Decreased platelet $^3$H-imipramine binding sites in classical migraine

DP GEANEY, MG RUTTERFORD, JM ELLIOTT, M SCHÄCHTER, KMS PEET, DG GRAHAME-SMITH

From the MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, UK

SUMMARY Patients with classical migraine investigated between attacks had significantly fewer platelet $^3$H-imipramine binding sites than control subjects and this finding was more marked in males than in females. There was no abnormality of binding characteristics of platelet 5-hydroxytryptamine receptors or of platelet $\alpha_2$-adrenoceptors. Because the reduced $^3$H-imipramine binding capacity was found in classical migraineurs who were investigated between attacks, it may reflect a predisposition to migraine rather than being a consequence of attacks.

Abnormalities of platelet aggregation, particularly increased aggregation, have been reported in migraineurs between attacks in response to different agents, including 5-hydroxytryptamine (5-HT) and adrenaline.\(^1\)\(^2\) In addition, the active uptake of 5-HT by the platelets of patients with migraine has been reported to be reduced between,\(^3\) during\(^4\) and after\(^5\) attacks of migraine. We have further investigated these abnormalities of platelet function by three separate radioligand binding studies to define the various platelet receptor sites involved. \(^3\)H-yohimbine binding to intact platelets was performed to label $\alpha_2$-adrenoceptors which mediate noradrenaline- and adrenaline-induced platelet aggregation.\(^6\)\(^7\) \(^3\)H-lysergic acid diethylamide (LSD) binding to platelet membranes was performed to label the receptor for 5-HT-induced shape change and aggregation.\(^8\)\(^9\) A site associated with the active uptake of 5-HT was labelled on intact platelets by $^3$H-imipramine.\(^10\)\(^11\)

Subjects and methods

Patients with classical migraine as defined by the criteria of the World Federation of Neurology Research Group\(^12\) were studied. They were investigated between attacks, at least 10 days after the last attack occurred. An interval of 10 days was used because this corresponds to the life-span of human platelets.\(^13\) Both patients and healthy age-matched control subjects had taken no drugs in the 10 days prior to investigation. Venous blood samples were taken between 0830 h and 1000 h for determination of the platelet binding affinity and capacity for $^3$H-imipramine, $^3$H-LSD and $^3$H-yohimbine.

$^3$H-Imipramine binding assay

$^3$H-Imipramine binding was performed by a modification of the method of Briley et al.\(^14\) Platelet-rich plasma was prepared by low speed centrifugation of venous blood anticoagulated with 1% ethylenediaminetetra-acetic acid (EDTA). This was then centrifuged at 1200 g for 7-5 min at 10°C to produce a platelet pellet which was resuspended in the incubation medium (0.1% EDTA, 150 mmol NaCl, pH 7-5) to give a final platelet concentration of approximately 4-8 x 10\(^7\) platelets/ml. Aliquots of the platelet suspension were incubated for 1 h at 2°C with $^3$H-imipramine, at six concentrations in the range 0.5-5 nM, and in the presence or absence of 1 µM fluoxetine. Incubations were terminated by rapid centrifugation (6500 g for 1 min). Specific binding was defined as the difference in $^3$H-imipramine binding occurring in the presence or absence of 1 µM fluoxetine. Fluoxetine was chosen as the inhibitor because, like imipramine, it is a potent inhibitor of 5-HT uptake in platelets, but it is structurally dissimilar to imipramine, and should thus inhibit only the specific binding of $^3$H-imipramine to the site related to 5-HT uptake.

$^3$H-yohimbine binding assay

This was performed according to a previously described method.\(^*\) The final platelet suspension was prepared as for $^3$H-imipramine. Aliquots of this suspension were incubated with $^3$H-yohimbine, at six concentrations in the range 1.5-15 nM, for 20 min at 37°C, and terminated by rapid centrifugation. Specific binding was defined by 5 µM phenotolamine.

$^3$H-LSD binding assay

This was performed as previously described.\(^*\) The platelet
Decreased platelet $^3$H-imipramine binding sites in classical migraine

pellet was prepared as for $^3$H-imipramine and was then
resuspended in hypotonic Tris-EDTA (5 mM Tris, 0.1%
EDTA, pH 7.5). The suspension was twice homogenised
and centrifuged at 30,000 g for 15 min. The membrane
pellet was suspended in incubation buffer (50 mM Tris,
120 mM NaCl, 5 mM KCl, 2 mM MgCl$_2$ and 0.05%
ascorbic acid, pH 7.3). The centrifugation was repeated
and the final pellet resuspended in the same incubation
buffer. Aliquots of the membrane suspension were
incubated with $^3$H-LSD, at six concentrations in the range
0.25–2.5 nM, for 4 h at 37°C. Incubations were terminated
by filtration under reduced pressure through Whatman
GF/F filters. Specific binding was defined by 300 nM
spiperone. Aliquots of the membrane suspension were
frozen at $-20^\circ$C and protein content later assayed by the
method of Lowry.\textsuperscript{14}

Statistical evaluation
The binding characteristics of $^3$H-imipramine, $^3$H-
yohimbine and $^3$H-LSD in each assay were determined by
non-linear regression analysis.\textsuperscript{14} Comparison of the bind-
ing capacity ($B_{\text{max}}$) and affinity ($K_d$) between groups were
made by Student’s two-tailed $t$ test.

Results

$^3$H-IMPRAMINE BINDING
Platelet $^3$H-imipramine binding characteristics are
shown in table 1. The binding capacity ($B_{\text{max}}$) was
significantly lower in patients with classical migraine
than in control subjects ($p < 0.05$). This difference
was more marked in the males ($p < 0.01$) than in the
females where it did not reach significance. This was
due to female controls having a lower mean binding
capacity than male controls, while male and female
classical migraineurs had similar mean values (fig).
In addition, five female classical migraineurs have
been studied within five days after an attack and had
a mean binding capacity which was lower than
female classical migraineurs between attacks and
significantly lower than female controls ($p < 0.05$).

Table 1 Platelet $^3$H-imipramine binding characteristics in
classical migraineurs and controls (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>$B_{\text{max}}$ (fmol/10$^8$ platelets)</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>24</td>
<td>1.27 ± 0.13</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>1.17 ± 0.21</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>1.34 ± 0.16</td>
</tr>
<tr>
<td>Classical Migraine: (between attacks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>24</td>
<td>1.70 ± 0.25</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>1.27 ± 0.23</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>2.01 ± 0.38</td>
</tr>
<tr>
<td>Classical Migraine: (&lt;5 days after attack)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>1.73 ± 0.51</td>
</tr>
</tbody>
</table>

* $p < 0.05$ than all controls.
† $p < 0.01$ than male controls.
‡ $p < 0.05$ than female controls.

Fig $^3$H-imipramine binding capacity in classical
migraineurs between attacks and in controls.

There was no significant difference in binding
affinity between the groups.
The ages were satisfactorily matched (male con-
trols: median 32 years, range 24–40 years; female
controls: median 36 years, range 18–49 years; male
classical migraineurs: median 32 years, range 17–50
years; female classical migraineurs: median 35
years, range 17–53 years).

Table 2 Platelet $^3$H-yohimbine binding characteristics in
classical migraineurs and controls (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>$B_{\text{max}}$ (fmol/10$^8$ platelets)</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>18</td>
<td>4.67 ± 0.26</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>4.53 ± 0.39</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>4.81 ± 0.35</td>
</tr>
<tr>
<td>Classical Migraine: (between attacks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>24</td>
<td>4.23 ± 0.21</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>3.97 ± 0.15</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>4.38 ± 0.33</td>
</tr>
</tbody>
</table>

Mean $B_{\text{max}}$ all classical migraineurs—mean $B_{\text{max}}$ all controls (95%
confidence limits) = 2.8 (±4.3 to 9.9) fmol/10$^8$ platelets.
**3H-YOHIMBINE BINDING**

Platelet 3H-yohimbine binding characteristics are shown in table 2. There was no significant difference in binding capacity or affinity between the groups.

**3H-LSD BINDING**

Platelet 3H-LSD binding characteristics are shown in table 3. There was no significant difference in binding capacity or affinity between the groups.

**Discussion**

We have found no difference between the binding characteristics of platelet 5-HT receptors or \( \alpha_2 \)-adrenoceptors in patients with classical migraine compared with control subjects. This suggests that the increased platelet aggregation reported in migraineurs to 5-HT and adrenaline is not due to a primary platelet receptor change.

We have found a significant reduction in the number of platelet 3H-imipramine binding sites in classical migraineurs between attacks compared with controls. 3H-imipramine binding sites are closely associated with 5-HT uptake sites. The active uptake of 5-HT by the platelets of patients with migraine has previously been found to be reduced between, during and after attacks of migraine. This reduced platelet 5-HT uptake in migraineurs may thus be at least partially due to a reduction in the number of platelet 5-HT uptake sites. As the reduction in 3H-imipramine binding capacity was found in classical migraineurs investigated at least 10 days after their last attack, and we have found no correlation between 3H-imipramine binding capacity and the time elapsed since the last attack (data not shown), this reduced capacity may reflect a predisposition to attacks of migraine rather than being a consequence of them.

It is noteworthy that we found that the reduction in 3H-imipramine binding capacity was more marked in male than in female migraineurs because the control values for females were lower than those of males. It is well documented that the prevalence of migraine in females is higher than in males and their lower control value for 3H-imipramine binding capacity may reflect an increased predisposition to migraine. Similar lower values of 3H-imipramine binding capacity in female controls than in male controls have been found previously in another study. While we are not aware of any report of sex differences in platelet 5-HT uptake, previous investigators have not found a close correlation between 5-HT uptake and 3H-imipramine binding within individual subjects.

We have also investigated female classical migraineurs within five days of an acute attack of migraine. Although the numbers are small, the mean value of 3H-imipramine binding capacity was lower than the female classical migraineurs between attacks and significantly lower than female controls. Whether this may be an immediate consequence of the attacks or may reflect the predisposition around the time of attacks is not yet clear.

5-hydroxytryptamine has previously been implicated in the aetiology of migraine for a number of reasons in addition to reduced platelet 5-HT uptake. Antagonists or mixed agonist/antagonists of 5-HT are effective in the prophylaxis or treatment of migraine, for example, methysergide, pizotifen and ergotamine. During attacks of migraine there is a reduction in plasma or platelet 5-HT and increased urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), a major metabolite of 5-HT. The reduced 3H-imipramine binding sites we have found in this study are consistent with such peripheral changes in 5-HT metabolism.

There is some evidence that classical migraine may be primarily a brain disorder with secondary vascular changes. In depressive illness platelet 5-HT uptake is also reduced, and 3H-imipramine binding sites are reduced in both platelets and in brain. The decreased 3H-imipramine binding sites in platelets of classical migraineurs may therefore reflect changes in 3H-imipramine binding sites in the brain.

It is not yet clear whether reduced 3H-imipramine binding capacity may be a primary cause of predisposition to attacks of migraine or is a secondary effect in response to another predisposing factor. Longitudinal investigations of platelet 3H-imipramine binding in classical migraineurs may throw further light on the aetiology of classical migraine.

We thank our colleagues in the Department of Neurology, Radcliffe Infirmary, for their cooperation in allowing us to study patients in their care.

---

**Table 3 Platelet 3H-LSD binding characteristics in classical migraineurs and controls (mean ± SEM)**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Bmax (fmol/mg protein)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>24</td>
<td>57.7 ± 3.7</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>54.6 ± 3.1</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>60.3 ± 6.3</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>Classical Migraine: (between attacks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>22</td>
<td>50.2 ± 2.2</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>47.3 ± 3.2</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>52.6 ± 2.9</td>
<td>0.58 ± 0.06</td>
</tr>
</tbody>
</table>

Mean Bmax all classical migraineurs—mean Bmax all controls (95% confidence limits) = 7.5 (–16.4 to 1.4) fmol/mg protein.
Decreased platelet \(^3\)H-imipramine binding sites in classical migraine

References

Decreased platelet 3H-imipramine binding sites in classical migraine.

D P Geaney, M G Rutterford, J M Elliott, M Schächter, K M Peet and D G Grahame-Smith

*J Neurol Neurosurg Psychiatry* 1984 47: 720-723
doi: 10.1136/jnnp.47.7.720

Updated information and services can be found at:
http://jnnp.bmj.com/content/47/7/720

These include:

**Email alerting service**
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/