Platelet 5-hydroxytryptamine is decreased in a preliminary group of depressed patients receiving the 5-hydroxytryptamine re-uptake inhibiting drug fluoxetine

V. C. MENYS, C. C. T. SMITH*, P. LEWINS†, R. D. T. FARMER‡ and M. I. M. NOBLE§

Department of Biological Sciences, The Manchester Metropolitan University, Manchester, U.K., *Department of Medicine, University College and Middlesex School of Medicine, London, U.K., †Gallions Reach Health Centre, London, U.K., and ‡Department of Community Health and §Academic Unit of Cardiovascular Medicine, Charing Cross and Westminster Medical School, London, U.K.

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1. In view of the importance of 5-hydroxytryptamine in coronary thrombosis, we wanted to know whether a potentially protective decrease in platelet 5-hydroxytryptamine could be achieved by treatment with an inhibitor of 5-hydroxytryptamine uptake, fluoxetine.

2. We studied 15 patients treated for psychiatric indications with fluoxetine, and compared the findings with those obtained with blood from 18 patients treated with amitriptyline and 13 controls previously treated for affective disorders.

3. Platelet-rich plasma 5-hydroxytryptamine levels were significantly decreased in the fluoxetine group (P<0.005) but not in the amitriptyline group compared with the control group.

4. Collagen-induced aggregation in whole blood anticoagulated with hirudin was measured by sequential single platelet counting. The contribution of 5-hydroxytryptamine was assessed from the effect of adding the 5-hydroxytryptamine specific antagonist ICI 170809. This contribution was significantly decreased in the fluoxetine group but not in the amitriptyline group compared with the control group.

5. It is concluded that platelet 5-hydroxytryptamine is indeed decreased by fluoxetine, and we would predict a protective effect of fluoxetine against coronary thrombosis.

INTRODUCTION

5-Hydroxytryptamine (5HT) is plentiful in blood platelets, the richest source outside the brain, and may be very important in the pathophysiology of arterial thrombosis [1]. The 5HT hypothesis of coronary arterial thrombosis proposes that 5HT released from activated platelets stimulates additional platelets via the 5HT₂ receptor. Under the conditions of high shear present at a coronary arterial stenosis, high local concentrations of 5HT occur as a result of the release mechanism. This mechanism acts as a positive feedback system leading to rapid thrombus growth and is completely inhibited by 5HT₂ receptor antagonism [1]. However, 5HT is not involved in the adhesion of platelets to the arterial wall, so that pharmacological interference with the 5HT system does not cause bleeding [1].

In the absence of 5HT₂ receptor antagonist drugs in clinical practice in the U.K., the most common clinical interference with 5HT arises from the use of 5HT re-uptake inhibiting drugs for the treatment of depression [2-4]. The purpose of such medication is to increase 5HT concentration in the neuronal synapses. Platelets differ from neurons in that they do not synthesize 5HT, but accumulate it after uptake from the blood. It does not necessarily follow that platelets would also show reduced 5HT content after antidepressant therapy, but we postulated that this might be observed in platelets, in the absence of bleeding. A point that might predict the opposite, i.e. an increase in platelet 5HT content, is the claim by Leonard and co-workers [3, 5-7] that selective 5HT re-uptake inhibitors actually increase 5HT uptake in depressed patients, in contrast to normal volunteers. The importance of the hypothesis of lowered platelet 5HT, if correct, is that lower concentrations of 5HT would be obtained from platelet activation at a coronary arterial stenosis in such patients; there could therefore be a lower incidence of coronary thrombosis in patients so treated. The present report concerns experiments designed to test the first part of this hypothesis.
MATERIALS AND METHODS

Patients

The study was based on a general practice serving a population of over 12,000 people. All patients were either being treated for affective disorders or had previously received drug treatment. The control group consisted of 13 patients (six males, seven females), median age 42 (interquartile range 36, 54) years, who had been treated with either amitriptyline, dothiepin or fluoxetine, and in each case treatment had been stopped at least 2 weeks previously [5 (2–16) weeks; median and range]. The patients treated with amitriptyline formed a second control group; this is not ideal because amitriptyline has some 5HT uptake inhibiting properties [8, 9], but this group forms those with the most commonly prescribed antidepressant. The amitriptyline group (n=18; nine males, nine females), aged 44 (39, 50) years, had been taking amitriptyline [50 (25, 75) mg/day; median and interquartile range] for 17 (12, 35) weeks. The fluoxetine group (n=15; four males, eleven females), aged 43 (41, 49) years, had been taking fluoxetine (20 mg/day) for 11 (7, 23) weeks. No patients were being prescribed aspirin or aspirin-like drugs and no patients reported having used such drugs in the previous 7 days.

Platelet-rich plasma 5HT

After venous blood sampling without occlusion, hirudin anticoagulation of blood was used before the preparation of a platelet-rich plasma (PRP) by centrifugation. Platelet counts in PRP were measured in a Coulter Thrombocounter. EDTA (5 mmol/l) was added to samples of hirudinized PRP, which were then stored at –20°C until assayed for 5HT. 5HT was measured in the plasma plus platelets of the PRP, using HPLC with electrochemical detection [10]. Platelet 5HT was expressed relative to the number of platelets in the PRP which was estimated using a Coulter counter. Aliquots of 125 μl of PRP were mixed with 875 μl of sodium chloride solution (154 mmol/l), 45 μl of perchloric acid (9.2 mol/l; final concentration 400 mmol/l) and 10 μl of n-acetyl-5HT internal standard (100 pmol) and centrifuged to sediment precipitated proteins. Supernatants were then injected into the chromatographic system which consisted of a Waters Model 510 pump (Waters Ltd, Watford, U.K.), a SPECAC Model 34000 injection valve fitted with a 100 μl loop (Analytical Accessories Ltd, Orpington, Kent, U.K.) and a Spherisorb S3 ODS2 analytical column (Phase Separations Ltd, Deeside, Clwyd, U.K.). Separation was achieved using an isocratic eluent system consisting of an acetate–citrate buffer containing EDTA (3 mmol/l) and 17.5% (vol/vol.) methanol [11]. A flow rate of 0.6 ml/min was maintained and detection of 5HT was achieved using an EDT Research electrochemical detector (EDT Instruments Ltd, Dover, Kent, U.K.) with a working potential of +0.72 V. The assay was validated with standards containing 5HT and internal standard (n-acetyl 5HT) which were run on the day of assay. Electrochemical detector responses for 5HT were linear up to a concentration of 1 μmol/l and the measurements are extremely reproducible. Intra- and inter-assay coefficients of variation for 5HT in plasma were 3.9% and 9.5% respectively (n=6). For platelet samples, intra- and inter-assay coefficients of variation for 5HT were 3.8% and 2.1% (n=6). The identities of 5HT peaks on the chromatograms were confirmed by 'spiking' samples with 5HT hydrochloride.

Platelet function

Although the study of stirred platelets in vitro underestimates activity in vivo under high shear conditions, a few such measurements were made. We used hirudin to anticoagulate blood samples, to maintain normocalcaemia, thereby avoiding artefacts associated with the use of citrate [12].

Materials

Collagen (Hormon Chemie, Munich, Germany), was obtained from Nycomed U.K., Birmingham, U.K. Recombinant desulphatohirudin HVI (CGP 39393), was kindly provided by Dr G. F. Pay, Ciba Pharmaceuticals, Horsham, U.K., and solutions were prepared as described elsewhere [13]. The 5HT3 receptor antagonist ICI 170809 [14], provided by Zeneca Pharmaceuticals, Alderley Park, Macclesfield, U.K., was freshly dissolved in DMSO, protected from light and diluted with saline. The final concentration of DMSO in blood PRP (0.01% vol/vol.) has been shown to have little or no effect either on spontaneous or collagen-induced aggregation in whole blood, or on collagen-induced aggregation in PRP, whereas the final concentration of ICI 170809 used (3.0 pmol/l) effectively abolishes 5HT-induced aggregation [15]. Aspirin (acetylsalicylic acid) and adrenaline bitartrate were obtained from Sigma Chemical Co., Poole, U.K., and solutions were prepared and used as described elsewhere [15]. Aspirin was used at a concentration (1.0 mmol/l) previously shown to inhibit collagen-induced thromboxane A2 (TXA2) synthesis by > 98% [13].

Blood samples

Blood was obtained from fasted subjects by venepuncture without venous stasis, and anticoagulated with CGP 39393 (200 units/ml). Platelet aggregation studies were commenced 30 min after blood collection and completed within 2 h. Aliquots of whole blood were dispensed for whole-blood aggregation studies, while the remainder was centrifuged at
Fluoxetine and platelet 5-hydroxytryptamine

room temperature for 15 min at 123 g for the isolation of PRP.

**Platelet aggregation in whole blood**

Aliquots of whole blood (0.5 ml) were preincubated for 3 min at 37°C, either with DMSO vehicle (0.1%, vol./vol.), aspirin (1.0 mmol/l), or with both aspirin and ICI 170809 (3.0 μmol/l).

After preincubation, aliquots were transferred to a Payton Aggregometer and with stirring at 1000 rev./min, collagen vehicle or collagen (0.3 μg/ml, corresponding to a plasma concentration of 0.5 μg/ml, assuming a haematocrit of 0.44) was added. After stirring for 3 min, samples were taken into glutaraldehyde (0.8%, vol./vol.) for platelet counting using a Coulter Thrombocounter (Coulter Electronics, Luton, U.K.), as described in detail elsewhere [13]. Spontaneous aggregation in stirred blood was calculated using the platelet count in unstirred blood. Collagen-induced aggregation was calculated using the platelet count in blood stirred with vehicle alone.

**Platelet aggregation in PRP**

Aliquots of PRP (0.4 ml) were preincubated for 5 min at 37°C, either with vehicle, aspirin or aspirin and ICI 170809, as for whole-blood aggregation studies. Aggregation induced by collagen (0.5–1.0 μg/ml) was studied using a Payton Aggregometer with a potentiometric recorder and calibrated using PRP (0 cm scale deflection) and platelet-poor plasma (16 cm scale deflection). After stirring at 1000 rev./min for 5 min, both the initial (maximal) rate (cm/min) of aggregation and the extent of aggregation (amplitude of the change in light transmission at 3 min after the initial decrease in light transmission) were determined. Where used, adrenaline (20 nmol/l) was added immediately before collagen. Platelet counts in PRP were determined using a Coulter Thrombocounter as described above.

**Statistical analysis**

Data are presented as medians (interquartile range) unless stated otherwise. Comparisons were made using either the Wilcoxon matched-pairs signed-ranks test or the Mann–Whitney U-test, as appropriate.

**RESULTS**

**PRP 5HT**

A significant decrease of 5HT in PRP was found in patients treated with fluoxetine, but not in those treated with amitriptyline (Fig. 1). The concentration of 5HT in PRP was significantly reduced in the patients receiving fluoxetine when compared both with controls (P < 0.005) and with patients treated with amitriptyline (P < 0.005).

**Platelet counts and spontaneous aggregation**

Results for the counts are given in Table 1. Platelet counts were similar, both in whole blood and PRP, in all groups. However, spontaneous platelet aggregation, occurring in blood stirred with vehicle alone, was significantly increased in the amitriptyline group [24 (13, 45)% median and interquartile range] compared with the control group [15 (6, 20)% P < 0.05], but not in the fluoxetine group [17 (4, 26), P not significant].

**Collagen-induced aggregation in whole blood**

With collagen (0.3 μg/ml in blood, corresponding to about 0.5 μg/ml in plasma), near-maximal aggregation occurred in all groups (medians 95–96%). Aspirin markedly inhibited aggregation induced by collagen in all groups by about 46% (data not shown). The relative contributions of TXA₂ and 5HT to collagen-induced aggregation were esti-
Table 2. Relative contributions of TXA₂, ADP and 5HT mediation to collagen-induced platelet aggregation in whole blood. The contributions of TXA₂ and 5HT to collagen-induced aggregation were estimated, using the differences between aspirin alone and vehicle control, and between aspirin alone and aspirin in combination with ICI 170809, respectively. The residual aggregation response with the latter combination was assumed to reflect ADP-dependent aggregation; for validation, see Menys [16]. Data are medians and interquartile ranges for the relative contributions (% net response); Statistical significance: *P < 0.01 compared with control, †P < 0.05 compared with amitriptyline.

<table>
<thead>
<tr>
<th></th>
<th>TXA₂</th>
<th>ADP</th>
<th>5HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47 (27, 61)</td>
<td>32 (30, 56)</td>
<td>12 (10, 20)</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>45 (21, 65)</td>
<td>40 (28, 59)</td>
<td>13 (6, 26)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>47 (21, 75)</td>
<td>46 (20, 65)</td>
<td>4 (1, 10)†</td>
</tr>
</tbody>
</table>

Rate of collagen-induced aggregation in PRP

Results are given in Table 3. The rate of aggregation with collagen (0.5 μg/ml) was decreased in the amitriptyline group (P < 0.05) but not in the fluoxetine group compared with the control group. However, aspirin retarded aggregation more markedly both in the amitriptyline (P < 0.05) and fluoxetine (P < 0.05) groups compared with the control group. Furthermore, although adrenaline enhanced aggregation in the presence of aspirin in all groups, this effect was significantly greater in the control group (P < 0.05) than in the amitriptyline and fluoxetine groups. On the other hand, 5HT contributed to the rate of aggregation both in the control group and in the amitriptyline group, but not in the fluoxetine group (Table 3).

DISCUSSION

This study demonstrates that 5HT re-uptake inhibition decreases the 5HT of PRP, reflecting decreased platelet 5HT. This finding of reduced platelet 5HT with chronic 5HT re-uptake inhibition is not established in the previous literature [2-7], but can be predicted from the fact that tritiated 5HT uptake is reduced in patients taking fluoxetine [16]. (It is curious that the same result was not obtained with another drug of this class, nisoxetine [17]). We predicted that 5HT depletion would result in less 5HT-dependent aggregation; this is confirmed for collagen-induced aggregation in stirred blood (Table 2). We were not able to test high shear conditions as would be applicable in coronary arterial stenoses, but the present results lead us to predict a lower incidence of coronary arterial thromboses in patients treated with 5HT re-uptake inhibiting antidepressive drugs.

We did not measure plasma 5HT concentrations in this study; they are known to be three orders of magnitude less than that of PRP, and affected by platelet activation during sampling. Gow et al. [18] have shown that virtually all the 5HT in PRP is accounted for by the 5HT in the platelets.

Platelets are the richest source of 5HT outside the brain; the 5HT is stored in the dense granules and is released from platelets through activation by collagen [19]. Blood platelets do not synthesize 5HT [20]; they accumulate 5HT through an active imipramine-sensitive mechanism in the first instance, thence by a reserpine-sensitive mechanism which permits accumulation in the dense granules [8]. Thus, there are close similarities between platelets and 5HT-rich neurons with respect to uptake, storage and release of 5HT [8, 21]. Platelets accumulate 5HT during their physiological life as shown by survival studies in dogs [22], labelling experiments in rats, rabbits and dogs [23] and 5HT clearance studies in man [24].

Imipramine-type 'tricyclic antidepressant' drugs are widely used in the treatment of affective disorders and decrease platelet 5HT in rabbits and man [25, 26], but these drugs are neither selective nor potent for 5HT uptake and it is unclear whether any such effect occurs at therapeutic levels in man. The present study suggests such an effect with amitriptyline (Fig. 1), and has been shown for desipramine [27]. More recently, both potent and selective inhibitors of 5HT uptake, such as fluoxetine, have been introduced for the treatment of affective disorders, but the present study is the first to our knowledge to document lower platelet 5HT levels (Fig. 1).

The normal plasma level of 5HT is very low, or near the detection limits of commonly used assays [28, 29]. Plasma levels in vivo rise when platelets aggregate [30]. Patients with atheromatous disease and diabetes mellitus exhibit a fall in platelet 5HT concentration and a rise in plasma 5HT concentration [31]. The 'plasma levels rise artefactually in vitro when there has been activation and aggregation of the platelets during blood sampling [32].

When combined with other agonists of platelet aggregation [32] such as ADP, collagen, adrenaline, noradrenaline and TXA₂, 5HT amplifies their effects in citrated PRP. This amplification reaction is inhibited by the 5HT₂ antagonist, ketanserin. A similar action of 5HT has been shown in both whole blood and PRP, in experiments in which hirudin was used to anticoagulate blood, both to maintain normocalcaemia and to avoid artefacts associated with the use of citrate [15]. There is a marked difference between the agonist potency of 5HT in conditions in vitro as studied here, where micromolar concentr-
Table 3. Rate of collagen-induced platelet aggregation in PRP. Data are medians (interquartile range) for the initial (maximal) rate of aggregation (cm/min) in PRP stirred with collagen (0.5 μg/ml). PRP was preincubated with aspirin alone or with both aspirin and ICI 170809. Adrenaline (20 nmol/l) was added immediately before collagen. Statistical significance: *P < 0.05 compared with aspirin alone; †P < 0.05 compared with aspirin and adrenaline.

<table>
<thead>
<tr>
<th>Collagen, 0.5 μg/ml</th>
<th>Vehicle</th>
<th>Aspirin</th>
<th>Aspirin + adrenaline</th>
<th>Aspirin + ICI 170809 + adrenaline</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>16 (14, 26)</td>
<td>4.4 (2, 14)</td>
<td>14.0 (7, 15)†</td>
<td>9.0 (6, 12)†</td>
</tr>
<tr>
<td>Amitryptiline</td>
<td>13 (8, 18)</td>
<td>1.6 (0, 4)</td>
<td>3.6 (1, 6)†</td>
<td>2.4 (0, 5)†</td>
</tr>
<tr>
<td>P compared with control</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<tr>
<td>Fluoxetine</td>
<td>15 (9, 24)</td>
<td>2.2 (1, 3)</td>
<td>4.8 (2, 6)†</td>
<td>3.4 (1, 6)</td>
</tr>
<tr>
<td>P compared with control</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tbody>
</table>

In the light of our findings, we consider that 5HT re-uptake inhibitors may be the most suitable drug treatment for depressed patients with cardiovascular disease. Antidepressants of other types have a reputation for cardiotoxicity [49], which is not shared to date by the 5HT re-uptake inhibitors. Theoretically we would predict that the latter may even be protective against thrombotic events; such a possibility warrants examination in an epidemiological study.

REFERENCES


