นิพนธ์ต้นฉบับ

Alteration of platelet 5-HT_2 serotonin receptors in migraine patients with analgesics overuse.

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Recent advances in the field of receptor pharmacology have revealed the significance of receptor plasticity in the pathogenesis of several drug-related neurological disorders. Excessive use of analgesics is known to induce headache deterioration in patients with primary headache, especially migraine. To assess the possibility of 5-HT_2 serotonin receptor plasticity in this condition, we investigated receptor binding by the platelet membrane in patients with analgesic-induced headache (AIH), migriane patients and non-headache controls. The technique involved radioligand binding with [phenyl-4 ^{3}H]-spiperone and kitanserin. A greater density of receptor number was found in patients with AIH and in non-headache controls (759.9 ± 60.9 and 787.1 ± 80.8 fmol/mg protein, respectively) as compared to migraine patients (510.1 ± 78.2 fmol/mg protein). The value of the dissociation equilibrium constant remained unchanged (1.77 ± 0.28, 2.61 ± 0.62 and 1.62 ± 0.48 nM for patients with AIH, migraine patients and non-headache controls, respectively). Based on these findings, we suggest that up-regulation of 5-HT_2 serotonin receptors may be a possible mechanism of headache transformation in patients with AIH.

Key words : *Migraine, Analgesics, Headache, Serotonin receptors, Platelets.*

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สุภาภรณ์ ปวงนิยม, อนันต์ ศรีเกียรติขจร, นัยพินิช คชภักดี, ปิยะรัตน์ โกวิทตรพงศ์. การเปลี่ยนแปลงของตัวรับซีโรโตนินบนผิวเกร็ดเลือดในผู้ป่วยโรคปวดศีรษะไมเกรนที่ใช้ยาแก้ปวดเกินความจำเป็น. จุฬาลงกรณ์เวชสาร 2539 กรกฎาคม; 40(7): 557-566

ความก้าวหน้าในสาขาวิชาเภสัชวิทยาของตัวรับแสดงให้เห็นว่า การเปลี่ยนแปลงของตัวรับสารสื่อประสาท มีความสำคัญในพยาธิกำเนิดของโรคทางระบบประสาทที่เป็นผลจากการใช้ยา ในปัจจุบันเป็นที่ยอมรับโดยทั่วไปว่าการใช้ยาแก้ปวดติดต่อกันเป็นเวลานาน มีผลให้อาการของโรคปวดศีรษะ โดยเฉพาะโรคไมเกรนมีความรุนแรงมากขึ้น เพื่อศึกษาการเปลี่ยนแปลงของตัวรับซีโรโตนินชนิด 5-HT_2 ในภาวะนี้ คณะผู้วิจัยได้ศึกษาลักษณะการจับติดของตัวรับบนผิวเกร็ดเลือดในผู้ป่วยโรคปวดศีรษะไมเกรนที่ใช้ยาแก้ปวดเกินขนาด, กลุ่มผู้ป่วยไมเกรน และกลุ่มควบคุม โดยใช้เทคนิกการจับติดของสารรังสี (radioligand binding technique) ผลการศึกษาพบว่าในกลุ่มผู้ป่วยโรคไมเกรนที่ใช้ยาแก้ปวดเกินขนาด และกลุ่มควบคุม มีความหนาแน่นของตัวรับซีโรโตนินชนิด 5-HT_2 บนผิวเกร็ดเล็อดสูงกว่ากลุ่มผู้ป่วยไมเกรน, อย่างมีนัยสำคัญ (759.9 ± 60.9,787.1 ± 80.8 และ 510.1 ± 78.2 fmol/mg protein, ตามลำดับ) ในขณะที่ค่าคงที่ของสมดุลการแยกตัว (dissociation equilibrium constant) ไม่มีการเปลี่ยนแปลง (1.77 ± 0.28, 2.61 ± 0.62 and 1.62 ± 0.48 nM สำหรับกลุ่มผู้ป่วยโรคไมเกรนที่ใช้ยาแก้ปวดเกินขนาด, กลุ่มควบคุม และกลุ่มผู้ป่วยไมเกรน, ตามลำดับ) จากผลการศึกษานี้ คณะผู้วิจัยตั้งสมมติฐานว่า การเพิ่มจำนวนของตัวรับซีโรโตนินชนิด 5-HT_2 อาจเป็นกลไกหนึ่ง ที่ทำให้เกิดการเปลี่ยนแปลงในลักษณะการดำเนินโรคของอาการปวดศีรษะที่พบในผู้ป่วยไมเกรนที่ใช้ยาแก้ปวดเกินความจำเป็น

As in other pain syndromes, overuse of analgesics is a common problem coexisting with headache. The tendency of analgesic overuse correlates directly with the frequency of headache.[1] Based on a recent survey in Thailand, the prevalence of daily analgesic consumption among chronic daily headache sufferers is 58.3%.[2] Besides other adverse effects, excessive use of analgesics has recently been recognized as a cause of deterioration in primary headache patients. Moreover, the analgesic overuse also interferes with the therapeutic efficacy of standard, usually effective, pharmacological and non-pharmacological treatment regimens, thus preventing expected improvement. This phenomenon has been recognized as headache transformation.[3]

Recent advances in the field of receptor pharmacology reveal the significance of receptor adaptation in the pathogenesis of several drug-related disorders. Although the mechanism underlying the headache transformation remains to be determined, an alteration of receptor function is one possibility. Serotonin (5-hydroxytryptamine, 5-HT) has long been implicated in migraine pathogenesis.[4] The beneficial effect of various serotonergic drugs, e.g. nortriptyline, pizotifen, etc., in the treatment of analgesic induced headache implies that serotonin also plays some roles in this condition.

Platelets have been shown to be a good model of serotonergic neurons, as both types of cells share many morphological, biochemical and pharmacological characteristics.[5] It has recently been demonstrated that the binding characteristics of 5-HT_2 receptors in platelets are a reliable reflection of those in the neurons of the central nervous system.[6] To investigate the possibility of 5-HT_2 serotonin receptor plasticity in analgesic-induced headache (AIH), we studied their saturation binding properties on platelet membranes. The technique involved quantitative radioligand binding assay, which is the most accurate method for characterization of the receptors.

Materials and methods

Subjects :

Ten migraine patients with a history of analgesic overuse visiting the Headache Clinic, Chulalongkorn University Hospital were enrolled in this study. All had a past history of migraine without aura, which eventually evolved into AIH. Details of their headache characteristics, including time of transformation, were reviewed. Organic causes of headache were excluded by physical examination and appropriate radiological investigations. Ten patients having migraine without aura and ten non-headache controls were also studied for comparison. Diagnoses of migraine and analgesic induced headache were based on the International Headache Societys criteria.(7) Apart from the patients with AIH, none of the subjects had received any medication for at least two weeks before blood sampling. All patients gave their consent to be included in the study after verbal discussion with them.

Platelet Membrane Preparation :

Blood (10-20 ml) was drawn from the antecubital vein and transferred into plastic tubes

containing 0.38% (final concentration) sodium citrate as anticoagulant. Platelet rich plasma (PRP) was prepared by centrifugation of anti-coagulated blood at 200 x g for 15 minutes. The platelet cells were lysed by adding PRP preparations with half of their original volume of a hypotonic medium (5 mM Tris HCl, pH 7.5) and homogenized for 15 seconds with a tissue homogenizer (Ultra Turrax T25), set at 13,500 rpm. The suspensions were centrifuged at 40,000 x g for 20 minutes at 4°C in a refrigerated centrifuge (Doupont, Sorvall RC 26 plus). The supernatant was decanted and the membrane pellet was resuspended in 20 volumes of ice cold, 50 mM Tris HCL, pH 7.5, and homogenized. The process was repeated twice with 50 mM Tris HCl for washing the membrane pellet. The membrane pellet was then resuspended into the incubation buffer (containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$ in 50 mM Tris HCl buffer, pH 7.5) to form the final membrane suspension for binding studies.

Radioligand Binding Assays :

The freshly prepared membrane was resuspended in 20 volumes of ice cold 50 mM Tris HCl salt buffer (pH 7.5) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$ and homogenized for 15 seconds with a tissue homogenizer set at 13,500 rpm. All binding assays were carried out by using sufficient membrane preparation to provide a tissue protein concentration between 0.1-0.3 mg/ml. Assays were performed by placing 400 µl of platelet membrane suspension into glass tubes containing 50 µl incubation buffer with or without the appropriate drug. Six to ten concentrations varying from 0.4-12 nM of (phenyl-4 ^3H)-spiperone (Amersham, UK) were added to the tissue suspension making a final incubation volume of 500 µl. The assay mixture was incubated at 37° C for 30 minutes, during which time equilibrium was reached. The reaction was then terminated by rapid filtration through glass microfiber filter (Whatman GF/C, Whatman International Ltd., Maidstone, UK) under vacuum. The filters were washed twice with ice cold Tris buffer. Receptor-bound radioactivity was counted in 5 ml of scintillation fluid containing Triton X 100/ toluene base fluor (1:3) by a scintillation counter (Beckman LS 1801). Specific (^3H)-spiperone binding, which was defined as the excess over blanks taken in the presence of 10 µM of ketanserin (Janssen Research Foundation, Belgium), accounted for 40-60% of the total binding. All experiments were performed in duplicate. The protein concentrations of the membranes were estimated by Lowry's method using bovine serum albumin as a standard.

Analysis of Receptor Binding Data

The saturation curve was analyzed by the method of Scatchard, and then using the non-linear least square regression analysis computer program (LIGAND) for analyzing the relationship between bound/free versus bound fraction. The data were expressed in dissociation equilibrium constant (K_d) and maximum number of receptor sites (B_{max}) as mean ± SEM. Statistical evaluation of the results was performed using mixed analysis of variance (ANOVA) and Students t-test. As the number of samples were

limited, the data were not normally distributed.

Results

Clinical Characteristics of Subjects:

Details of the clinical profiles of the subjects are summarized in Table 1. Patients with AIH were generally older and had been subjected to headache attacks for a longer time. Their headache frequencies were daily. Depressive symptoms, i.e. sleep difficulty, anorexia, loss of concentration, low self-esteem, etc. were reported in almost all instances. All patients with AIH daily used analgesics and the average amount of analgesic consumption was 25.1 tablets/week. Among various forms of analgesics, acetaminophen was the most common drug abused.

Table 1. Clinical profiles of three groups of patients.

	Control	Migraine	AIH
Total patients	10	10	10
male	4	1	1
female	6	9	9
Age (years)			
mean	26.2	29.9	32.8
range	20-32	21-45	22-48
Migraine history (years)			
mean	-	4.1	7.1
range	-	1-15	1-20
Daily headache history (years)			
mean	-	-	1.8
range	-	-	0.5-4
Headache frequency	-	1.7 day/week	daily
Monthly analgesic consumption (tablets)	-	12.4	104.6

5-HT_2 Serotonin Receptor Binding :

The saturability of specific (^{3}H)-spiperone binding to the platelet membrane was determined as a function of various (^{3}H)-spiperone concentrations at 37°C. The results indicated that platelet membrane contained a single population of saturable and high affinity 5-HT_2 serotonin receptor binding sites.

The result revealed the B_{max} values for patients with AIH, migraine patients and non-headache controls to be 759.9 ± 60.9, 510.1 ± 78.2 and 787.1 ± 80.8 fmol/mg protein, respectively.

The K_d values for the three groups were 1.77 ± 0.28, 2.61 ± 0.62 and 1.62 ± 0.48 nM, respectively. The difference of the B_{max} values between migraine patients and patients with AIH was statistically significant ($p<0.02$), whilst no significant difference was observed between patients with AIH and non-headache controls. No significant difference in K_d values among the three groups was evident.

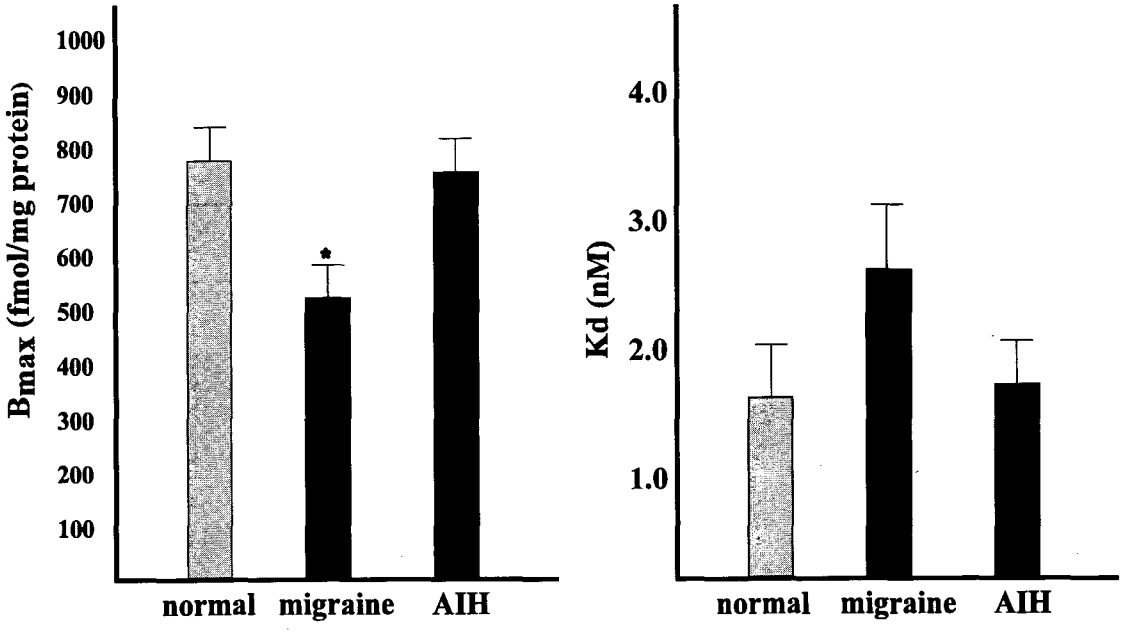

Figure 1. Saturation parameters of [Phenyl-^{3}H]-spiperone binding on platelet membrane from patients with AIH, migraine and non-headache controls. A greater density of receptor numbers (B_{max}) was demonstrated in patients with AIH as compared to migriane patients (* $p < 0.02$), whereas values for the dissociation equilibrium constant (K_d) remained unchanged.

Discussion

In our study, alterations of 5-HT_2 serotonin receptor binding characteristics were demonstrated in two groups of migraine patients, i.e. in those with or without medication overuse. A down regulation of 5-HT_2 receptor, as evidenced by a decrease in B_{max} value, was demonstrated in patients with migraine without aura as compared to non-headache controls. This finding corresponds to previous studies using the same model.[8,9] However, such change has been altered in migraine patients complicated by AIH. As all patients with AIH had a previous history of migraine, it is reasonable to assume that they, in fact, had concentrations of 5-HT_2 receptors on platelet membrane similar to those

of migraine patients before they developed AIH. The finding that their B_{max} values were similar to non-headache controls, can be attributed to the regular intake of analgesics producing up-regulation of receptors, and can be viewed as an increase in their numbers on the platelet membrane.

Serotonin plays an important role in the pathogenesis of migraine, acting as a vasoactive substance and as a pain modulatory transmitter. Among a number of contradictory findings regarding the concentration of 5-HT in platelets and plasma, the finding of a decrease in platelet 5-HT content during the attack of migraine without aura with a concomitant rise of 5-HT in platelet-free plasma has been confirmed.[10] This ictal rise in plasma 5-HT has been proposed to be a self defense mechanism of the body, in view of the fact that the exogenous administration of 5-HT is able to abort migraine attacks.[11] It is well accepted that the concentration of neurotransmitters plays an important role in receptor adaptation. Recently, a negative correlation between blood or platelet 5-HT and receptor binding capacity on platelet membrane was demonstrated. Based on this assumption, one may draw the conclusion that the down-regulation of 5-HT_2 receptor demonstrated here is an adaptive process responding to the ictal rise of plasma 5-HT. Serotonin exerts its various physiological effects via a vast diversity of receptor subtypes. [12] Generally, the 5-HT_1 family exerts an inhibitory effect, while the 5-HT_2 family stimulates. Regarding nociception, recent advances in the field of 5-HT receptor pharmacology show that the different 5-HT receptor subtypes play different roles.[13] Several lines of evidence suggest that stimulation of 5-HT_1 receptor subtypes in the spine can elicit analgesia. On the other hand, stimulation of 5-HT_2 receptors potentiates nociceptive transmission, possibly by enhancing the release of algogenic peptides from primary afferents. This hypothesis has been deduced from the observation that behavioral responses to the intrathecal administration of 5-HT_2 agonist can be blocked by substance P receptor blockers.[14]

A relationship between 5-HT_2 receptors and clinical headache has previously been observed. A decreased amount of this receptor in the elderly has been proposed to be a mechanism for improvement or total disappearance of migraine in this age group.[15] Several 5-HT_2 receptor antagonists possess a beneficial effect in migraine prophylaxis. Thus, the decrease in 5-HT_2 receptor reported here may contribute to a refractory period in the migraine cycle. Interestingly, such down-regulation of 5-HT receptors has also been observed in the brain tissue of animals acutely treated with acetylsalicylic acid.[16] This result implied a relationship between analgesic use and modification of the central serotonergic system. On the other hand, an increase in receptor numbers observed in patients with AIH may result in a hyperalgesic state and the development of chronic daily headache.

How do simple analgesics alter 5-HT neurotransmission ? Traditionally, it was believed that non-narcotic analgesics act peripherally by blocking cyclo-oxygenase enzyme and consequent inhibition of prostaglandin synthesis.

However, several lines of investigation have implied that inhibition of locally synthesized prostaglandins per se does not satisfactorily explain the analgesic effect of these agents.[17] On the contrary, recent in vivo evidence strongly suggests that for some of these agents, centrally mediated analgesia may also be achieved by additional mechanisms, which depend on serotonin transmission. Local injection of diclofenac into nucleus raphe magnus, a major serotonergic cell group, produced a more pronounced antinociceptive effect as compared to subcutaneous, intrathecal or intracerebroventicular injection.[18] The analgesic effect of diclofenac was significantly attenuated in animals lesioned with 5,7-dihydroxytryptamine, the specific serotonergic neurotoxin. Pretreatment with para-chlorophenylalanine, a tryptophan hydroxylase inhibitor, profoundly antagonized the antinociceptive effect of this analgesic.[19] These experiments indicate that a reduction of the bulbo-spinal 5-HT neurotransmission, regardless of the mechanism, attenuates the antinociceptive effect of diclofenac. Moreover, subcutaneous administration of this agent reduced both brainstem and spinal cord 5-HT, indicating an increased release of 5-HT in these region after injection.[19] Based on this evidence, one may hypothesize that diclofenac, and possibly other analgesics as well, exert their antinociceptive effect by increasing the release of 5-HT from the rapheospinal pathway. Therefore, chronic analgesic overuse may deplete 5-HT in the central nervous system. Such derangement has recently been demonstrated in patients with AIH, as evidenced by a decrease in platelet 5-HT concentration.[20]

Based on the above assumption of an analgesic-induced central 5-HT depletion, the 5-HT_2 receptor can consequently be up-regulated. Such receptor up-regulation may lead to a hyperalgesic state. This hypothesis corresponds to the finding of Pini, et al of an increase in 5-HT receptor numbers in rat cortical and pontine membranes after being chronically treated with the pyrazole derivative, phenazone.[21]

Taken together, we suggest that migraine patients may have some defects, by some unknown mechanisms, in the endogenous 5-HT dependent pain control system. Chronic analgesic overuse may interfere with this already abnormal system by depleting the amount of transmitter and eventually up-regulating the post-synaptic 5-HT_2 receptor. This receptor up-regulation may be an explanation of headache transformation observed in patients with AIH.

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