1 nM and the $^3$H-5-HT concentration was 0.5 μM.

**Statistical methods**

The results have been analyzed using the paired t-test for treatment effects. The values are denoted, e.g., $B_{\text{max I}}$ before treatment, $B_{\text{max II}}$ after 3 weeks of treatment and $B_{\text{max III}}$ after 6 weeks of treatment. Differences between 3 and 6 weeks are denoted $B_{\text{max III}} - II$ etc. Data are expressed as mean ± SD.

**Results**

The mean values ± SD of all parameters examined during 6 weeks of fluoxetine treatment in the depressed patients are listed in Table 1, including results of statistical analysis.

**Plasma concentrations**

The mean elimination half-life after long-term administration of fluoxetine is 2–7 days and that of norfluoxetine is about 1 week (Benfield et al., 1986). The majority of patients had reached steady-state levels of both compounds after 3 and 6 weeks of treatment (Fig. 1).

There was a significant increase in plasma concentrations of both fluoxetine and norfluoxetine between 3 and 6 weeks of treatment (cf. Table 1). It has been reported that fluoxetine has concentration-dependent kinetics (Benfield et al., 1986) which might explain the continued increase between 3 and 6 weeks of treatment. Those patients who were treated with 60 mg fluoxetine/day all had higher plasma concentrations after 3 weeks than the patients treated with 40 mg/day. Included in Fig. 1 are data from one patient in whom plasma concentrations were measured during 3 weeks after withdrawal of fluoxetine.

**$K_i$ values**

The $K_i$ values for fluoxetine and norfluoxetine were calculated from the IC$_{50}$ values of the inhibition potency of $^3$H-imipramine binding and 5-HT
TABLE 1

PLATELET $^3$H-IMIPRAMINE BINDING, 5-HT UPTAKE, 5-HT CONTENT AND PLASMA CONCENTRATIONS OF FLUOXETINE AND THE METABOLITE NORFLUOXETINE INCLUDING CPRS SCORES DURING 6 WEEKS OF FLUOXETINE TREATMENT

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After 3 weeks</th>
<th>After 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_{\text{max}}$</td>
<td>988 ± 141 (14)</td>
<td>785 ± 117 (11)</td>
<td>818 ± 157 (9)</td>
</tr>
<tr>
<td>$K_d$</td>
<td>1.17 ± 0.30 (14)</td>
<td>1.97 ± 0.67 (11)</td>
<td>1.91 ± 0.55 (9)</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>34.76 ± 13.72 (10)</td>
<td>6.24 ± 5.16 (10)</td>
<td>22.93 ± 19.58 (6)</td>
</tr>
<tr>
<td>$K_m$</td>
<td>0.77 ± 0.26 (10)</td>
<td>1.50 ± 0.55 (10)</td>
<td>5.71 ± 3.73 (6)</td>
</tr>
<tr>
<td>5-HT</td>
<td>0.923 ± 0.501 (11)</td>
<td>0.103 ± 0.100 (9)</td>
<td>0.046 ± 0.023 (8)</td>
</tr>
<tr>
<td>FLU</td>
<td>661 ± 218 (10)</td>
<td>805 ± 213 (10)</td>
<td>1003 ± 380 (9)</td>
</tr>
<tr>
<td>NorFLU</td>
<td>28.8 ± 7.1 (14)</td>
<td>22.5 ± 8.2 (13)</td>
<td>11.8 ± 9.5 (9)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. Figures in parenthesis indicate numbers of patients. $B_{\text{max}}$ is given in fmol/mg protein and $K_d$ in nM, $V_{\text{max}}$ in pmol/10⁸ platelets/min and $K_m$ in μM, 5-HT content in nmol/10⁸ platelets, plasma concentrations of FLU and NorFLU are in nmol/l.

*: Significantly different compared to before treatment.
**: No significant difference compared to 3 weeks of treatment.
*: No significant difference compared to before treatment.
**: Significantly different compared to 3 weeks of treatment.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

uptake according to the formula of Cheng and Prusoff (1973). The results are listed in Table 2. The inhibiting potency of fluoxetine is approximately of the same order for $^3$H-imipramine binding and 5-HT uptake. In both cases the affinity of the metabolite was about four times weaker than that of the parent compound. Both compounds inhibited 5-HT uptake in a mixed antagonist manner, i.e., $V_{\text{max}}$ was decreased and $K_m$ increased in the in vitro studies (data not shown) (Malmgren and Wägner, 1990).

$^3$H-Imipramine binding

After 3 weeks of fluoxetine treatment there was a significant decrease in $B_{\text{max}}$ of about 20% ($B_{\text{max}}$ II−I: $-213 ± 205$, $n = 11$, $t_{10} = 3.45$, $P < 0.01$) with a concomitant significant increase in $K_d$ of about 48% ($K_d$ II−I: $0.84 ± 0.85$, $n = 11$, $t_{10} = 3.29$, $P < 0.01$). Following 3 more weeks of treatment there was no further significant change in $B_{\text{max}}$ ($B_{\text{max}}$ III−II: $52 ± 193$, $n = 9$, $t_8 = 0.81$, NS) or in $K_d$ ($K_d$ III−II: $-0.026 ± 0.760$, $n = 9$, $t_7 = 0.100$, NS). After 6 weeks of treatment $B_{\text{max}}$ was still significantly lower than before treatment ($B_{\text{max}}$ III−I: $-195 ± 230$, $n = 9$, $t_6 = 2.54$, $P < 0.05$) while $K_d$ was significantly higher ($K_d$ III−I: $0.76 ± 0.72$, $t_6 = 3.17$, $P < 0.05$). The results are shown in Table 1 and Fig. 2 and 3.

There was a positive correlation between $K_d$ and the plasma concentrations of fluoxetine and norfluoxetine, which was statistically significant only for norfluoxetine after 6 weeks of treatment (cf. Table 3). No correlation was found between $B_{\text{max}}$ and plasma concentrations.

5-HT uptake

There was a significant decrease in $V_{\text{max}}$ by about 85% after 3 weeks of treatment ($V_{\text{max}}$ II−I: $-31.30 ± 15.21$, $n = 8$, $t_7 = 5.820$, $P < 0.001$) with a slight increase in $K_m$ ($K_m$ II−I: $0.620 ± 0.751$, $n = 8$, $t_7 = 2.338$, $P < 0.05$). After 3 more weeks of treatment there was a tendency to an increase in $V_{\text{max}}$ ($V_{\text{max}}$ III−II: $15.06 ± 19.91$, $n = 6$, $t_5 = 1.852$, $P < 0.10$) which did not reach statistical significance, but $K_m$ continued to significantly increase ($K_m$ III−II: $4.262 ± 3.598$, $n = 6$, $t_5 = 2.902$, $P < 0.05$).

Thus, after 6 weeks of treatment $K_m$ was significantly increased compared to pretreatment values ($K_m$ III−I: $4.90 ± 3.84$, $n = 6$, $t_5 = 3.126$, $P < 0.01$) while $V_{\text{max}}$ was not significantly different from pretreatment values ($V_{\text{max}}$ III−I: $14.18 ± 28.39$, $n = 6$, $t_5 = 1.224$, NS). The results are shown in Figs. 4 and 5. In one patient 5-HT uptake was completely blocked, i.e., the active uptake was equal to the passive uptake, so no
Fig. 1. Plasma concentrations of fluoxetine (top) and norfluoxetine (bottom) during 6 weeks of treatment.

calculation was possible.

As expected there was a significant negative correlation between $V_{max}$ and the plasma concentrations of FLU and NorFLU after 3 and 6 weeks of treatment (Table 3).

**TABLE 2**

<table>
<thead>
<tr>
<th>$K_s$ VALUES (nmol/l) OF FLUOXETINE AND NORFLUOXETINE ON PLATELET $^3$H-IMIPRAMINE BINDING AND 5-HT UPTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3$H-imipramine binding</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Fluoxetine</td>
</tr>
<tr>
<td>Norfluoxetine</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD in a series of experiments ($n$).
TABLE 3

INTERCORRELATION COEFFICIENTS BETWEEN CHANGES IN 3'H-IMIPRAMINE BINDING AND 5-HT UPTAKE PARAMETERS DURING FLUOXETINE TREATMENT

<table>
<thead>
<tr>
<th>Changes between</th>
<th>0–3 weeks</th>
<th>3–6 weeks</th>
<th>0–6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B_{\text{max}} \text{ and } K_d )</td>
<td>0.23 (11) NS</td>
<td>0.21 (9) NS</td>
<td>-0.89 (9) ***</td>
</tr>
<tr>
<td>( V_{\text{max}} \text{ and } K_m )</td>
<td>0.69 (8) *</td>
<td>0.70 (6) NS</td>
<td>0.79 (6) NS</td>
</tr>
<tr>
<td>( B_{\text{max}} \text{ and } V_{\text{max}} )</td>
<td>0.6 (8) NS</td>
<td>0.84 (6) *</td>
<td>0.83 (6) *</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate numbers of patients.
* \( P < 0.05; ** P < 0.01; *** P < 0.001. \)

Fig. 4. Platelet 5-HT uptake (\( V_{\text{max}} \)) during fluoxetine treatment.

Fig. 5. Platelet 5-HT uptake (\( K_m \)) during fluoxetine treatment.

treatment (5-HT II - I: \(-0.764 \pm 0.452 \text{ nmol/10}^8\text{ platelets, } n = 8, t_7 = 4.81, P = 0.002\) which further significantly decreased after 3 more weeks of treatment (5-HT III - II: \(-0.035 \pm 0.036 \text{ nmol/}10^8\text{ platelets, } n = 8, t_7 = 4.67, P = 0.002\) )

TABLE 4

INTERCORRELATION COEFFICIENTS BETWEEN DIFFERENT PLATELET PARAMETERS AND DRUG PLASMA CONCENTRATIONS BEFORE AND AFTER 3 AND 6 WEEKS OF FLUOXETINE TREATMENTS

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After 3 weeks</th>
<th>After 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B_{\text{max}} \text{ and } K_d )</td>
<td>-0.18 (11) NS</td>
<td>0.71 (11) *</td>
<td>-0.29 (9) NS</td>
</tr>
<tr>
<td>( V_{\text{max}} \text{ and } K_m )</td>
<td>0.23 (7) NS</td>
<td>0.72 (10) *</td>
<td>0.74 (6) NS</td>
</tr>
<tr>
<td>( K_d \text{ and FLU} )</td>
<td>0.46 (10) NS</td>
<td>0.60 (9) NS</td>
<td>0.87 (9) **</td>
</tr>
<tr>
<td>( K_d \text{ and NorFLU} )</td>
<td>0.60 (9) NS</td>
<td>0.60 (9) NS</td>
<td>0.87 (9) **</td>
</tr>
<tr>
<td>( V_{\text{max}} \text{ and FLU} )</td>
<td>-0.70 (9) *</td>
<td>-0.79 (6) NS</td>
<td>-0.76 (6) NS</td>
</tr>
<tr>
<td>( V_{\text{max}} \text{ and NorFLU} )</td>
<td>-0.78 (9) *</td>
<td>-0.79 (6) NS</td>
<td>-0.76 (6) NS</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate numbers of patients.
* \( P < 0.05; ** P < 0.01. \)
Fig. 6. Platelet 5-HT concentrations during fluoxetine treatment expressed as nmol/10^8 platelets (a) and as nmol/mg protein (b).

10^8 platelets, n = 7, t^2 = 2.55, P < 0.05. The results are shown in fig. 6.

We have noted that expressing 5-HT concentrations in nmol/mg protein, instead of per platelet number, gives more reproducible data. In the present study there was not enough material for doing so in all subjects. For comparison the results are both in nmol 5-HT/10^8 platelets (Fig. 6a) and in nmol 5-HT/mg protein (Fig. 6b). In one subject no 5-HT concentration was detectable after 6 weeks of treatment. No correlations between baseline or changes in 5-HT concentrations and the plasma concentrations of fluoxetine or norfluoxetine were found.

**Clinical correlates**

Correlations between the decrease in CPRS rating and changes in platelet parameters were sometimes observed but no relevant statistically significant correlations could be established. No significant correlations between platelet parameters or changes in platelet parameters and clinical response were found.

**Discussion**

In the present study we have shown that chronic treatment with the selective 5-HT uptake blocker fluoxetine caused significant changes in all three platelet 5-HT parameters examined: 5-HT concentrations, 5-HT uptake and imipramine binding.

The platelet 5-HT content decreased significantly in all treated patients in line with most antidepressant treatments (Lemberger et al., 1978; Wirz-Justice and Puehringer, 1978; Ross et al., 1980; Corona et al., 1982; Kenny et al., 1983; Poirier et al., 1985; Sarrias et al., 1987; Timmerman et al., 1987; Mårtensson et al. 1990).

Consistent with our investigations in vitro, fluoxetine decreases the V_{max} and increases the K_m of platelet 5-HT uptake. The decrease in V_{max}, which was seen in all patients, was negatively correlated with the plasma concentrations of fluoxetine and the metabolite norfluoxetine. Similar findings have been reported for other antidepressants, such as citalopram (Bjerkenstedt et al., 1985), zimeldine and clomipramine (Ross et al.,
1980), zimeldine and amitriptyline (Coppen et al., 1979), fluvoxamine (Wood et al., 1983) and fluoxetine (Lemberger et al., 1978). The increase in $K_m$, which was most pronounced after 6 weeks of treatment, was not, however, present in all patients and in fact in two patients $K_m$ remained unchanged. Due to limitations in available blood amounts, the highest substrate concentration used for determination of the 5-HT uptake was only 1.5 $\mu$M of $^3$H-5-HT. The high $K_m$ values are thus subject to a large error, and should hence be interpreted with some caution.

Mixed antagonist inhibition has been reported for several other selective 5-HT uptake blockers such as clomipramine (Lingjaerde 1979a) and fluvoxamine (Wood et al., 1983) in the treatment of depressed patients and for sertraline (Philips et al., 1988) and PK5078 (Kenny et al., 1983) in healthy control studies. Citalopram has been shown to have mixed antagonist properties in vitro (Lingjaerde et al., 1979b) which might explain the total 5-HT blockade observed in the study of depressed patients by Beving et al. (1985). A mixed antagonist action does not, however, seem to be a property of all selective 5-HT uptake blockers. Treatment with zimeldine caused only a significant increase in $K_m$, while treatment with alaproclate did not affect either $B_{max}$ or $K_m$ (Åberg-Wistedt et al., 1985). In the latter study it was suggested that the lack of effect could be due to the scheme of dosage, since alaproclate has a rather short half-life (about 6 h) and the drug was given only once a day in the morning, i.e., no plasma concentrations of alaproclate were measurable at the time of blood sampling (prior to drug intake) (Åberg-Wistedt et al., 1985).

Also consistent with the investigations in vitro, fluoxetine treatment caused a decrease in $B_{max}$ of platelet $^3$H-imipramine binding in the majority of patients (9/11). An explanation for the observed decrease in $B_{max}$ and increase in $K_d$ could be that fluoxetine and its metabolite were still present in the platelet membranes which will affect the binding analysis. The magnitude of the decrease in vivo was, however, less than would be expected from in vitro data. Since the plasma concentrations of fluoxetine and norfluoxetine were much higher than their respective $K_i$ values in vitro, about 80% inhibition of $^3$H-imipramine binding would be expected if all drug residues remained in the platelet membranes compared to the observed 20% decrease. However, the decrease was not seen in all patients and no correlations were found between $K_d$ and the plasma concentrations of fluoxetine or norfluoxetine after 3 weeks of treatment indicating that most of the compounds were removed during the washing procedure of the platelet membranes. Only the plasma concentration of norfluoxetine after 6 weeks of treatment was significantly correlated with $K_d$.

Despite the continued increase in plasma concentrations of fluoxetine and norfluoxetine between 3 and 6 weeks of treatment there was no further decrease in $B_{max}$ of $^3$H-imipramine binding or $V_{max}$ of 5-HT uptake. If anything, both $B_{max}$ and $V_{max}$ seemed to increase, while the affinity of the 5-HT uptake carrier continued to decrease. Thus, in six of the nine patients there was an increase in $B_{max}$ and in four of six there was an increase in $V_{max}$, although not significantly so. Still, the net effect was that $B_{max}$ remained significantly lower after 6 weeks of fluoxetine treatment compared to pretreatment levels and the reduction in $B_{max}$ was significantly negatively correlated with the increase in $K_d$. Similarly, after 6 weeks of treatment, there was a tendency for $V_{max}$ to increase to pretreatment levels and this increase was positively correlated with the increase in $K_d$.

Currently we have no simple explanation for these findings. Since the platelet population is already renewed to a large extent after 3 weeks of treatment (assuming a platelet turnover of 7–14 days (Ebbe, 1971)) it is not likely that the distribution of young and older platelets can account for the present findings. Unless fluoxetine changes platelet turnover, the proportion of new platelets (with more 5-HT and $^3$H-imipramine binding sites (Arora and Meltzer, 1982, 1984)) would remain unchanged. In vitro, fluoxetine is a potent inhibitor of platelet aggregation and this might affect the platelet distribution between whole blood and PRP during fluoxetine treatment (Malmgren and Wågner, 1990).

Another possibility is that clinical recovery itself might lead to an increase in $B_{max}$ or $V_{max}$ (Healy et al., 1983; Suranyi-Cadotte et al., 1984; Langer et al., 1986; Maj et al., 1988) which would mask a further reduction in these parameters pro-
duced by the presence of fluoxetine. No significant correlations between the platelet parameters or changes in these parameters and clinical outcome could be established, but this might be explained by the low number of patients. Previously we reported that $B_{\text{max}}$ increased after treatment with the selective 5-HT uptake blockers alaproclate and zimeldine while there was no change in affinity (Wagner et al., 1987). However, the patient sample was rather small and most patients were responders and no correlations with clinical outcome were observed. In agreement with the present results, a decrease in $B_{\text{max}}$ of imipramine binding was observed after clomipramine treatment (Mellerup and Plenge, 1986; Poirier et al., 1987; Mårtensson et al., 1990). It was suggested that this decrease could be explained by a drug-induced destruction of the binding sites (Mellerup and Plenge, 1986).

Although the number of patients after 6 weeks of treatment was too small to draw any firm conclusions it appears that fluoxetine has different acute and chronic effects on platelet imipramine binding and 5-HT uptake mechanisms, while still reducing platelet 5-HT content very markedly during continuous treatment.

The fact that the reduction in platelet 5-HT content paralleled the decrease in $V_{\text{max}}$ after 3 weeks of treatment but not after 6 weeks indicates that factors other than 5-HT uptake blockade per se are of importance for the regulation of platelet 5-HT content. Furthermore, the decrease in $V_{\text{max}}$ but not the decrease in 5-HT content was closely correlated with the plasma concentrations of fluoxetine and norfluoxetine. The rate of 5-HT uptake inhibition does not always coincide with the reduction rate of platelet 5-HT concentrations (Keuny et al., 1983) and this time discrepancy is even more apparent when these parameters are studied after withdrawal of antidepressant treatment (Ross and Åberg-Wistedt, 1983).

The time courses of changes in $^3$H-imipramine binding and 5-HT uptake parameters appear to be closely parallel, however. In view of the suggestion that the IMI binding site is involved in the regulation/modulation of 5-HT uptake (Barbaccia et al., 1983; Sette et al., 1983; Meyerson et al., 1987), it might be of interest to note that although no significant intraplatelet correlations between $^3$H-imipramine binding and 5-HT uptake parameters were found, changes in these parameters during fluoxetine treatment were significantly positively associated.

Conclusions

In conclusion, fluoxetine treatment caused substantial alterations in platelet $^3$H-imipramine binding, 5-HT uptake and 5-HT content, and these changes were dependent on the duration of the treatment period. These observed effects after fluoxetine treatments as well as after treatment with other selective 5-HT uptake blockers indicate that selection of the right time schedule for blood sampling as well as monitoring plasma concentrations of the parent drug (including active metabolites) are extremely important. The observation that a certain plasma concentration of fluoxetine inhibited 5-HT uptake as could be expected from the in vitro data ($K_i$ values) suggests that in vitro data including drug plasma concentrations can to some extent be used to predict acute or short-term pharmacological effects on different platelet 5-HT parameters in clinical studies. However, these parameters should be used with caution and are most likely not useful for predicting long-term effects of antidepressant treatment. The findings of associations between changes within several platelet parameters during fluoxetine treatment point towards the relevance of longitudinal studies, rather than comparing baseline values.

Acknowledgements

Financial support was provided by the Swedish Medical Research Council (5454, 7763, 9121), the Söderström-König Foundation, the Torsten and Ragnar Söderberg Foundation, funds from the Swedish Medical Society, the Lundbeck Foundation for Psychopharmacological Research, Magnus Bergvalls Stiftelse, Stiftelsen Lars Hiertas Minne, and funds from the Karolinska Institute. The assistance of Mr. Mats Samuelsson at the psychiatric ward of the Karolinska hospital and the staff at the psychiatric ward of Huddinge hospital are greatly acknowledged. Eli Lilly Company is acknowledged for providing the fluoxetine and norfluoxetine compounds used in the in vitro
studies, for providing fluoxetine and placebo capsules for clinical use, for measuring the drug plasma concentrations and for valuable information during the study.

References


Lingjaerde, O. (1979b) Inhibitory effect of clomipramine and related drugs on the serotonin uptake in platelets: more
complicated than previously thought. Psychopharmacology 61, 245–249.


