CHAPTER V

Discussion

This study utilized transmission electron microscopy (TEM) to evaluate possible morphological abnormalities of platelets in patients with aminergic disorders. The results revealed platelet morphological abnormalities in serotonergic (migraine, depression) and dopamine system (Parkinson's disease) disorders.

In migraine, the results confirmed the link between migraine and platelet abnormalities (Malmgren and Hasselmark, 1989; Hantington, 1989; D'Andrea, 1995). In accordance with previous demonstrations of D'Andrea et al. and Riddle et al. (1989), a statistically significant increase in platelet dense granules in migraine patients was observed in this study. Such an increase was found in both resting and activated platelets.

Dense granules constitute the specific class of platelet granules (Bull, 1966) which serve as a storage site for 5-HT, a non- metabolic pool of nucleotides and calcium (Crawford and Scrutton, 1994). The combination of these substances results in a product which electron beams cannot penetrate. This inherent electron opacity is the ultrastructural hallmark of the dense granules and allows their recognition in ultrathin sections of platelets.

Based on the finding of an increase in the number of platelet dense granules observed in migraine patients, one can expect elevated level of 5-HT in these patients. However, D'Andrea et al. (1994) and Srikiatkhachorn and Anthony (1996) demonstrated that the mean basal platelet values for 5-HT in migraine without aura (MwA) did not significantly differ from those of two controls. A significant increase of the 5-HT basal level was observed only in migraine with aura (MA). Since the number of platelet dense granules was increased in MwA, it is possible that the concentration of 5-HT per dense granule may be lower than that of the controls.

D'Adrea et al. hypothesized that the increase of platelet dense granules in migraine may be caused by the decreased secretion rate of platelet dense granules. Confirmed by morphological findings, the platelet ultrastructure revealed no evidence of platelet activation or secretion in the migraine group (D'Andrea et al., 1989; Riddle et al., 1989). In vitro data regarding platelet secretion indicated that after activation by either collagen or PAF platelets obtained from MA group showed increased 5-HT secretion. No significant difference of 5-HT secretion was observed in platelets from the MwA group when compared to normal controls (D'Adrea et al., 1994).

In this study, the open canaliculi system was studied measuring the number of dilated canaliculi. This membrane system participates in either

uptake entry of external elements into platelets or release of granule contents to the exterior (Ware and Coller, 1994). The results indicated that platelets obtained from the migraine group showed no difference in the canalicular system during the resting state. However, after glass surface activation an increased number of dilated canals was observed in this group. This may imply that platelets of patients with MwA are hypersensitive to glass surface activation compared with controls.

Several studies proposed there to be a disorder of mitochondrial energy metabolism in migraine (Montagna et al.,1988; Welch and Ramadan, 1995). The disturbance of energy metabolism in this condition was demonstrated in brain, muscle, and platelets (Welch et al., 1989; Barbiralli et al., 1990, 1992). Platelets require ATP to support the mechanochemical and ion transport activities in order to maintain their discoid shape in the resting state, and their exocytotic as well as morphological features in the activated state. Platelets possess a pattern of energy generating pathways comparable to that found in other cells. Their mitochondria contain the enzymes of the tricarboxylic acid cycle and of fatty acid oxidation together with the components required for coupled oxidative phosphorylation (Spector, 1987; Crawford and Scrutton, 1987, 1994). In the resting state, most of the ATP is generated by oxidation of the long chain fatty acids, whereas glycolysis becomes the major source of ATP in stimulated platelets.

Several studies indicated the reduction of enzyme activity in mitochondrial platelets of patients with migraine, especially important enzymes of the respiratory chain such as NADP-dehydrogenase, citrate synthase, and cytochrome-c-oxidase (Montagna et al., 1988; Welch et al., 1969; Bresolin, 1991; Babirani et al., 1992). Furthermore, muscle biopsies of migraine patients showed ragged-red fiber, on Gomori trichrome stains, and subsarcolemmal clusters of giant mitochondria with concentric cristae and paracrystalline inclusions, on EM (Montagna et al., 1988). However, the present study did not demonstrate any significant changes in platelet mitochondria in migraine patients. The requirement of ATP for maintenance of cell activity in platelets is perhaps less than that in muscle or brain. It is possible that mitochondrial dysfunction could not lead to structural change in platelets of this group.

Comorbidity of migraine and depression has consistently been described. Biologically, both conditions are associated with altered levels of 5-HT. During a migraine attack, low levels of 5-HT and possibly a low Vmax for 5-HT uptake, have been reported. Both phenomena are also observed in depression.

In this study, platelets obtained from the migraine with depression group were also examined by TEM. They showed ultrastructural abnormalities which differed from both normal control and migraine groups.

In comparison with the controls, the number of platelet dense granules did not show any significant difference. However, a significant decrease in the number of platelet dense granules was demonstrated in this group compared to migraine patients.

Various evidence indicated abnormalities of 5-HT metabolism in depression. Platelet 5-HT uptake studies revealed a significant decline in Vmax, which correlated well with the depressive symptom (Meltzer and Arora, 1991). A significant decrease in ^{3}H-imipramine binding sites was described in unmedicated depressive patients compared to controls. (Briley et al., 1980; Suronyi-codotte et al., 1985; Meltzer and Arora, 1991; Owens et al., 1996). These biochemical and phamacological abnormalities reflect a decrease of platelet 5-HT uptake and may lead to diminished 5-HT storage.

In this study, platelets of migraine patients showed increased numbers of dense granules. However, simultaneous affliction with depression and migraine lead to a further defect in the 5-HT uptake system. Hypofunction of the 5-HT uptake mechanism may lead to further diminution of 5-HT in platelet dense granules, as well as a decline in the number of platelet dense granules in migraine with depression.

On the other hand, a lower number of platelet dense granules may result from the increase of 5-HT secretion. Analysis of the canalicular system

in this group supports this hypothesis. The significantly higher number of dilated canals either in resting or activated platelets compared to controls indicated hyperactivity of this membrane system which can lead a hyperactive secretion process. In comparision to the migraine group, platelets of patients with migraine and depression showed a higher number of dilated canals. However, this increase did not attain statistic significance. Furthermore, recent studies revealed higher PRP serotonin levels in untreated depression than in controls (Lerva et al., 1996).

Hypersensitivity of platelets found in migraine patients with depression may be related to other abnormalities of platelet response previously reported by this group. Mc Bride et al. (1987) found significantly increased 5-HT_2 binding in platelets of depressed patients. Such findings were recently confirmed by Owen et al. (1996), who suggested that an increase in 5-HT_2 receptor binding in depressed patients was more likely related to a genetic abnormality than to an alteration of the central nervous system 5-HT status. Increased of 5-HT_2 binding in depression was also supported by an increased functional response of the 5-HT receptor as measured by phosphoinositide turnover(Meltzer and Arora, 1991; Rarege et al., 1996).

Morphological abnormalities of platelets obtained from patients with Parkinson's disease (PD) were observed in this study. In comparison with the controls, a higher number of dense granules was demonstrated in the PD

group in resting platelets. On the contrary, such increase became normal in glass surface activated platelets. Individual data revealed that almost all platelets of the PD group showed a decrease in the number of dense granules after being exposed to glass surface compared to resting platelets.

Besides 5-HT, dopamine (DA) is apparently stored in platelet dense granules (Stahl and Meltzer,1978). 5-HT and dopamine are antagonist with regard to platelet accumulation. Several studies of amine uptake by subcellular storage granules in platelets indicated that dopam ine had a lower affinity for ATP than 5-HT. Regarding these results, the accumulation of dopamine seemed to be lower than that of 5-HT (Boullin and O'Brien, 1970; Da Prada and Pletscher; 1969).

For quite some time, studies in 5-HT or dopamine metabolism in PD have used platelets as a neuronal model. Boulin and O'Brien (1970) studied the DA uptake and storage capacity in PD by incubating platelets with excess DA for 90 minutes. Their results showed that the equilibrium concentration of DA was lower in the PD group compared to controls. As dopamine accumulation appeared normal in untreated patients, they concluded that uptake was secondary to the levadopa treatment. On the contrary, Barbeau et al. (1975) demonstrated a decrease in the ability of platelet uptake of ^{14}C-dopamine in both treated and untreated PD patients.

This defect could be due to a reduced amount of available or circulating dopamine due to an impaired sympathetic or adrenal dopamine metabolism. Another alternative explanation was that the platelet pump mechanism for dopamine is insufficient in PD. This hypothesis was supported by a recent study of platelet ^{3}H-dopamine uptake. A significant decrease of dopamine uptake was demonstrated in untreated PD patients (Rabey et al.,1995).

5-HT, another component of platelet dense granules, has also been studied in the PD group. Conflicting results were demonstrated. In 1972, Yamaguchi and associates found that the 5-HT content of PRP of patients with PD did not differ from that of the controls. On the contrary, a decrease in platelet 5-HT has been reported by other groups (Bouling and O'Brien, 1972; Kembel et al., 1995). No significant difference as to 5-HT uptake and 3H-imipramine binding sites was demonstrated in platelets obtained from PD patients (Bouling and O'Brien, 1972; Sano et al., 1991).

In this study, the higher number of dense granules in resting platelets of the PD group may be caused by the compensatory process of the cell in response to the lower level of endogeneous 5-HT and dopamine. The reduction could be due to either a defect of the uptake system or hypersecretion of both substances.

In the present study, activated platelets of PD patients demonstrated a decrease in the number of dense granules compared to resting platelets. The reduction reflects hypersecretion of platelets in this group. Platelet DA hypersecretion has previously been demonstrated by pharmacological methods using ^{14}C-DA as a substrate (Boullin and O'brien, 1975). Dilated canaliculi in resting/activated platelets shown in this study also support the hypothesis of hyposecretion in the PD group.

Recent investigations of platelets of the PD group have shown defects in mitochondrial oxidative phosphorylation. Low activity of complex I, II, and III was reported (Parker et al., 1989; Krige et al., 1992; Haas et al., 1995). The results of this study did not support mitochondrial abnormality since morphologically, that organelle was normal in size and number. Shoffner et al. (1991) described physiological changes of mitochondria in muscle of PD patients without morphological correlation. Possibly mitochondrial dysfunction in PD cannot cause structural changes in platelets.

In addition, platelets apparently are functionally abnormal in PD. In a case control study, Struct et al. (1990) demonstrated a significantly lower incidence of stroke and myocardial infarction in PD than non-PD controls. Sharma et al. (1991) reported a 32% decrease in platelet aggregation utilizing adenosine diphosphate as activating substance and a 60% decrease utilizing epinephrine in PD patients compared to the age/sex

matched controls. The results of this study could theoretically be related to a decrease in platelet aggregation in PD. The reduction in number of alpha granules in resting platelets was demonstrated in the PD group. The reduction of von Willebrand factor, fibrinogen, PF-4 and β-thromboglobulin (pivotal substance for platelet aggregation) in the matrix of alpha granules, may explain the decrease of platelet aggregation in the PD group. Moreover, aggregation of platelets is a calcium dependent function (Hourani et al.,1991). It is possible that structural changes in the canalicular system in PD may affect the intracellular release of calcium from the closely associated tubular system and this could account for the alteration in the aggregating capacity of platelets (Parker et al., 1994)