CHAPTER II

Background

Alterations of the central aminergic system are responsible for the pathogenicity of various neurological and psychiatric diseases. However, a direct assessment of the central aminergic function is difficult and requires sophisticated equipment, e.g. PET scan etc. Therefore, platelets have been proposed as a good subject for the study of aminergic neurons from these reasons. First, they are easily accessible. Secondly, they can be readily obtained as a pure cell suspension. Thirdly, they are capable of manufacturing, storing, releaseing and taking up monoamine (Hourani and Cusack 1991; Malmgren and Hasselmark, 1989)

Hence, several studies concerning the pathogenetic mechanism of amine-related disorders have been carried out using platelets. In this study, the diseases caused by abnormalities in the serotonin and dopamine systems were selected.

Platelet studies in serotonergic disease

Serotonin (5-hydroxytryptamine, 5-HT) constitutes a major neurotransmitter involved in the control of numerous functions of the central

nervous system, such as mood, aggression, pain, anxiety, sleep, memory, eating behavior, temperature control, endocrine regulation and motor behavior. Various psychiatric disorders such as schizophrenia, mania, depression, aggressive and self-injurious behavior, obsessive compulsive disorders and behavioural disorders in geriatric patients have been linked to impaired central 5-HT functions.

Migraine and depression were selected in this study, since both are associated with a depletion of 5-HT in the central nervous system.

Platelet studies in migraine

Several biochemical and functional studies have been performed in an attempt to clarify specific platelet disorders occuring in migraine. The platelet function was more extensively investigated in aggregation in platelet rich plasma (PRP) applying the optical principle. Based on in vitro studies using this technique, increased aggregation, either spontaneous (Hanington,1981) or after administration of 5-HT (Hilton and Cumng, 1984) adenosine diphosphate, ADP (Deshmukh and stirling,1977; Kalendovsky and Austin, 1975), platelet activating factor, PAF (Andrew et al., 1993), and collagen (Tozzi et al., 1995) have been observed during migraine attacks and\or in headache-free periods. These results, however, are still controversial as they could not be reproduced in other studies (Hilton and Cumings, 1972;

Krunglak et al., 1984; Hanin et al., 1985; Riddle et al., 1988). Moreover, when chemically evoked platelet aggregation was studied using collagen, the extent of aggregation, was either similar to that observed in control subjects (Joseph et al., 1986; D' Andria et al., 1994a) or significantly reduced (D' Andria et al., 1994b).

The involvement of an altered platelet reactivity in migraine seems to be supported by an increased in vitro platelet responsiveness to 5-HT and also to biologically active agents like adenosine diphophate (ADP), prostaglandins, and catecholamines (D' Andrea et al., 1986; Kitano et al., 1994). To verify whether this alteration is the consequence of an abnormal lipid composition, Vecino et al. studied the phospholipid specimen and the cholesterol/phospholipid ratio in platelets of patients suffering from migraine. The proportion of five main platelet phospholipid components was also normal. These results suggested that platelet hyperactivity in migraine was not due to an altered lipid component of those cells (Vecino et al., 1996). In addition, the levels of β-thromboglobulin and platelet factor 4, two specific proteins released by platelet α-granules, were increased in the plasma of migraineurs (La Mancusa et al., 1991; Leira et al., 1991). Higher level of basal cytosolic platelet calcium (Ca^{2+}), the pivotal second messenger coupling platelet stimulation and response, has been also reported in the migraine group (Joseph et al., 1988). These results might be considered as an expression of in vivo platelet activation and granule hypersecretion in

patients with migraine. However, in vitro studies on platelet secretion in migraine with aura (MA) and migraine without aura (MwA) demonstrated that both PAF and collagen-induced 5-HT secretion was significantly increased in platelets of patients with MA compared with both those of controls and patients with MwA. By constrast, collagen induced PF4 secretion was significantly lower in platelets from patients with MA (D' Andrea et al., 1994). PAF-induced PF4 secretion was significantly increased in platelets of patients with both MwA and MA. These results might indicate increased platelet secretion of 5-HT from dense granules apparently to be specific for MA.

Several studies of 5-HT metabolism in migraine found that migraine patients had low level of plasma 5-HT and high level of 5-hydroxyindolacetic acid (5-HIAA), the major 5-HT metabolite, between attacks, whereas during attacks the plasma 5-HT level was higher and the metbolite level was lower than during attack-free periods (D'Andrea et al., 1989,1994; Ferrari, 1989). Unlike 5-HT, neuron platelets do not synthesize 5-HT but have an active uptake mechanism. Serotonin is transported into the dense granules by two specific carrier-mediated systems located at the plasma membrane, e.g. imipramine binding sites, and the dense granule membrane (Crawford and Scrutton, 1994). Studies of the two transport systems in migraineurs platelets found that both 5-HT uptake sites and ^{3}H-imipramine binding sites were decreased in number (Dalsgaard-Nielsen and Genefke, 1974; Marazziti,

1994). Moreover, Govitrapong et al. (1992) demonstrated a decrease of 5-HT_2 receptors on the platelet plasma membrane in the migraine group.

Another element noted in platelets and considered similar to that found in the brain is represented by the enzymes monoamine oxidase (MAO) and phenosulfotransferase (PST). MAO is a membrane-bound mitochondrial enzyme which exists as two distinct forms, MAO-A and B with different inhibition (Johnston, 1968) and substrate (White and Tansik, 1979) specificity.

MAO type A inactivates 5-HT, noradrenaline, adrenaline, tyramine (partly) and also dopamine in the intestinal mucosa, while type B inactivates tyramine, tryptamine phenylalanine and to a lesser extent dopamine. Platelet MAO is of the B type (Anthony, 1987). Two forms of PST are present in both platelets and in the central nervous system. One acts, preferentially on monoamine substrates such as catecholamine and 5-HT, and is thermolabile (TL). The other prefers phenol and phenolic compounds, is thermostable (TS) and the endogeneous substrate is unknown (Glover et al., 1983). Some authors found a reduction of both enzyme activities in platelets obtained from migraine patients, apparently caused by a functional rather than numeric change of the enzyme molecules (Ferrari et al., 1989; Marazziti et al., 1994).

Sangiorgi et al. (1994) discovered abnormalities in mitochondrial respiratory chain enzyme activities in platelets. They found a significant decrease of NADP-dehydrogenase, citrate synthase, and cytochrom-c-oxidase activities in platelets of migraine sufferers. The impairment of energy metabolism has been observed in braine and muscle of migraine patients (Montagna et al., 1988; Welch et al., 1969; Bresoline, 1991; Barbiroli et al., 1992)

Platelet studies in depression

Depression is considered to be associated with an impairment of 5-HT neurotransmission. Studies in patients with major depession demonstrated various abnormalities of serotonergic processes. The most direct evidence was a decrease in the level of 5-HT, the 5-HT major metabolite 5-hydroxyindolacetic acid (5-HIAA), the 5-HT receptor, and the ^{3}H imipramine binding site in post mortem specimen from depressed patients or suicides (Meltzer and Nash, 1988; Meltzer and Arora, 1991).

Platelets had also been studied in depressed patients. The finding of an increase in the platelet 5-HT_2 binding site (Cowen et al., 1993; Owen et al., 1996) in major depression further increased interest in the possible use of platelets for identifying disturbances of serotonergic functions in depression.

Several epidemiologic studies demonstrated an association between migraine and psychiatric disorders, primarily depression and anxiety (disorders) (Jarman et al., 1990; Merikangas et al., 1990). An association between major depression and migraine has been shown in subjects selected at random from a community sample (Paulin et al., 1985). Merikangas et al. (1988) studied patients with major depression and noticed a significant association between migraine and depression in both patient and their relatives. Furthermore, Garvey et al. (1983) investigated the headache pattern of patients with major depression. They had a headache rate similar to controls during their non-depressed phases but this was increased during episodes of depression. An increased prevalence of depression among migraine patients, as well as a higher incidence of migraine among patients receiving treatment for depression has been reported (Garvey et al., 1983; Merikangas et al., 1988). Based on the tyramine test, a marker for endogeneous depression, depression in patients with migraine seems unlikely to be secondary to migraine per se (Jarman et al., 1990). In addition, Brueslau et al. (1994) reported a bidirectional influence of the association between migraine and depression, with each disorder increasing the risk for first onset of the other.

Although research to date has focused primarily on the migraine-depression comorbidity, little is known about the mechanism that links the two

disorders. Disturbance in the serotonergic system, a biochemical feature common to both migraine and depression, is a possible mechanism.

Platelet studies in the dopaminergic system

Parkinson's disease (PD) is a neurodegenerative disorder clinically characterized by tremor, muscle rigidity, bradykinesis, and postural instability. The pathology of PD includes the degeneration of pigmented neurons, in par compacta of the substantia nigra and the locus celureus. The degeneration of dopaminergic cells in the substantia nigra results in central doparminergic deficiency in PD (Jellinker, 1987).

Previous reports indicated platelets to be physiologically abnormal in PD. The most important finding was that of Parker et al. (1989). They discovered a mitochondria complex I, NADH ubiquinone oxidoreductase, deficiency in platelets of PD patients. Studies in enriched mitochondrial fractions of blood had also attained the same results (Krige et al., 1992) . Moreover, Haas et al (1995) demonstrated that in addition to complex I deficency , complex II/III activity was also reduced by 20 % in PD compared to age/sex-matched controls. Since Parker et al.'s observation, mitochondrial dysfunction had been reported in brain (Schpira et al., 1996) and muscle (Shoftner et al., 1991) of patients with PD. This abnormality has been proposed to be important in the pathogenesis of this disease. Besides

mitochondrial abnormality, MAO activity in blood platelets, almost exclusively of the B-type, had been studied by several groups, but results were still an controversial. This platelet enzyme activity has been reported to be either increased (Bonucelli, 1990; Jarman, 1993), decreased (Steventon, 1990) or even unchanged (Mann et al., 1983; Kuiper et al., 1992; Ahlskog et al., 1996). Certain methodological factors might account for these disparate findings (Sandler, 1993) .

5-HT and dopamine, the amine stored in dense granules of blood platelets, had been studied in the PD group. Raisman et al. (1986) found that ^{3}H imipramine binding was decreased in the brain of patients with PD. Such a decrease was also demonstrated in platelets of this group, but these differences were not significant (Sano et al., 1991), whereas Rabey et al. demonstrated a significant decrease of DA uptake by platelet storage granules in PD (Rabey et al., 1993).

The above review indicated that platelets were abnormal in function and/or biochemical property in migraine, depression and Parkinson's disease. These abnormalities have also been correlated with the abnormalityies found in brain of each disease. However, there have been few morphological studies of platelets in these disorders. Riddle et al. (1989) used transmission electron microscopy to investigate selected aspects of the platelets response (surface activation as well as aggregation) and

quantify cytoplasmic organelles within the cytoplasm of platelet obtained from both healthy controls and migraine patients. The results showed no difference as to number of circulating platelets, degree of surface activation, amount of aggregate formation or percent of hyperactive platelet populations. However, platelets of migraine sufferers exhibited a significantly greater number of dense granules compared to control platelets. Platelets obtained from migaine patients appeared structurally altered (Riddle et al., 1989). Furthermore, ultrastructural studies of platelets in both migraine with aura and migraine without aura show the same structural alterations. They found platelet-dense bodies, the storage organelles for serotonin, increased in both migraine groups, paticularly in migraine with aura (D' Andrea, 1989).

In addition, Factor et al. (1993) tried to discover peripheral diagnostic markers for Parkinson's disease from a morphological point of view, utilizing transmission electron microscopy. The result showed the presence of numerous large intracytoplasmic vacuoles, which orginated from the open canaliculi system, in both treated and untreated PD patients. Even though no abnormality was found in relation to mitochondria, storage granules and glycogen, they concluded that platelets in PD were morphologically abnormal.

In this study, we employed transmission electron microscopy to examine the fine structural details of platelets obtained from migraine patients without depression, migraine patients with depression and patients with PD.

Ultrastructural abnormalities in platelets of each disorder, if present, could ultimately be useful in understanding platelet activation in each condition better and it might also reflect abnormalities of aminergic neurons in the brain.