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Abnormal Platelet Aggregation Response in Huntington's Disease*

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Summary. Platelet aggregation response to epinephrine, dopamine, serotonin, adenosine diphosphate, arachidonic acid, and collagen was examined in seven patients with Huntington's disease and nine of their relatives. All patients, except for two cases that were in terminal states, showed enhanced response to all the stimulants, especially to dopamine and epinephrine. The platelet aggregation response in many relatives also deviated from the normal limit.

The relationship between platelet aggregation abnormality in Huntington's disease and the pathophysiology of the disease was discussed from the view of a generalized membrane defect hypothesis in Huntington's disease, and of disturbed cathecholamine metabolism, both in the CNS and periphery.

A possibility that platelet aggregation response examination will be a useful screening test of offspring at risk was proposed.

Key words: Huntington's disease – Platelet aggregation response – Epinephrine – Dopamine – Screening test

Introduction

It has been suggested that the human platelet could serve as an appropriate model for the transport, metabolism, and release of neurotransmitters by CNS neurons. Stahl (1977) has extensively reviewed the usefulness and limitation of platelet investigation for the study of biogenic amines in psychiatric and neurologic diseases. According to him, the platelet membrane has more similarities to the serotonergic synaptosome than the cathecholaminergic one. But, recently attention has been given to the catecholamines (CA), such as dopamine (DA) and noradrenaline, with reference to uptake and release by the platelet membrane (Peyer and Pletscher 1981). From this point of view, it seems interesting to

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Table 1. Cases of patients with Huntington's disease and their relatives

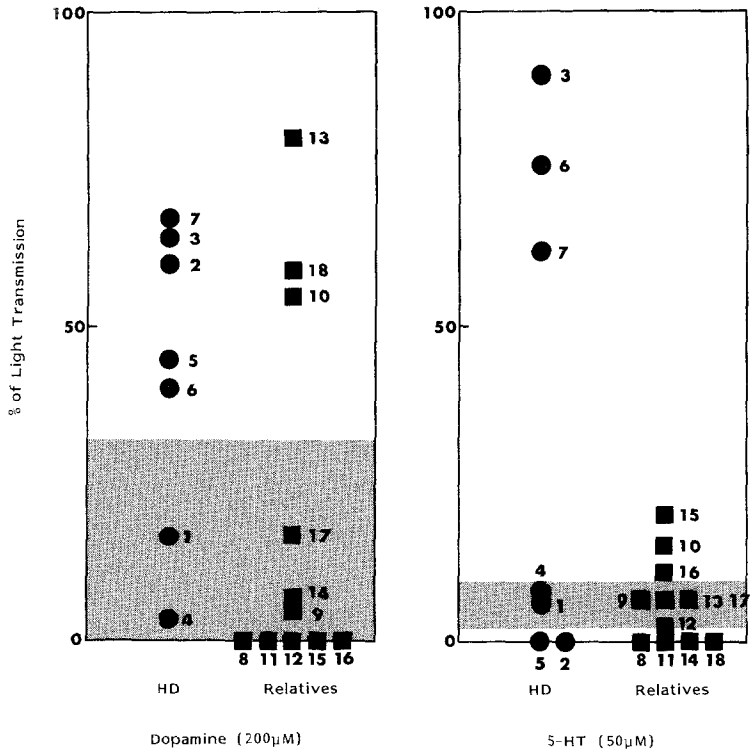
Case/Age/Sex	Family	Age of onset (year)	Duration of illness (year)	Treatment and its duration (year)
Cases with Huntington's disease				
1/42/M	A	24	18	thalamotomy (17)
2/43/F	B	39	4	haloperidol, cloxazolozepam (4)
3/46/F	B	30	16	floropipamide → haloperidol (10)
4/52/F	A	35	17	floropipamide → haloperidol (13)
5/57/M	C	47	10	haloperidol (0.3)
6/59/F	D	53	6	thioridazine (1.5)
7/66/M	B	46	20	floropipamide → (-)
	case 1 and 4 are siblings			
	case 2 and 3 are nieces of case 7			
Cases of relatives				
8/14/M	B	son of case 2		
9/17/F	B	daughter of case 2		
10/18/F	B	daughter of case 3		
11/20/M	B	son of case 3		
12/26/M	C	son of case 5		
13/28/F	C	daughter of case 5		
14/32/M	B	son of case 7		
15/37/F	B	sister of case 2		
16/41/F	B	sister of case 2		
17/47/M	B	brother of case 3		
18/54/M	D	brother of case 6		

examine platelets from patients with Huntington's disease (HD) in which the central biogenic amine disorder is postulated as a pathophysiological one (Barbeau 1979). Recently, Butterfield and Markesbery (1981) have been intensively studying the erythrocyte membrane in HD, on the basis that HD is associated with a generalized membrane defect. As the platelet membrane also plays an important role in aggregation (Ohki et al. 1980, Nathan et al. 1980), an investigation of platelet aggregation response in HD seems a valuable tool in searching for a fundamental pathology of the disease. The present study will describe the results of platelet aggregation response (PAR) to various stimulants in patients with HD and their relatives.

Subjects and Methods

Patients with HD (3 males, 4 females, mean age ± S.D.: 52.1 yrs ± 9.0) are shown in Table 1; they exhibited apparent hereditary traits of HD and hyperkinetic hypotonic symptoms. In addition, 7 offspring (4 males, 3 females, mean age ± S.D.: 22.1 yrs ± 6.6) and 2 male siblings were also tested. As normal controls, 18 volunteers (10 males, 8 females, mean age ± S.D.: 32.4 yrs ± 8.8) were examined. Because all HD cases except for cases 1 and 7 were examined under neuroleptic medication, the drug effects on PAR were evaluated in 13 schizophrenic patients under neuroleptic therapy (7 males, 6 females, mean age ± S.D.: 32.4 yrs ± 12.2). Informed consent was obtained in all cases.

A blood sample (9 ml) was taken between 10 and 13 h after drug administration with a plastic syringe containing 1 ml of 3.8% sodium citrate. Platelet rich plasma (PRP) was prepared by centrifugation of the citrated blood at 150 *g*, 10°C, for 10 min, and platelet poor plasma (PPP)



Figs. 1–5. Platelet aggregation response (PAR) to each stimulant in patients with Huntington's disease (HD) and their relatives. Shadowed area represents the normal range of platelet aggregation response in controls with 99% confidence limit

Fig. 1. PAR to dopamine and serotonin in patients with HD and their relatives

was obtained by centrifugation at 1500 *g*, 4°C, for 10 min. Platelet aggregation ability in response to each stimulant was shown by light transmission (%), using an aggregometer (Sienco). As a stimulant, $10^{-10} \sim 4 \times 10^{-6}$ g/ml of epinephrine (EP), 200 μM of dopamine hydrochloride (DA) (Wako Pure Chem.), 50 μM of serotonin (5-HT) (Aldrich Chem.), 20 nM ~ 10 μM of adenosine diphosphate (ADP) (Sigma), 40 $\mu M \sim 8$ mM of arachidonic acid (AA) (PL Chem.), $10^{-9} \sim 10^{-5}$ g/ml of collagen (COL) (Hormon Chem.) was added to PRP, and the maximum light transmission was measured at room temperature.

Platelet number in PRP was determined, using a Coulter Counter Model Z-BI. Mean platelet volume was computed from the volume distribution (1.85–13.94 μm^3). Student *t* test was used for statistical analysis, and the normal range of PAR in controls was plotted as 99% confidence limit on the graph. All mean values were represented together with a standard deviation.

Results

All patients with HD, except for 2 cases from family A, showed elevated PAR to DA (Fig. 1). The enhanced response to DA was seen in 3 of 9 relatives (Fig. 1). Of the 7 patients with HD 2 showed enhanced PAR to 5-HT (Fig. 1), and their response to DA also rose above normal limits. Responsiveness of platelet aggre-

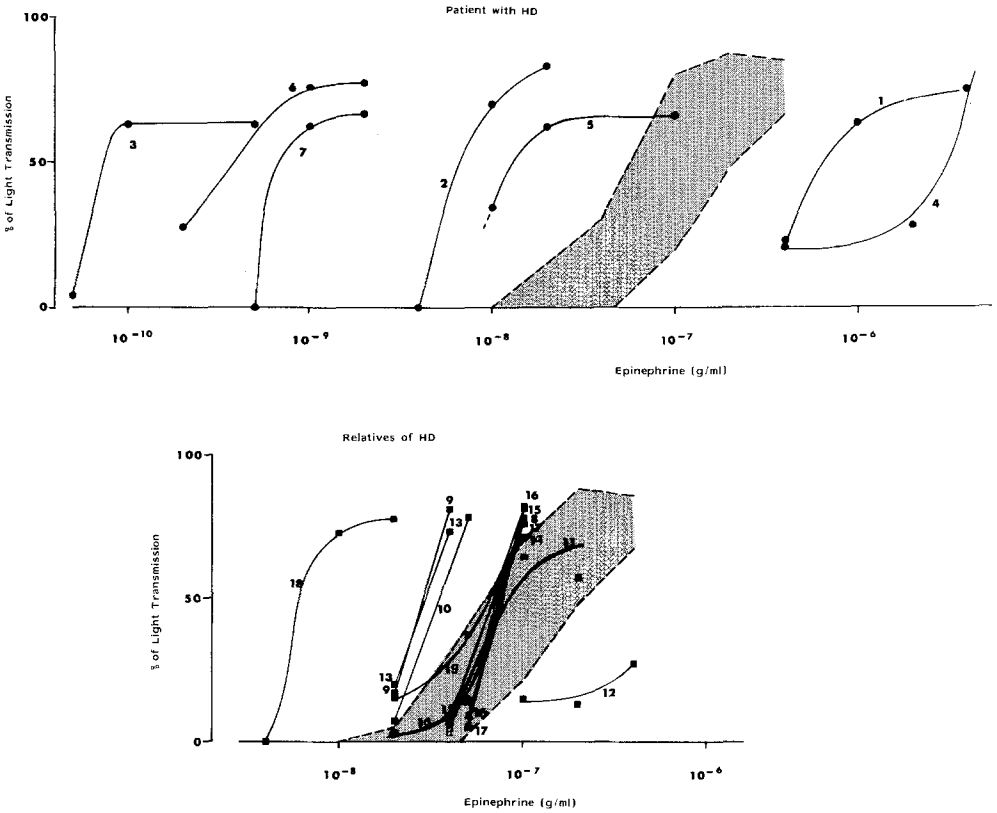


Fig. 2. PAR to epinephrine in patients with HD and their relatives

gation to EP (Fig. 2), ADP (Fig. 3), and COL (Fig. 4) in 5 patients from families B, C, and D was increased. Conversely, the responsiveness in 2 patients from family A was lower than that of the normal limit. Responsiveness to EP (Fig. 2), ADP (Fig. 3), COL (Fig. 4), and AA (Fig. 5) deviated from the normal range in many relatives of HD patients. Two offspring (cases 10 and 13) showed elevated PAR to almost all stimulants, and need to be observed to see if choreic symptoms develop.

No remarkable changes in the response pattern of PAR were seen in any of the cases. No relationship between the behavior of PAR and clinical variables, such as age, duration of illness and age of onset, was recognized. Non-medicated patients did not show a different aggregation response in comparison to those receiving medication. The maximum percentage light transmission of non-medicated normal volunteers and of schizophrenic patients under neuroleptic therapy is shown in Table 2. No statistically significant difference was seen between these subjects.

There was no significant difference in platelet number between patients with HD ($516{,}800 \pm 67{,}850$ cell/μl, $n = 6$, mean age 52.1 yrs ± 9.0) and controls ($424{,}250 \pm 27{,}400$ cell/μl, $n = 5$, mean age 51.0 yrs $\pm$ 2.9). The platelet volume decrease with age (Table 3). The platelet volume of patients with HD was smaller in comparison with that of age-matched controls ($P < 0.001$).

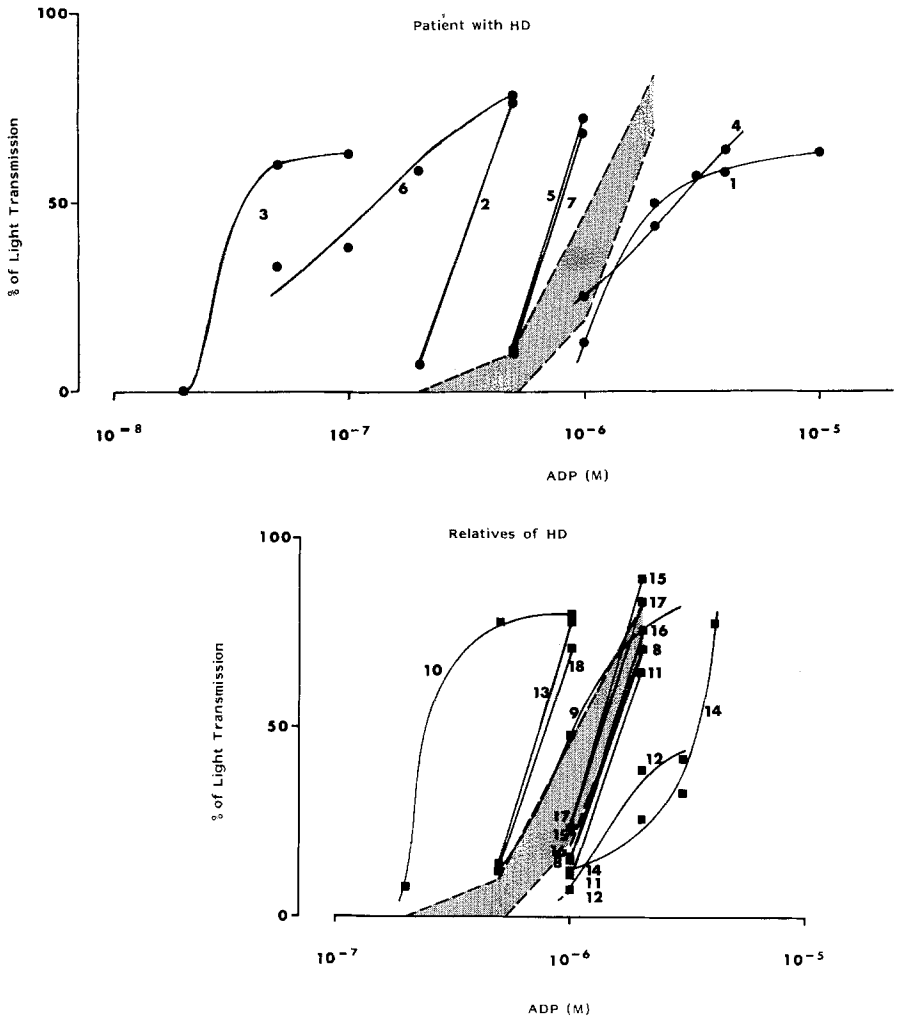


Fig. 3. PAR to ADP in patients with HD and their relatives

Discussion

Until now, there have been few platelet studies in HD. Aminoff et al. (1974) first showed an increased uptake of DA and 5-HT by PRP from HD. McLean and Nihei (1977) reported increased uptake of DA but not of 5-HT by platelets from HD. Some studies did not reveal any differences in amine uptake by HD platelets (Butterworth et al. 1977; Omenn and Smith 1978; Bonilla et al. 1978; Diez-Ewald et al. 1980). Elevated platelet monoamine oxidase activity was found only in male patients with HD by Mann and Chiu (1978), but Belendiuk et al. (1980) found elevated levels in both sexes and in their offspring at risk.

The present study showed that PAR in HD apparently deviated from normal limits. The same direction of PAR was not observed in all families, but the

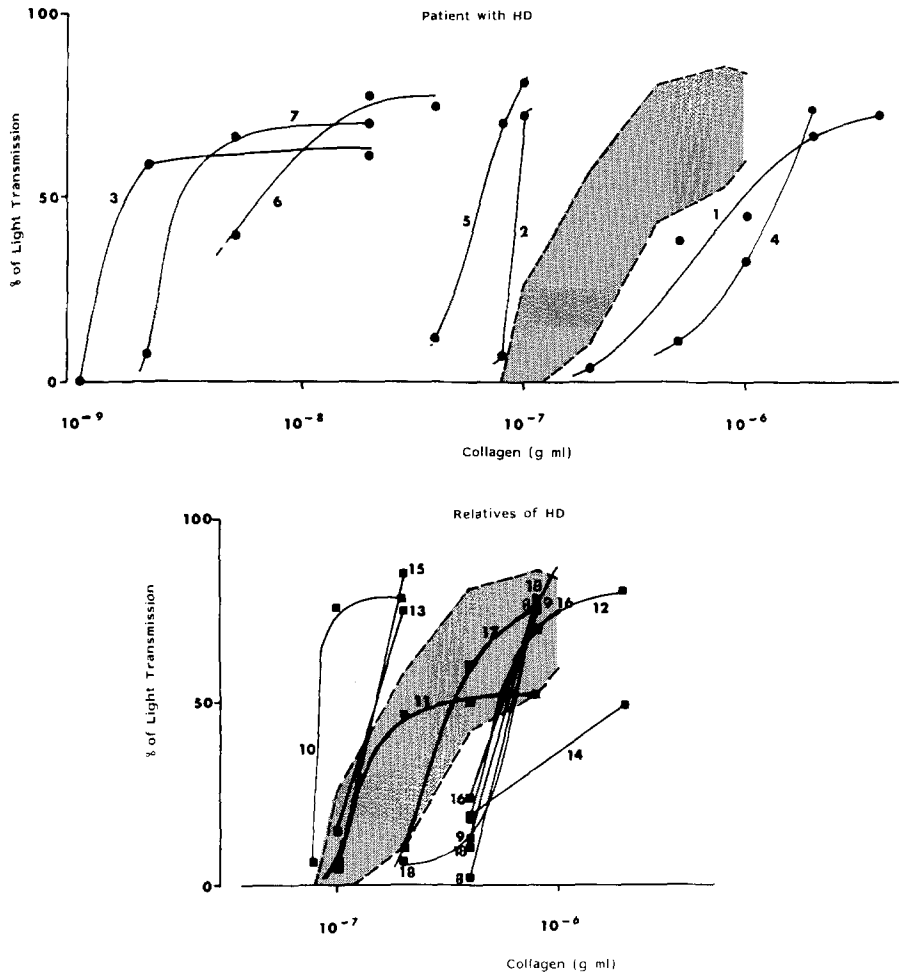


Fig. 4. PAR to collagen in patients with HD and their relatives

response was depressed in 2 cases from family A and was elevated in the other 5 cases from families B, C, and D. Until now, there has been no clue to explain the discrepancy of the findings in each family from hereditary or symptomatic viewpoints. Only circumstantial evidence common to cases 1 and 4 can be pointed out, i.e., both cases have been confined to their beds for 5 years and are in the terminal state. As an experimental study has shown that nutritional inadequacies cause lowering of PAR, a nonspecific decrease of PAR in cases 1 and 4 could be associated with their general condition.

Our results are not consistent with those reported by Diez-Ewald et al. (1980) who showed decreased PAR in 13 patients with HD. The discrepancy seems to have resulted from different methods and analysis of the data. In their experiment, PAR was examined only at a certain concentration with each stimulant, and the concentration which Diez-Ewald et al. used was higher than those used in this study. This resulted in a skew to the right of the graph at the lower concen-

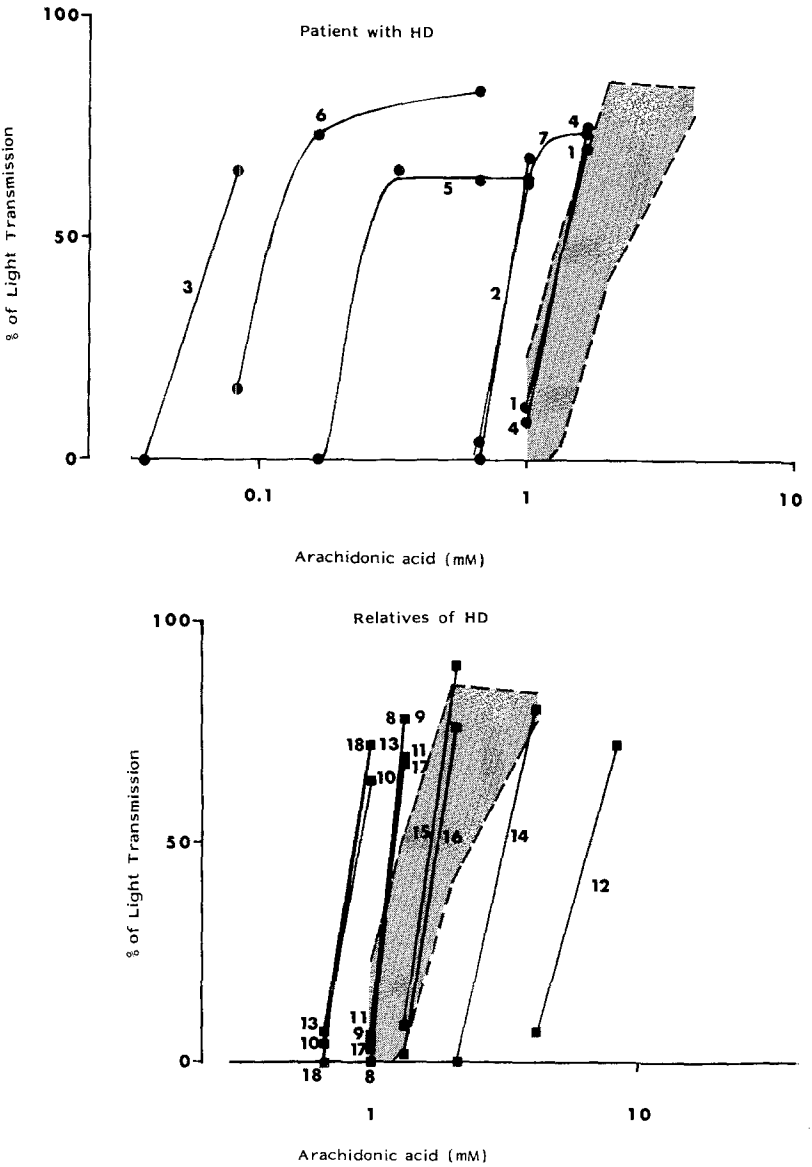


Fig. 5. PAR to arachidonic acid in patients with HD and their relatives

trations. Also their experimental condition could only detect decreased and normal PAR but not increased PAR. On the other hand, the present study has checked PAR with each stimulant under various concentrations and thus represents the true nature of PAR in HD.

The present study showed a nonspecific change of PAR to every stimulant in patients with HD. The evidence implies that a disturbance in a basic mechanism of platelet aggregation exists in HD. Recently, some studies have been performed under a hypothesis that a generalized membrane defect plays an important role

Table 2. Results of platelet aggregation response to each stimulant in normal volunteers and schizophrenic patients receiving neuroleptic medication. No statistically significant difference was seen between the two groups

		Light Transmission (%)	
Inducers	conc.	Control (n=18)	Schizophrenia (n=13)
5-HT	50 μM	6.0 ± 1.4	14.3 ± 5.7
Dopamine	200 μM	14.3 ± 6.1	19.7 ± 8.2
Epinephrine	4×10^{-8}(g/ml)	13.6 ± 6.1	14.5 ± 8.4
	10^{-7}	45.9 ± 8.8	49.8 ± 11.7
	2×10^{-7}	68.2 ± 6.8	74.0 ± 6.8
	4×10^{-7}	76.1 ± 3.3	80.3 ± 1.4
Arachidonic acid	1.0 (mM)	8.8 ± 5.1	0.8 ± 0.8
	1.3	27.1 ± 8.4	18.3 ± 9.9
	2.1	63.0 ± 7.8	71.1 ± 7.7
	4.2	81.3 ± 1.3	79.1 ± 1.4
Collagen	10^{-7}(g/ml)	11.1 ± 5.3	8.9 ± 6.9
	2×10^{-7}	33.8 ± 8.3	26.0 ± 10.8
	4×10^{-7}	62.3 ± 6.7	58.1 ± 9.8
	8×10^{-7}	69.4 ± 5.8	71.4 ± 3.8
ADP	5×10^{-7}(M)	5.8 ± 1.7	5.8 ± 1.7
	10^{-6}	32.5 ± 2.4	22.3 ± 1.8
	2×10^{-6}	76.6 ± 2.4	80.1 ± 1.9

Table 3. Mean platelet volume (μm^3)

Age of subject (yrs)	Control	HD
20–29	7.29 ± 0.103 $(n = 6)$	
30–39	7.13 ± 0.072 $(n = 5)$	
40–59	6.97 ± 0.075 $(n = 5)$	6.43 ± 0.080* $(n = 6)$

* $P < 0.001$

in HD. Butterfield and Markesberg (1981) found an abnormality in a protein on the membrane surface of erythrocytes in HD patients using electron spin resonance and labeling with MAL-6. They speculated that changes in a membrane glycoprotein could reach small neurons in the basal ganglia, resulting in neuronal loss and atrophy. Thus when considering that a generalized membrane defect underlies HD, nonspecific change in PAR as shown in the present study becomes understandable. A survey of neuropathologic changes in HD (Bruyn et al. 1979) indicated the existence of predilection at both histologic and molecular levels (Pathoklise–Vogt). Medium and small neurons are particularly involved. A primary, and not a secondary, neuronal death and atrophy are seen in the cerebral and cerebellar cortex, neostriatum and pallidum, thalamus, subthalamus and hypothalamus. The lesional selectivity cannot be explained solely by the generalized membrane defect. Although PAR in patients with HD was uniform

and nonspecific to all stimulants in the present study, the magnitude of the response was different among the stimulants. There were more cases that had enhanced PAR to DA than to 5-HT. The degree of PAR deviation from normal limits was most striking when platelet aggregation was induced by EP as compared with ADP, AA, and COL. From the finding of elevated PAR in patients with HD, especially to CA, an increased number of sensitivity of the catecholamine receptor on the platelet membrane can be postulated. Studies on neurochemical analysis of brain CA in HD victims have not reached any consensus (Kaiya et al. 1981). However, on the basis of the clinical observation that L-Dopa (DA precursor) elicits a choreatic movement and haloperidol (DA blocker) suppresses the symptom, overfunction of DA in the HD brain is suggested. This consideration does not contradict an increased function of CA receptor on the platelet membrane. That is, platelets of patients with HD may reflect the abnormality of CA synaptosome in the brain.

The mean volume of the platelet particle was significantly smaller in HD than in age-matched controls. The mean volume in normal controls decreased with age. In this context, one should take into consideration that a premature senescence occurs in HD, in spite of some contrary findings (Bruyn et al. 1979; Butterfield and Markesbery 1981).

Although we have discussed a relationship between abnormal PAR and pathophysiology in HD, the problem remains unclarified because of the obscurity of the physiological mechanism in platelet aggregation. Be that as it may, the remarkable deviation of PAR in patients with HD and many of their relatives should be kept in mind. The possibility that PAR examination may predict the development of HD in relatives at risk deserves further study.

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