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

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SUMMARY Patients with classical migraine investigated between attacks had significantly fewer platelet ^3H -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 α_2 -adrenoceptors. Because the reduced ^3H -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 during⁴ and after⁵ 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. ^3H -yohimbine binding to intact platelets was performed to label α_2 -adrenoceptors which mediate noradrenaline- and adrenaline-induced platelet aggregation.^{6,7} ^3H -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 ^3H -imipramine.^{10,11}

Subjects and methods

Patients with classical migraine as defined by the criteria of the World Federation of Neurology Research Group¹² 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 ^3H -imipramine, ^3H -LSD and ^3H -yohimbine.

^3H -Imipramine binding assay

^3H -Imipramine binding was performed by a modification of the method of Briley *et al.*¹⁰ 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 $4-8 \times 10^7$ platelets/ml. Aliquots of the platelet suspension were incubated for 1 h at 2°C with ^3H -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 ^3H -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 ^3H -imipramine to the site related to 5-HT uptake.

^3H -yohimbine binding assay

This was performed according to a previously described method.⁶ The final platelet suspension was prepared as for ^3H -imipramine. Aliquots of this suspension were incubated with ^3H -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 phenolamine.

^3H -LSD binding assay

This was performed as previously described.^{8,9} The platelet

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pellet was prepared as for ^3H -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 MgCl₂, 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 ^3H -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°C and protein content later assayed by the method of Lowry.¹⁴

Statistical evaluation

The binding characteristics of ^3H -imipramine, ^3H -yohimbine and ^3H -LSD in each assay were determined by non-linear regression analysis.¹⁵ Comparison of the binding capacity (B_{max}) and affinity (*Kd*) between groups were made by Student's two-tailed *t* test.

Results

^3H -IMIPRAMINE BINDING

Platelet ^3H -imipramine binding characteristics are shown in table 1. The binding capacity (B_{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 ^3H -imipramine binding characteristics in classical migraineurs and controls (mean $\pm$ SEM)

	<i>n</i>	B_{max} (fmol/10 ⁶ platelets)	<i>Kd</i> (nM)
Controls:			
All	24	141 $\pm$ 8	1.27 $\pm$ 0.13
Male	10	154 $\pm$ 10	1.17 $\pm$ 0.21
Female	14	132 $\pm$ 10	1.34 $\pm$ 0.16
Classical Migraine: (between attacks)			
All	24	121 $\pm$ 6*	1.70 $\pm$ 0.25
Male	10	121 $\pm$ 6†	1.27 $\pm$ 0.23
Female	14	122 $\pm$ 9	2.01 $\pm$ 0.38
Classical Migraine: (<5 days after attack)			
Female	5	100 $\pm$ 10‡	1.73 $\pm$ 0.51

* $p < 0.05$ than all controls.

† $p < 0.01$ than male controls.

‡ $p < 0.05$ than female controls.

Fig ^3H -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 controls: 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 ^3H -yohimbine binding characteristics in classical migraineurs and controls (mean $\pm$ SEM)

	<i>n</i>	B_{max} (fmol/10 ⁶ platelets)	<i>Kd</i> (nM)
Controls:			
All	18	49.5 $\pm$ 2.5	4.67 $\pm$ 0.26
Male	9	49.7 $\pm$ 2.7	4.53 $\pm$ 0.39
Female	9	49.3 $\pm$ 4.4	4.81 $\pm$ 0.35
Classical Migraine: (between attacks)			
All	24	52.3 $\pm$ 2.3	4.23 $\pm$ 0.21
Male	9	53.0 $\pm$ 3.3	3.97 $\pm$ 0.15
Female	15	51.9 $\pm$ 3.1	4.38 $\pm$ 0.33

Mean B_{max} all classical migraineurs—mean B_{max} all controls (95% confidence limits) = 2.8 (–4.3 to 9.9) fmol/10⁶ 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 α_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.¹¹ The active uptake of 5-HT by the platelets of patients with migraine has previously been found to be reduced between,³ during⁴ and after⁵ 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 males¹⁶ 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.¹⁷ 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.¹⁸

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.¹⁹ During attacks of migraine there is a reduction in plasma or platelet 5-HT^{20, 21} and increased urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA),^{20, 22} 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.²³ In depressive illness platelet 5-HT uptake is also reduced,^{18, 24} and ³H-imipramine binding sites are reduced in both platelets^{17, 18, 25} and in brain.^{26, 27} 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 $\pm$ SEM)

	n	B _{max} (fmol/mg protein)	K _d (nM)
Controls:			
All	24	57.7 $\pm$ 3.7	0.58 $\pm$ 0.03
Male	11	54.6 $\pm$ 3.1	0.58 $\pm$ 0.05
Female	13	60.3 $\pm$ 6.3	0.57 $\pm$ 0.04
Classical Migraine: (between attacks)			
All	22	50.2 $\pm$ 2.2	0.62 $\pm$ 0.05
Male	10	47.3 $\pm$ 3.2	0.68 $\pm$ 0.08
Female	12	52.6 $\pm$ 2.9	0.58 $\pm$ 0.06

Mean B_{max} all classical migraineurs—mean B_{max} all controls (95% confidence limits) = 7.5 (–16.4 to 1.4) fmol/mg protein.

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