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Studies on Human Blood Platelets in Affective Disorder

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Abstract. Platelets were examined to enable a simultaneous investigation to be made of indolylamine and electrolyte metabolism in affective disorder.

No significant differences were detected in either platelet membrane ATPase or adenyl cyclase specific activity in any of the groups of patients studied, when compared with appropriate controls. A reduced V_{max} and $\bar{y}$ for the 5-hydroxy-tryptamine uptake process into platelets was observed in both unipolar and bipolar depressed groups. The K_m for this process was not significantly different in any of the patients from that found in control subjects.

Lithium therapy was shown not to influence significantly any of the platelet parameters examined.

It is suggested that membrane enzyme changes found in some peripheral cells in patients suffering from affective disorder, i.e. reduced $Na^+ + K^+$-ATPase activity in erythrocytes in depression, is not common to all peripheral cells and may or may not reflect central nervous system changes.

Key words: Affective disorder – Platelets – ATPase – Adenyl cyclase – 5-HT uptake – Lithium

The aetiology of affective illness has been associated with disturbances in electrolyte (Coppen, 1967; Baer et al., 1970), catecholamine, and indolylamine metabolism (Schildkraut, 1965; Ashcroft et al., 1972). Investigation of such disturbances has involved measurement of urinary and CSF levels of biogenic amine metabolites and electrolytes. In addition, erythrocytes from patients have been used to measure ATPase specific activities and intracellular electrolytes.

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Ashcroft et al. (1973) found levels of 5-HIAA in CSF to be low in unipolar depressives, but normal in bipolar depressives and manic patients. However, no change in 5-HIAA levels occurred on recovery from unipolar depression, nor was there any abnormality in tryptophan-5-hydroxylase activity. No defect in tryptophan transport was detected after giving patients a loading dose of tryptophan followed by measurement of 5-HIAA and tryptophan in CSF. Ashcroft et al. (1973) therefore concluded that CSF levels of amine metabolites are not related to mood changes. They considered that in unipolar depression a change in functional release of 5-HT occurs, without a change in synthetic capacity. In bipolar depression and mania, they hypothesised that a change in receptor sensitivity occurs.

On the other hand, Naylor et al. (1973) have suggested that depression is associated with a reduction in $Na^+ + K^+$-ATPase activity, a reduction in active sodium transport, and an increase in intracellular sodium concentration.

Sneddon (1973) and Stahl (1977) have proposed the blood platelet as more useful than the erythrocyte as a peripheral cell model for the central aminergic neurone. Tuomisto and Tukianen (1976) carried out an investigation on 5-HT uptake and the effect of tricyclic antidepressants on uptake in blood platelets obtained from depressive patients. They found a reduction in initial uptake of 5-HT with a decrease V_{max} but with a normal K_m value. After four weeks' treatment with a tricyclic antidepressant, the V_{max} was increased to its normal value, whereas the K_m was increased above normal. In vitro, addition of the drug to the platelet suspension led to the K_m being increased without a concomitant V_{max} change. They concluded that the V_{max} change was associated with recovery from depression and was not a drug effect. Tuomisto and Tukianen state that their patients were 'newly hospitalised with a diagnosis of depression.' It was decided to extend their

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observations and to examine patients who had been classified into unipolar and bipolar depressed, bipolar manic, and unipolar and bipolar well groups.

It was also decided to measure ATPase activities. Work already carried out (Hesketh, 1976) has shown that in depressed patients (especially unipolar), a reduction in erythrocyte $Na^+ + K^+$-ATPase specific activity occurs in comparison to controls. On treatment with lithium and subsequent recovery, $Na^+ + K^+$-ATPase specific activities returned to control values and Mg^{2+}-ATPase specific activities increased above normal values. It was found that the change in $Na^+ + K^+$-ATPase is due to the recovery process, but that the Mg^{2+}-ATPase change is solely a drug effect; i.e., lithium-treated patients who had not recovered from their depression had reduced $Na^+ + K^+$-ATPase activities but increased Mg^{2+}-ATPase activities.

It has been proposed that a monoamine-receptor abnormality occurs in certain depressed patients (Ashcroft et al., 1972). Wang et al. (1974) undertook a study of platelet adenyl cyclase responses to noradrenaline and PGE in male depressed patients. It was decided to extend their study by measuring platelet adenyl cyclase activity in male and female patients suffering from affective disorder and by measuring the action of lithium on adenyl cyclase.

In summary, we designed our investigation to bridge the gap between electrolyte, catecholamine, and indolylamine studies by using the platelets from various subgroups of patients suffering from affective illness.

Materials and Methods

Clinical Data. All patients were referred from within the Royal Edinburgh Hospital, which serves a population of 470,000. Clinicians withheld active treatment from acutely ill patients until venesection took place, using only a barbiturate for sedation if necessary. The authors were able to process specimens on any day of the week to avoid unacceptable delay before any patient received specific therapy. Patients who had ingested a tricyclic antidepressant, neuroleptic, or benzodiazepine within two weeks of venesection were excluded. Patients had been diagnosed as suffering from an affective disorder by the referring clinician. They were assessed also by a research psychiatrist using the glossary developed for the Wing Present State Examination (Wing et al., 1974) on the basis of the criteria described by Feighner et al. (1972) for affective disorders. A previous episode of mania or hypomania requiring neuroleptic treatment defined a patient as bipolar. Patients receiving lithium were assessed as to whether they were ill or recovered. Hospital and MRC Unit staff were used as a source of control material, and to the best of our knowledge they had no family history of psychiatric disorder nor were they exposed to prescribed drugs.

Clinical Sampling. Patients and controls took only a light breakfast on the morning of sampling, but otherwise there was no dietary control. The patients and controls receiving lithium took no medication on the morning of sampling.

Measurement of Plasma Lithium Concentrations. Plasma lithium concentration was estimated by flame atomic absorption at 670 nm using a Perkin Elmer 360 atomic absorptiometer.

Blood Sampling. After applying a venous tourniquet, cubital venous blood was withdrawn through a B-D Yale 20-g $1^1/_2$ microlance into a B-D plastipak sterile polypropylene syringe. Nine-millilitre units of blood were immediately transferred to the bottom of an all-polythene 10-ml stoppered tube that contained as anticoagulant 1 ml of 3.8% sodium citrate. The tubes were gently inverted thrice to ensure mixing of the sodium citrate with the blood.

Preparation of Human Platelet-Rich Plasma. A paper tissue was used to clean the top inner surface of the 10-ml tubes containing blood. This was to prevent erythrocyte contamination of the plasma under preparation. The tubes were centrifuged using a low speed head in a Mistral 2L centrifuge, for 30 min at 800 rpm (125 g) at room temperature to produce platelet-rich plasma (Lingjaerde, 1971).

Preparation of Human Platelet Membranes. For any one sample, 4 ml platelet-rich plasma (PRP) was dispensed into a 10-ml all-polythene tube using an Eppendorf pipette with a polypropylene pipette tip. To this was added 4 ml of 0.38% sodium citrate. The tube was gently inverted three times. Sodium citrate was added merely to decrease the viscosity of the plasma, thus enabling the platelets to be spun down readily. The tubes were centrifuged using a low-speed head in a Mistral 2L centrifuge at 2,000 g for 30 min at 4° C. This produced a platelet pellet at the bottom of the tube. The supernatant (platelet-poor plasma) was decanted off and the tubes were inverted and allowed to drain for 10 min onto a piece of tissue paper. At the end of this time a piece of tissue paper was used to wipe the inner surface of the tube to prevent plasma contamination. 100 µl 0.1% Ttriton × 100 was added to the pellet and a smooth-edged glass rod was used to mix. Two millilitres of distilled water were added and the mixture was then whirlymixed for approximately 30 s. The tubes were then placed on their sides at −10° C for approximately 30 min, or until the mixture had frozen solid. The tubes were then removed and allowed to thaw at room temperature. They were then whirlymixed for 30 s and returned to the deep freeze for a further 30 min. When they had been removed from the deep freeze and allowed to thaw in a similar manner as before, an aliquot was removed for an assay of adenyl cyclase. The remaining sample was diluted five times. This solution was used for measuring ATPase specific activities and protein content.

Estimation of ATPase Specific Activity. $Na^+ + K^+$-ATPase (Na^+-pump ATPase), Mg^{2+}-ATPase and $Ca^{2+} + Mg^{2+}$-ATPase specific activities were measured in a platelet-membrane preparation according to the method used by Hesketh et al. (1977). The ATPase specific activities are given in units of nmol P_i liberated/h/mg protein.

Estimation of Adenyl Cyclase Specific Activity. Adenyl cyclase specific activities were measured in the incubation media described by Krishna et al. (1968), with the exception that unlabelled ATP was used in the experiments. The reaction was stopped by placing the incubation tubes (Eppendorf tubes) in a heating block at 80° C for 10 min. The tubes were then spun in an Eppendorf centrifuge for 6 min. An aliquot of the supernatant was then used to measure the concentration of cyclic AMP present. This was done using a cyclic AMP assay kit obtained from the Radiochemical Centre, Amersham. The adenyl cyclase specific activities are given in units of nmol cAMP produced/10 min/mg protein.

Measurement of 5-HT Uptake. Uptake was measured in aliquots of PRP. Uptake was measured at 37° C and 4° C. The difference between the two values was taken as a measure of active uptake of 5-HT into the platelets. 100 µl PRP was added to 3.9 ml of prewarmed (37° C) or ice-cold (4° C) Krebs-Henseleit bicarbonate buffer (pH 7.4), containing various concentrations of ^{3}H-5-HT [5-Hydroxy (G-^{3}H)] tryptamine creatinine sulphate from the Radiochemical Centre, Amersham, specific activity adjusted to 50 mCi/mmol. Uptake was measured at 5-