Effects of Calcium Antagonists on Serotonin-Dependent Aggregation and Serotonin Transport in Platelets of Patients with Migraine

T. G. Pukhal'skaya, O. A. Kolosova,* M. Yu. Men'shikov, and A. M. Vein***

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Flunarizine and cinnarizine (IC_{50} 6.8×10^{-6} and 2.8×10^{-5} M, respectively) inhibited ^{3}H-serotonin uptake by platelets. In higher doses, they blocked serotonin-induced platelet aggregation and stimulated ^{3}H-serotonin release from these cells. Imipramine did not affect serotonin-releasing effects of preparations. In all patients cinnarizine was more potent in inhibiting serotonin uptake, and in half of the patients cinnarizine displayed higher activity as an inductor of serotonin release.

Key Words: *migraine; platelets; aggregation; serotonin uptake and release; flunarizine; cinnarizine*

Clinical and experimental observations indicate that serotonin is involved in the pathogenesis of migraine. Platelets of patients with migraine attract much attention in studies of the pathogenesis of this disease because serotoninergic structures (5-HT_2 receptors and serotonin transport systems), and the mechanisms of serotonin secretion by platelets and neurons are similar [8,13]. In patients with migraine, the content of 5-HT_2 receptors mediating serotonin-dependent platelet aggregation [6] and the number of imipramine-binding sites related to the system of serotonin uptake are much lower than in healthy individuals [5].

Ca^{2+} antagonists interact with the serotonin system in platelets and other cells. Verapamil and diltiazem inhibit serotonin-induced platelet aggregation by blocking 5-HT_2 receptors [14]. Verapamil competitively inhibits serotonin uptake mediated by serotonin transport systems in platelets and neurons [7]. Studies of the interaction between Ca^{2+} antagonists and serotoninergic structures in platelets will reveal structural and functional peculiarities of the serotonin system in patients with migraine. Here we evaluated the effects of Ca^{2+} antagonists flunarizine and cinnarizine on serotonin-dependent platelet aggregation and serotonin uptake and release from platelets of men with migraine without aura (common migraine) and healthy donors.

MATERIALS AND METHODS

We examined 8 men with common migraine at the period without migraine attacks and 8 healthy men. Migraine headaches corresponded to the criteria proposed by the Classification Committee of the International Headache Society.

Blood from the cubital vein was collected into a tube containing 130 mM sodium citrate (pH 7.4) in a blood-anticoagulant ratio of 1:9. Platelet-rich plasma (PRP) was obtained by centrifugation at 190*g* for 15 min. Platelet aggregation was measured at 37°C under constant mixing using a Biola 230 high-resolution two-channel laser aggregometer (Institute of Experimental Cardiology, Russian Cardiology Research-and-

Institute of Pharmacology, Russian Academy of Medical Sciences; *Department of Nervous Diseases, M. I. Sechenov Moscow Medical Academy; **Institute of Experimental Cardiology, Russian Cardiology Research-and-Production Complex, Russian Ministry of Health, Moscow

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Production Complex) [4]. Platelets were preincubated with various doses of test preparations at 37°C for 3 min, and serotonin in a concentration of 5×10^{-6} M was added. Serotonin-induced platelet aggregation was evaluated from changes in the mean radius of platelet aggregates. The concentration of platelets in PRP was 200 000/μl.

^{3}H-Serotonin uptake and release from platelets were analyzed as described elsewhere [1]. Platelets were washed in buffer A containing (in mM): 150 NaCl, 2.7 KCl, 0.37 Na_2HPO_4, 1 $MgCl_2$, 5 glucose, and 10 HEPES-NaOH (pH 6.5) with 0.35% bovine serum albumin (fraction V) and then in buffer B of the same composition containing 1 mM $CaCl_2$ (pH 7.4). To study serotonin uptake, platelets were incubated with ^{3}H-serotonin (100 nM, 18 Ci/mmol) in the absence (control) or presence of flunarizine and cinnarizine in various concentrations at 37°C for 20 min. To evaluate serotonin-releasing effects of these preparations, 20 μl platelet suspension preincubated with ^{3}H-serotonin was resuspended in 400 μl buffer B containing various concentrations of flunarizine or cinnarizine (with or without 10^{-4} M imipramine) and incubated at 37°C for 15 min. After incubation, platelets were filtered through Millipore filters (0.45 μ) and washed with cold 0.9% NaCl. Radioactivity was estimated by liquid radiometry.

The results were analyzed by Student's *t* test.

RESULTS

Flunarizine was equally potent in inhibiting ^{3}H-serotonin uptake by platelets from patients and healthy subjects: the concentrations of this agent corresponding to 50% inhibition (IC_{50}) were 6.8×10^{-6} and 7.1×10^{-6} M, respectively. Cinnarizine more effectively inhibited this process in patients than in healthy men (IC_{50} 5×10^{-6} and 2.8×10^{-5} M, respectively, $p<0.01$, Table 1). The increased sensitivity of platelets from patients with common migraine to cinnarizine is probably due to higher affinity of serotonin transporter for this agent. It can not be excluded that a higher potency of cinnarizine as the inhibitor of serotonin uptake is related to changes in the sensitivity of second messengers in platelets from patients with common migraine. Recent studies showed that secondary messengers are involved in the regulation of serotonin uptake by platelets and other cells [2,11].

The concentrations of flunarizine and cinnarizine causing a 50% release of ^{3}H-serotonin (EC_{50}) from platelets of healthy donors were 6.5×10^{-5} and 3.8×10^{-4} M, respectively. Flunarizine and cinnarizine induced ^{3}H-serotonin release from platelets of healthy donors independently on the presence of imipramine in the incubation medium (data not shown). Platelets from patients with common migraine differed in their sensitivity to serotonin-releasing effects of cinnarizine. In 4 patients, ^{3}H-serotonin release was induced by far lower concentrations of cinnarizine (EC_{50} 2.5×10^{-5} M, $p<0.01$, Tables 1 and 2) than in healthy donors, while in other 4 patients these differences were not found (EC_{50} 4.0×10^{-4} M). Probably, the system of serotonin uptake in not involved in the mechanisms of its release, because the concentrations of flunarizine and cinnarizine inducing serotonin release were an order of magnitude higher than those modulating serotonin uptake. The fact that imipramine (inhibitor of serotonin uptake) had no effect on preparation-induced ^{3}H-serotonin release also indicate that the serotonin transport system is not involved in this process. Previous studies showed that flunarizine in a concentration of 10^{-4} M replaced ^{3}H-ketanserine in its binding sites on human platelets [12]. Two types of high-affinity binding sites for ketanserine were identified in platelets: 5-HT_2 receptors mediating serotonin-dependent aggregation and nonserotoninergic acceptors con-

TABLE 1. Effects of Cinnarizine on ^{3}H-Serotonin Uptake and Release from Platelets of Patients with Common Migraine and Healthy Donors (% of Control, $M\pm m$)

Cinnarizine concentration, M	Uptake		Release		
	healthy donors (n=10)	patients (n=8)	healthy donors (n=10)	patients	
				group 1+	group 2++
5×10^{-4}	0	0	27.0±3.1	11.0±1.2*	28.0±1.6
5×10^{-5}	24.0±1.2	0	91.0±2.4	32.0±2.3*	93.0±2.7
5×10^{-6}	93.0±8.8	47.0±1.5*	103.0±2.5	96.03±2.7	
5×10^{-7}	97±6	90.0±3.4	107.0±2.6	98.0±2.3	
5×10^{-8}	107.0±7.9	94.0±5.1	102.0±1.8	96.0±1.9	
5×10^{-9}	106.0±7.9	103.0±6.4	—	—	

Note. *p<0.01 compared to healthy donors. +Patients 1-4 and ++patients 5-8 (see Table 2).

TABLE 2. Effects of Cinnarizine on ^{3}H-Serotonin Uptake (Numerator) and Release (Denominator) from Platelets in Patients with Common Migraine (% of Control)

Patient, No.	Cinnarizine concentration, M					
	5×10^{-4}	5×10^{-5}	5×10^{-6}	5×10^{-7}	5×10^{-8}	5×10^{-9}
1	0/18	0/54	54/106	91/102	97/103	107/—
2	21/8	27/22	36/84	82/93	93/86	105/—
3	6/6	9/32	42/91	85/96	82/92	108/—
4	0/10	0/21	58/108	96/102	105/102	100/—
5	0/29	0/90	49/89	88/106	97/98	107/—
6	0/22	0/95	51/101	94/101	96/98	98/—
7	0/31	0/89	49/94	98/98	101/100	100/—
8	0/29	0/98	38/102	85/89	87/95	102/—

trolling serotonin release from platelets [9,10]. The fact that ^{3}H-serotonin release from platelets and replacement of ketanserine from binding sites occur in the presence of the same flunarizine concentrations (10^{-4} M) attests to affinity of this agent for nonserotoninergic acceptors controlling serotonin release from platelets. In addition, chemical structures of cinnarizine and flunarizine are similar (the only difference is that the molecule of cinnarizine lacks 2 fluorine atoms). Therefore, cinnarizine also can display affinity for ketanserine acceptors controlling serotonin release from platelets.

Flunarizine and cinnarizine dose-dependently inhibited (with equal potency) serotonin-induced platelet aggregation in patients with common migraine (IC_{50} 3.5×10^{-4} and 1.7×10^{-4} M, respectively) and healthy donors (IC_{50} 2.8×10^{-4} and 2.5×10^{-4} M, respectively). Therefore, the sensitivity of platelet 5-HT_2 receptors to cinnarizine and flunarizine in patients with common migraine remains unchanged. Platelet aggregation was studied in the plasma, while serotonin release was assessed in buffer solution. Therefore, it is difficult to compare the potencies of flunarizine and cinnarizine as inhibitors of platelet aggregation and inductors of serotonin release.

Thus, flunarizine and cinnarizine change functional activity of the serotonin system in platelets. The sensitivity of platelets to cinnarizine in patients with common migraine differs from that in healthy individuals probably due to increased affinity of receptors and acceptors involved in the regulation of serotonin uptake and release. Since the mechanisms of serotonin uptake and release from platelets and neurons are similar, it can be suggested that the sensitivity of systems regulating serotonin transport in neurons of patients with common migraine changes similarly. We assume that in these patients, structural changes in receptor and acceptor molecules regulating serotonin uptake and release from neurons and platelets are responsible for pathological sensitivity to some endogenous and exogenous compounds, including cinnarizine. Therefore, these compounds produce pathological effects on serotonin uptake and release from platelets and neurons and cause migraine attacks by inducing its release.

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