Platelet ^{3}H Ketanserin Binding in Tension-type Headache

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Objective.—The present study was undertaken to investigate the alterations in platelet 5-HT$_2$ receptor binding in patients with tension-type headache.

Background.—Serotonin (5-HT) has an important but complex role in pain modulation. The involvement of serotonin in tension-type headache has been investigated by studying serotonin in peripheral blood, but results have been inconclusive. There are, however, only a few investigations in which the status of platelet serotonin transporters has been studied by ^{3}H imipramine and ^{3}H paroxetine. The present study was undertaken to investigate alterations in platelet 5-HT$_{2A}$ receptors using ^{3}H ketanserin as a ligand.

Methods.—Platelet ^{3}H ketanserin binding was studied in 14 patients with tension-type headache and in 15 healthy controls. The binding characteristics, equilibrium dissociation constant and maximal number of binding sites were determined by Scatchard analysis.

Results.—There was no change in the equilibrium dissociation constant in the patients with headache as compared to the control group, but subgroup analysis revealed that patients with tension-type headache with a headache index of less than 360 had a significantly lower equilibrium dissociation constant as compared to those with a headache index of more than 360; there was a significant correlation between the equilibrium dissociation constant and the headache index. A significant decrease was observed in the maximal number of binding sites in tension-type headache. No correlation was observed between the maximal number of binding sites and age, duration of illness, or headache intensity.

Conclusions.—The findings of the present study show that there is a decrease in the number of binding sites of 5-HT$_{2A}$ receptors in some patients with tension-type headache, suggesting postsynaptic serotonergic dysfunction and the involvement of serotonin in that group.

Key words: platelets, tension-type headache, ^{3}H ketanserin, 5-HT$_2$ receptors

Abbreviations: TTH tension-type headache, 5 HT 5-hydroxytryptamine (serotonin), CTTH chronic tension-type headache, PRP platelet rich plasma, Kd equilibrium dissociation constant, B_{max} maximal number of binding sites

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Tension-type headache (TTH) is the most common form of primary headache with a prevalence of more than 40% among all headache disorders and about a 35% incidence in the normal population.[1] The pathogenesis of TTH is poorly understood but appears to be multifactorial and both peripheral and central factors may be involved.[2] The pathophysiological mechanisms involved in central sensitization in TTH have been reviewed recently.[3] Serotonin (5-hydroxytryptamine, 5-HT) is a widely distributed neurotransmitter that has an important but complex role in pain modulation. The central antinociceptive mechanisms of 5-HT are complex and often difficult to investigate in clinical studies. The circulating platelets in the peripheral blood constitute an important dynamic reservoir of 5-HT and are able to rapidly ac-

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cumulate and secrete 5-HT and, thus, they have aroused considerable interest in their possible involvement in TTH. The biogenic amine functions of the platelets closely resemble those of serotonergic neurons.[4,5] The platelets can be obtained by a simple venipuncture, so they form a readily available tool to study the various serotonergic mechanisms.

The role of 5-HT in TTH has been investigated by the study of 5-HT in the peripheral blood, however the results are inconclusive. Platelet 5-HT has been reported to be decreased,[6-9] normal,[10-12] or increased,[13,14] while plasma 5-HT has been found increased during headache-free periods,[14,15] normal during headache,[11,12] or normal during headache-free periods and increased during headache.[16] The platelet 5-HT uptake index, calculated as the ratio between platelet 5-HT and plasma 5-HT levels, was significantly lower in patients with chronic tension-type headache (CTTH).[10] No release of platelet 5-HT was observed when platelets from TTH were incubated with plasma collected from patients with migraine during an attack.[17]

The platelet 5-HT uptake has been found to be increased or decreased.[10,18] Serotonin binds in a saturable and specific manner to high-affinity binding sites. The 5-HT uptake site or serotonin transporter is a membrane protein and can be labelled by ^{3}H imipramine and ^{3}H paroxetine. The results of 2 studies in which platelet ^{3}H imipramine binding has been evaluated in patients with TTH have shown variable results,[18,19] while ^{3}H paroxetine binding has been reported to be normal in patients with CTTH.[20] In vitro binding of 5-HT to the surface of lymphocytes and monocytes in patients with TTH is impaired with a complete loss of high-affinity binding sites.[21]

The role of 5-HT in the regulation of nociception depends on the 5-HT receptor subtype involved.[22] Seven distinct classes or families of 5-HT receptors have been delineated. The 5-HT$_2$ receptors belong to the G protein coupled receptor superfamily and exerts its cellular function by stimulation of phosphoinositol hydrolysis. These receptors are expressed more in the postsynaptic membrane. The most commonly used radioligand for 5-HT$_2$ receptors is ^{3}H ketanserin which predominantly labels the 5-HT$_{2A}$ subtype.[23]

A decreased affinity of platelet 5-HT$_2$ receptors in migraine has recently been reported by us.[24] The present study has been undertaken to investigate status of platelet 5-HT$_{2A}$ receptors using ^{3}H ketanserin as a ligand in patients with TTH.

SUBJECTS AND METHODS

Fourteen patients with TTH attending the Headache Clinic in the neurology outpatient department of King George's Medical College were studied. The diagnosis of TTH was based on the criteria of the International Headache Society.[25] A detailed history was taken according to a predesigned questionnaire and a complete physical examination was performed. The severity of headache was measured by calculating the headache index (number of attacks per month by severity of pain by duration of pain in hours). The severity of pain in the attacks was graded using a 3-point scale: 1, mild headache, does not interfere with the patient's daily routine; 2, moderate headache, interferes with the daily routine but patient can continue with work; 3, severe headache, patient has to take bed rest. Fifteen healthy controls comprising of paramedical staff and relatives of the patients were also evaluated. The participants' characteristics are shown in Table 1. The mean age of the controls was about a decade higher than that of the patients. There was a greater number of men in the control group as the patients usually came with a male relative (husband or brother).

None of the subjects included in the study were suffering from hypertension, diabetes mellitus, ischemic heart disease, or any psychiatric illness which may affect 5-HT metabolism. Food items (bananas,

Table 1.—Participant Characteristics

Feature	Patients (n = 14)	Controls (n = 15)
Age, mean ± SEM, y	28.79 ± 2.84	39.53 ± 2.47
Ratio of men to women	4:10	11:4
Type of headache, No.		
Episodic	6	0
Chronic	8	0
Duration of disease, mean ± SEM, y	1.50 ± 0.41	—
Headache index, mean ± SEM	617.14 ± 55.73	—
Positive family history, No.	1	0

chocolates, nuts, etc) containing 5-HT were forbidden for at least 14 days prior to collection of blood samples and drugs like aspirin, psychotropics, or antidepressants were not allowed for at least 7 days prior to collection.

Collection of Blood Samples.—Nine milliliters of blood was collected by venipuncture from the antecubital vein using a disposible plastic syringe with a 20-gauge needle between 9 and 10 AM and immediately transferred to plastic tubes containing 1 mL of 3.8% sodium citrate. It was gently mixed and transported to Industrial Toxicological Research Centre within 30 minutes of collection for biochemical analysis.

Preparation of Platelet Membrane.—The method of Khanna et al was followed for preparation of platelet rich plasma (PRP) and platelet membrane.[26] The blood was centrifuged at 200*g* for 10 minutes. The supernatant PRP was transferred into another plastic tube and centrifuged at 12000*g* for 10 minutes at 4°C. The platelet pellet obtained was washed twice with 5 mM Tris HCI buffer (pH 7.4) and the platelet suspension was homogenized and centrifuged at 50000*g* for 10 minutes at 4°C. The platelet membrane fraction was finally suspended in Tris HCI buffer (50 mM, pH 7.5).

Platelet Counts.—For platelet counts, 0.1 mL of PRP was taken out in plastic tubes and 1.9 mL of formyl citrate was added to it. Following this, platelets were counted in a Naebeur hemocytometer in duplicate.

Receptor Binding Assay.—Binding assay of ^{3}H ketanserin to platelet membrane was carried out as described by Oshuka et al.[27] Briefly, binding tubes containing assay buffer (50 mM Tris HCI, pH 7.5), platelet membrane protein (150-300 μg/tube), and radioligand, in a final volume of 1 mL, in triplicate were incubated for 60 minutes at 4°C. The experiments were performed using varying concentrations of ^{3}H ketanserin (0.25 nM to 9 nM). Nonspecific binding was determined using cinanserin (1×10^{-5} M). Following incubation, the contents of the tubes were rapidly filtered over glass fiber filters (Whatman GF/B) using a cell harvester (Brandel, USA). The filter sheets were washed twice with 5 mL chilled buffer, dried, and filter discs were transferred into scintillation vials. Scintillation fluid containing 2,5-diphenyloxazole (PPO), (1,4-bis-2,5-phenyl-oxazolyl)-benzene (POPOP), naphthalene, methanol, toluene, and dioxan was added and vials were counted on the LKB Wallac Rack Beta II scintillation counter at counting efficiency of 40% for ^{3}H.

Assay of Protein Content.—Protein was determined following the method of Lowry et al using bovine serum albumin as reference standard.[28]

Chemicals and Source.—^{3}H ketanserin (61.5 Ci/mM) was procured from M/s NEN, USA and cinanserin from M/s RBI, USA. All other chemicals used in the assay were of analytical grade and obtained from a local source.

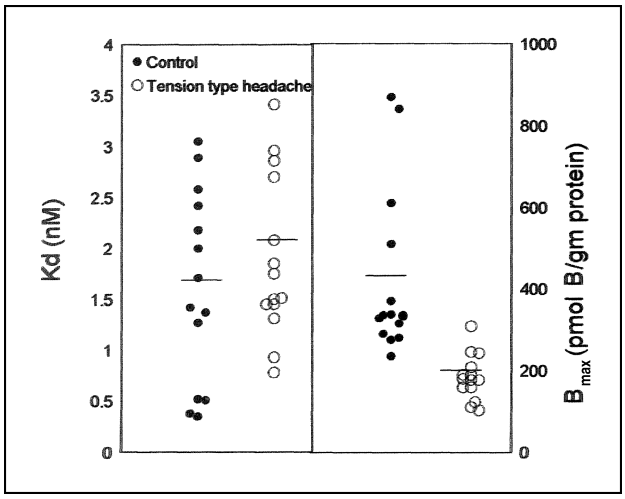
Data Analysis.—The data were analyzed by the method of Scatchard,[29] plotting bound/free (B/F) versus bound (B) and finally analyzed by the nonlinear regression analysis, and the equilibrium dissociation constant (Kd) and the maximal number of binding sites (B_{max}) were obtained. The values of Kd and B_{max} are expressed as mean±SEM.

Statistical Analysis.—The Student *t* test was used to compare the mean values between patients with TTH and controls. A univariate analysis of the various clinical features with the binding characteristics (Kd, B_{max}) was done. The value of the correlation coefficient was also calculated for the binding characteristics with age, duration of disease, time since last attack, and severity of headache (determined by headache index). A *P* value less than .05 was considered significant.

RESULTS

The platelet count in PRP in the patients with TTH (2.75±0.6 cells/$10^{8}mm^{3}$) and controls (2.79±0.07 cells/$10^{8}mm^{3}$) did not show any significant difference. The binding data of 5-HT_{2A} in the control group was analyzed with respect to sex and age. There was no significant difference in the mean values of Kd and B_{max} in men and women in the control group (Kd, men 1.44±0.28 nM; women 2.36±0.34 nM; B_{max}, men 404.91±54.23 pmol B/gm protein; women 451.76±139.73 pmol B/gm protein). Moreover, there was no correlation between age and Kd ($r = 0.16$) or age and B_{max} ($r = 0.27$).

There was no difference in the Kd, however the B_{max} was significantly lower in patients with TTH as compared to controls (Figure). Univariate analysis of



Scatterplot showing equilibrium dissociation constant (Kd) and maximal number of binding sites (B_{max}) of platelet ^{3}H ketanserin binding in patients with tension-type headache and in control subjects. A significant decrease in the B_{max} was observed in tension-type headache ($P < .05$), whereas the Kd showed no significant difference.

platelet ^{3}H ketanserin binding with clinical features of TTH did not reveal any significant difference with age, sex, duration of illness, family history, and type of headache (Table 2). There was no correlation between platelet ^{3}H ketanserin binding with age and duration of illness (Table 3). The Kd was, however, significantly less in those with a headache index of less than 360 as compared to those with a headache index of 360 or more ($P<.05$). There was also a significant correlation between Kd and headache index ($r = 0.54$).

COMMENTS

Serotonergic neurons play a major role in the regulation of pain in the central nervous system (CNS). The antinociceptive effects of 5-HT in the CNS are mediated via many different receptor subtypes, eg, 5-HT_1, 5-HT_2, and 5-HT_3 receptors.[22] Blood platelets have many similarities with the serotonergic nerve endings and are considered to be a model for serotonergic neurons.[30] There are recent data confirming an intraindividual correlation between brain and platelet 5-HT_2 receptor affinity, which suggest that platelet 5-HT_2 receptor affinity appears to be regulated at the cellular level by blood serotonin and that the binding characteristics of the 5-HT_2 receptor in brain cortex synaptosomes corresponds to that on platelets.[31] Studies undertaken by Cook et al have suggested that both brain and platelet 5-HT_{2A} receptors are encoded by a single copy gene which further strengthens the commonality between platelet and brain 5-HT_{2A} receptors.[32]

The findings of the present study indicate that there is a decrease in the number of 5-HT_2 receptor binding sites in patients with TTH, while there is

Table 2.—Univariate Analysis of Platelet ^{3}H Ketanserin Binding With Clinical Features of Tension-type Headache*

Feature	No. of Patients	Kd, nM	B_{max}, pmol B/gm Protein
Age, y			
<30	8	1.86 ± 0.33	187.38 ± 16.40
≥30	6	1.95 ± 0.28	177.17 ± 28.97
Sex			
Male	4	1.39 ± 0.32	212.75 ± 17.93
Female	10	2.10 ± 0.25	171.00 ± 18.80
Duration of illness, y			
<2	9	1.69 ± 0.26	193.33 ± 10.80
≥2	5	2.27 ± 0.35	164.20 ± 38.33
Headache index			
<360	2	0.86 ± 0.08†	216.50 ± 28.59
≥360	12	2.07 ± 0.21	177.33 ± 6.62
Family history			
Positive	1	0.78	245
Negative	13	1.98 ±0.21	178.15 ± 15.27
Type of headache			
Episodic	6	1.38 ± 0.18	199.67 ± 14.60
Chronic	8	2.29 ± 0.28	170.38 ± 23.62

*Values of Kd (equilibrium dissociation constant) and B_{max} (maximal number of binding sites) are mean ± SEM.
†$P < .05$ as compared to patients with tension-type headache having a headache index ≥360.

Table 3.—Correlation of Platelet ^{3}H Ketanserin Binding With Clinical Features of Tension-type Headache*

Feature	Kd	B_{max}
Age	0.34	−0.14
Duration of illness	0.04	−0.32
Headache index	0.54†	−0.12

*Kd indicates equilibrium dissociation constant and B_{max} maximal number of binding sites.
†$P < 0.05$.

no change in the affinity of the receptors to 5-HT. No significant difference between the Kd and B_{max} was observed in patients with episodic tension-type headache (ETTH) and CTTH in the present study. A decrease in imipramine binding sites in patients with ETTH was reported by Marazitti et al,[19] while Hannah et al found no difference in the platelet ^{3}H imipramine binding sites in patients with TTH as compared to controls.[18] Headache frequency was not reported in the latter study. Using tritiated paroxetine as the binding ligand, Bendtsen and Mellerup found the number of binding sites to be normal in patients with CTTH.[20]

Extrapolation of the findings of the present study in the platelets to central serotonergic neurons would suggest that there is a reduced number of binding sites as a result of the deficiency of 5-HT in the serotonergic synapses, thus providing supporting evidence to the central nociceptive theory of pain proposed by Sicuteri et al.[33] Altered peripheral 5-HT metabolism may reflect similar changes in the CNS since there are several indicators that peripheral and central serotonin turnover rates are modulated by a common pacemaker. Abnormal peripheral serotonin levels in endogenous depression are markedly influenced by antidepressant drugs that interfere with 5-HT receptors of platelet and the CNS suggesting a parallelism of peripheral and central changes,[34,35] as also indicated by animal experiments.[36]

The exact mechanism for the reduction in the number of platelet 5-HT receptor binding sites is not clear, but may be due to plasma factors acting as competitive inhibitors or an alteration in the configuration of the binding site. A biochemical marker for the diagnosis of TTH or migraine without aura (ie, common migraine) would be extremely useful because sometimes it is difficult to clinically differentiate the 2 conditions. Platelet 5-HT_2 receptor binding is unlikely to be of any help in this regard because a decrease in B_{max} has also been found in patients with migraine.[19] However, the shared biochemical abnormality (ie, alteration in the 5-HT_2 receptor binding) may suggest that common migraine and tension headache may have a common pathogenesis or may be the 2 ends of a spectrum of headache disorders.[37]

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