SHORT REPORT

Platelet catecholamines in cluster headache

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Abstract

Platelet tyrosine and catecholamine (CA) content was measured in cluster headache sufferers during the different phases of the illness. Compared with controls, cluster headache sufferers had lower platelet levels of norepinephrine (NE) and epinephrine (E) in all phases of the syndrome. Tyrosine levels were increased significantly during the cluster headache attack. We suggest that these results provide biochemical evidence of sympathetic nervous system (SNS) hypofunction in cluster headache.

The clinical features of excessive lachrymation, nasal rhinorrhoea and stuffiness, sweating, Horner's syndrome, changes in blood pressure and sinus arrhythmia in cluster headache (CH) patients suggest autonomic impairment.¹ Physiological and pharmacological studies have supported the clinical evidence.^{2,3} Norepinephrine (NE), the principal neurotransmitter of the sympathetic nervous system (SNS) is released into plasma from vesicles at nerve terminals present in the vicinity of the blood vessels.⁴ Platelet CA content may be an index of SNS function over prolonged time periods⁵ because the platelet does not synthesise NE or E but takes up these catecholamines from the plasma for storage in dense bodies.⁶ We report measurements of platelet CA content in CH.

Materials and methods*Patients and controls*

Thirty three patients (30 male, three female), ranging in age from 22 to 64 years (mean 43), with a diagnosis of CH⁷ were admitted to the Vicenza Hospital for this study. After informed consent was obtained, platelet levels of NE and E were measured in 25 patients in remission, 16 in the cluster period (but outside an attack) and 11 during an attack. Eight in remission were studied subsequently in a cluster period as well as in an attack. Platelet levels of tyrosine were measured in 23 patients during the remission and cluster period and in 14 during the attack. Twelve patients were studied in all phases of the illness. All measures were simultaneously evaluated in 22 patients during the remission period, 15 during the cluster period and 11 during the attack. Only in seven patients were all three measures obtained at

every stage of the illness. Results were compared with 15 control subjects (13 male, two female) age range from 25-55 years (mean 41). All subjects were free from drugs known to affect platelet function for at least two weeks before the study, and O₂ inhalation was the only therapy administered to the patients during attacks of CH.

Norepinephrine and epinephrine assay procedure

Subjects were resting in a supine position in a quiet room when blood samples were drawn. Platelets were obtained from 20 ml blood as previously described.⁸ The platelet pellet was sonicated in 1 ml perchloric acid, centrifuged and the supernatant placed in a filtration column (Baker 10-SPE) containing 25 mg acid-washed alumina (INC Biomedicals) and 1 ml of 1.5 M TRIS buffer, pH 8.6. After the column was washed with HPLC grade water the catecholamines were separated from the alumina by passing a 200 µl aliquot of 0.1 M perchloric acid through the column.

NE and E were detected by HPLC using coulometric detection. The system consisted of a Rheodyne injector (Model 7125), Waters pump and ESA 5100A coulometer detector together with an ESA model 5021 conditioning cell and an ESA model 5011 analytical cell. The potentials for detector 1, detector 2 and the conditioning cell were +0.05, -0.30 and +0.40 V respectively. (An ESA HR-80 column was used for the separation). The mobile phase consisted of 0.0575 M monobasic sodium phosphate, 1.25 mM heptanesulphonic acid and 0.215 mM EDTA, adjusted to pH 3.6 with phosphoric acid and combined with 3% HPLC grade methanol. A 50 µl sample was injected directly onto the column. The retention times for NE and E were 1.9 and 3.1 minutes respectively. Dihydroxybenzylamine was used as an internal standard.

Tyrosine assay procedure

Blood (8 ml) was drawn from subjects who were in the supine position and placed into plastic tubes 1:10 dilution containing citrate (3.8%) as the anticoagulant mixture. Platelet rich plasma (PRP) was obtained after the centrifugation of blood at 165 g for 15 minutes. Platelets were counted using a platelet analyser BAKER-810. One ml of PRP was centrifuged at 2500 g for 15 minutes to obtain the platelet pellet. The pellet was resuspended in 1 ml of saline solution, with 30 mg of sulfosalicylic acid added to deproteinise plate-

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Table 1 Platelet tyrosine levels in control subjects and cluster headache patients

	N	Tyrosine*	t test vs controls	t test between groups
Controls	15	0.080 (0.043)		
Remission period (RP)	23	0.076 (0.041)	ns	ns vs CP
Cluster period (CP)	23	0.086 (0.059)	ns	p < 0.001 vs PA
Painful attack (PA)	14	0.156 (0.067)	p < 0.001	p < 0.002 vs RP

*Values are expressed as nm/10¹⁰ platelets.

lets and then sonicated for 55 minutes. The preparation was then centrifuged for five minutes at 12000 g and a 50 µl aliquot of supernatant was injected onto a Millipore/Waters HPLC connected with a Perkin-Elmer fluorescence spectrometer. Tyrosine was calculated by utilising norvaline as an internal standard.

The statistical significance of differences in tyrosine, NE and E between groups was tested by Student's *t* test. The Wilcoxon Ranked Sum test was used to determine the difference between CH and normal controls because of an unequal distribution of values between groups.

Results

There were no significant differences in platelet tyrosine levels (nm/ 10¹⁰ platelet) between control subjects and CH patients in remission or in the cluster period (table 1). Platelet tyrosine levels were increased significantly during an attack compared with controls and asymptomatic patients in remission or in a cluster period. The results were confirmed in the 12 patients studied sequentially in all phases of the condition [0.17(0.03) attack vs 0.111 (0.07) remission, 0.07 (0.04) cluster period, p = 0.01]. Compared with the control subjects, the mean values of NE and E in the platelet of CH sufferers were significantly lower in all stages of the illness (table 2). Also, no significant differences were found among groups of patients studied at different phases of CH or in eight patients sequentially studied in all phases of the condition.

Discussion

Platelet catecholamines were low in all stages of CH. We suggest these findings may reflect a

generalised systemic state of SNS hypofunction.⁶ Low platelet CA could be also explained by excessive release during platelet activation.⁸ Platelet activation is reversed during attacks, however, and release of platelet 5-HT, also contained in dense bodies, is not characteristic of cluster headache. Nevertheless, a normal platelet dense body content must be documented to discount this alternative hypothesis confidently. The normal tyrosine measurements show that substrate deficiency cannot account for low platelet CA levels.

High platelet tyrosine during an attack was unexpected. An increased demand for tyrosine incorporation into structural proteins occurs because of increased protein phosphorylation⁹ when platelets are activated as in CH patients in remission or in the cluster period outside an attack.⁸ When during an attack platelet activation is reversed and tyrosine utilisation is correspondingly decreased, platelet levels may become increased transiently until previously enhanced tyrosine synthesis or uptake is down-regulated.¹⁰ This hypothesis and other potential mechanisms require investigation in separate experiments.

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Table 2 Norepinephrine and epinephrine platelet levels in control subjects and cluster headache patients†

	N	Norepinephrine*	Patients vs controls**	Epinephrine*	Patients vs Controls***
Controls	15	3.45 (0.74)		0.83 (0.75)	
Remission period	25	2.17 (0.50)	p < 0.001	0.28 (0.25)	p < 0.002
Cluster period	16	2.26 (0.61)	p < 0.005	0.22 (0.17)	p < 0.01
Painful attack	11	2.22 (0.90)	p < 0.001	0.19 (0.11)	p < 0.01

*Values are expressed as ng/10¹⁰ platelets

**Unpaired t-test

***Rank sum test

†No difference was found among the means of patients in the different phases of cluster cycle.