BIOPHYSICS AND BIOCHEMISTRY

Serotonin-Induced Platelet Aggregation and Release of ^{3}H-Serotonin in Patients with Migraine

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Serotonin-induced platelet aggregation in patients with migraine without aura is less pronounced than in healthy individuals. In 50% of the patients the platelet serotonin transport system is characterized by increased sensitivity to serotonin: serotonin induced a more potent (by 26%) release of ^{3}H-serotonin from platelets, while the serotonin transport inhibitor imipramine blocked this effect. Thus, the serotonin transport system in patients with migraine differs from that of healthy individuals.

Key Words: *migraine; platelet; aggregation; serotonin transport*

Clinical and experimental studies have shown that serotonin (5-HT) is involved into the pathogenesis of migraine. It has been shown that the content of 5-HT in platelets decreases during migraine attack [3,6,7]; however, the mechanisms of this phenomenon remain unclear. Platelets from patients with migraine are characterized by increased aggregative response to ADP and adrenaline [2,10,12] and enhanced release of 5-HT in response to tyramine induction [4]. Aggregation in response to 5-HT is less studied. In patients with migraine normal or enhanced platelet aggregation in response to 5-HT has been observed [5,11,14]. On the other hand, the number of 5-HT_2 receptors mediating 5-HT-induced platelet aggregation is considerably decreased in these patients in comparison with the normal, their affinity being unchanged [9]. The 5-HT transport system in patients with migraine also differs from the normal. It has been shown that platelets from patients are characterized by a lower number of imipramine binding sites (B_{max}) in comparison with healthy individuals [8,12]. The 5-HT transport system mediates its release from platelets in response to some activators, in particular, 5-HT [1, 15]. Bearing in mind the peculiarities of the 5-HT transport system and increased 5-HT release from platelets in patients with migraine, we assume that the 5-HT transport system is responsible for pathological release of 5-HT from platelets in these patients.

The aim of the present study was to compare platelet aggregation and 5-HT release in response to 5-HT induction in healthy individuals and in patients with migraine without aura.

MATERIALS AND METHODS

Eight migraine male patients without aura in the interictal period and 8 healthy men were examined.

Experiments were carried out on platelet-rich plasma (PRP) obtained from patients receiving no drugs for 7-10 days. Blood was drawn from the ulnar vein, diluted 9:1 with 130 mM sodium citrate (pH 7.4), and centrifuged at 190*g* for 15 min. After the PRP was collected, the bottom portion was recentrifuged (200*g*, 15 min), and platelet-depleted plasma (PDP) was prepared. To measure platelet

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aggregation 300-μl aliquots of PRP were transferred into 5-mm round-bottom glass cuvettes. Measurements were performed at 37°C and constant stirring using a Biola-230 dual-channel laser aggregometer (Russian Cardiology Research-and-Production Center). This device simultaneously records light transmission of cell suspension and the mean radius of platelet aggregates by the method of light transmission fluctuation analysis. Platelet count in the PRP was determined before each experiment and adjusted to 200,000 cells/μl with PDP. Platelet aggregation induced by 5-HT (5×10^{-6} M) was evaluated by recording changes in the mean radius of platelet aggregates.

To study ^{3}H-5-HT release from platelets, blood obtained as described above was diluted 9:1 with 3.8% sodium citrate and centrifuged at 190*g* for 15 min; PRP was then recentrifuged at 650*g* for 10 min. Isolated platelets were washed in buffer A containing 150 mM MaCl, 2.7 mM KCl, 0.37 mM Na_2HPO_4, 1.0 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES-NaOH (pH 6.55) and 0.35% bovine serum albumin (fraction V), centrifuged and resuspended in buffer B the same components as buffer A plus 1.0 mM $CaCl_2$ (pH 7.4). The platelet suspension was incubated with 100 nM ^{3}H-serotonin for 20 min at 37°C, washed, and resuspended in buffer B. An aliquot (20 μl) of this suspension was incubated with 400 μl buffer B containing 5-HT, imipramine, or both agents for 15 min at 37°C and then filtered through Millipore filters (0.45 μ). The filters were washed with cold 0.9% NaCl and radioactivity was measured.

The data were processed statistically using the Student *t* test.

RESULTS

Figure 1 shows the size of platelet aggregates formed in PRP from migraine patients and healthy donors in response to 5-HT induction. The mean radii (*R*) for patients and donors were 1.77 and 4.11, respectively. In patients, 5-HT induced weaker platelet aggregation in comparison with the control ($p<0.05$). In one patient, platelet aggregation activity was similar to that of healthy donors ($R=3.57$). Seemingly, platelets from patients vary in their aggregation response to 5-HT. In the present study we compared functional activity of 5-HT_2 receptors, but not their number and affinity for 5-HT. Published data suggest that the number of 5-HT_2 receptors in migraine patients decreases below the normal level, but their affinity remains unaffected [9], which probably results in a weaker platelet aggregation response to 5-HT in patients. These findings disagree with the data on increased aggregation response to 5-HT in migraine patients [5,11]. This may be due to the fact that in the present study platelet aggregation was evaluated from the magnitude and the rate of changes in the mean aggregate radius, while in previous studies this process was quantified by measuring the amplitude and changes in light transmission through platelet suspension. 5-HT induced the formation of very small aggregates. Hence, the magnitude and the rate of changes in the mean aggregate radius more adequately reflect the intensity of platelet aggregation, since light transmission depends not only on the size of aggregates but also on the platelet shape.

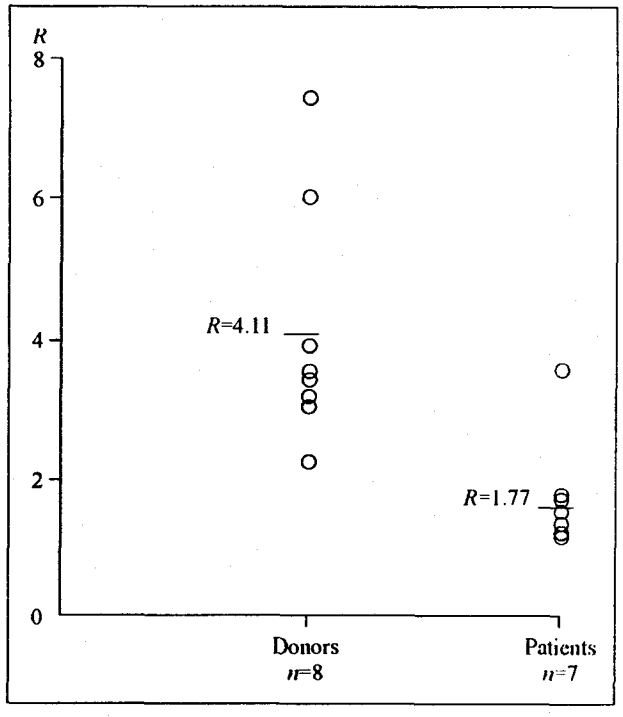

Fig. 1. Radii of platelet aggregates induced by serotonin in migraine patients and healthy donors. *R* is the mean radius of platelet aggregates. Final concentration of serotonin in the sample is 5×10^{-6} M.

The effect of 5-HT on the release of ^{3}H-5-HT from platelets of migraine patients and healthy donors are illustrated in Table 1. In a concentration range of 5×10^{-9}-5×10^{-7} M 5-HT had no effect on the release of ^{3}H-5-HT from platelets of healthy donors, while in concentrations of 5×10^{-6} and 5×10^{-5} M it induced the release of 10% ^{3}H-5-HT ($p<0.05$). Platelets isolated from patients differed in their ability to release ^{3}H-5-HT in response to 5-HT (Table 1). In some patients it was similar to that of healthy donors: ^{3}H-5-HT induced the release of 10% ^{3}H-5-HT in concentrations of 5×10^{-6} and 5×10^{-5} M and was ineffective in concentration of 5×10^{-9}-5×10^{-7} M. In other patients, 5-HT stimulated the release of ^{3}H-5-HT by 35-38% in con-

TABLE 1. Release of ^{3}H-5-HT from Platelets in Response to 5-HT in Patients and Donors (% of ^{3}H-5-HT in Platelet Suspension the Absence of Test Agents)

Patients	Concentration of 5-HT, M									
	5×10^{-9}		5×10^{-8}		5×10^{-7}		5×10^{-6}		5×10^{-5}	
	without imipramine	imipramine, 10^{-4} M	without imipramine	imipramine, 10^{-4} M	without imipramine	imipramine, 10^{-4} M	without imipramine	imipramine, 10^{-4} M	without imipramine	imipramine, 10^{-4} M
Sh.	84.0	101.0	87.3	109.3	83.0	97.9	92.6	107.5	83.9	111.7
K.	97.9	92.8	97.5	85.9	105.4	96.0	96.3	92.2	90.3	87.8
L.	75.2	122.0	84.0	112.0	80.3	112.1	80.0	115.6	79.0	94.6
M.	94.7	109.6	98.0	94.1	90.2	97.6	89.2	107.8	87.2	101.1
U.	99.5	111.5	82.4	126.8	108.6	107.9	80.1	111.0	63.8	96.7
P.	95.8	135.2	86.0	103.9	87.6	99.7	76.0	106.4	64.4	93.1
Kh.	94.3	102.4	80.2	99.1	64.8	103.4	58.9	94.7	62.1	94.7
V.	89.7	98.5	97.2	103.5	88.5	102.2	85.7	100.0	64.0	93.9
Mean... (n=8, $M\pm m$)	91.3±2.88		89.1±2.5		91.9±4.1		85.7±2.8		85.1±2.41[1]	
									63.6±2.52[2]**	
Donors (n=8, $M\pm m$)	105.9±2.38		104.9±1.71		108.0±1.7		91.5±0.69*		91.7±2.06*	

Note. [1]Patients Sh., K., L., and M.; [2]patients U., P., Kh., and V. *$p<0.05$, **$p<0.02$ compared with the control.

centrations of 5×10^{-5}, which surpassed the normal level by 26% ($p<0.02$). In one patient 5-HT in concentrations of 5×10^{-7} and 5×10^{-6} M induced a release of 36 and 41% ^{3}H-5-HT, respectively in comparison with the normal level. In patients, imipramine (10^{-4} M) blocked the release of ^{3}H-5-HT from platelets in response to 5×10^{-9}-5×10^{-5} M 5-HT (Table 1). Since imipramine is an effective inhibitor of the 5-HT transport system, it can be concluded that the release of ^{3}H-5-HT from platelets in response to 5-HT is mediated by this system. Our findings suggest that the 5-HT transport system in platelets of migraine patients is highly susceptible to 5-HT and responsible for its pathological release. Although abnormalities in the 5-HT transport system were found only in half of migraine patients, we assume that in all patients this system is characterized by increased sensitivity to some exogenous or endogenous agents, i.e., for each patient an individual trigger agent modulating functional activity of the 5-HT transport system and inducing 5-HT release can be identified. Therefore, individual trigger agents different in their chemical nature, produce the same effect in all patients: they induce uncontrolled, i.e., pathological release of 5-HT from platelets.

Thus, our findings suggest that the 5-HT transport systems in migraine patients and healthy donors are different. Moreover, in migraine patients without aura, the 5-HT transport system plays an essential role in the pathogenesis of 5-HT release from platelets.

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