Platelet monoamine oxidase activity in megaloblastic anaemia

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SUMMARY Platelet monoamine oxidase activity has been measured in 17 patients with megaloblastic anaemia due to either vitamin B₁₂ or folate deficiency, and in 20 healthy subjects. There was a highly significant increase in patients compared with controls. In two patients, platelet activity decreased following successful treatment. A significant correlation between platelet activity and the severity of bone marrow megaloblastic change, assessed by the deoxyuridine suppression test and bone marrow morphology, was also observed. If the change in activity also occurs in the nervous system, this may contribute to the mental disturbance associated with vitamin B₁₂ or folate deficiency.

The widespread mitochondrial enzyme monoamine oxidase (MAO) metabolises a broad range of biologically active monoamines thought to be important for the normal functioning of the brain. The enzyme exists in two forms with different substrate specificity: MAO A predominantly oxidises 5-hydroxytryptamine and noradrenaline, whereas MAO B acts on phenylethylamine and *tele*-methylhistamine. Dopamine can be a substrate for both forms. As the human brain enzyme is inaccessible, platelet activity has been extensively studied as an indicator of possible enzyme involvement in certain diseases of the central nervous system, although the human platelet possesses only the B form. A decrease in platelet activity has been found in schizophrenia,¹ an increase in depression,² and transient changes in migraine³ and alcoholism.⁴ Here we extend a preliminary abstract of Latt and her colleagues,⁵ suggesting that platelet MAO activity is raised in megaloblastic anaemia.

Methods

Seventeen patients (9 male, 8 female) with megaloblastic anaemia due to vitamin B₁₂ or folate deficiency, and 20 normal blood donors (10 male, 10 female), were studied. In all 17 patients, the mean corpuscular volume was greater than 100 fl (range 101-128 fl). The haemoglobin levels ranged from 5.7 to 12.0 g/dl in the nine males and from 4.6 to 10.8 g/dl in the eight females. Megaloblastic change was

determined by morphological examination of May-Grünwald-Giemsa stained marrow smears, and the severity was graded + to +++ (+ mild changes; +++ florid changes). Iron stores were present in all cases. Deoxyuridine (dU) suppression of tritiated thymidine uptake tests were carried out on bone marrow samples from nine patients, as described by Killman⁶ and Metz *et al.*⁷ Serum vitamin B₁₂ and folate concentrations were measured by microbiological assay using *Euglena gracilis* and *Lactobacillus casei*, respectively.

Platelets were prepared in EDTA as described previously.³ MAO was assayed using ¹⁴C-phenylethylamine (PEA), as described previously,³ except that the reaction was terminated with 2 M citric acid (0.1 ml) and the product was extracted into toluene (3 ml). Protein was assayed by Lowry's method,⁸ and all measurements were carried out blind and in duplicate. Enzyme activity is expressed in units consisting of nmoles PEA oxidised/mg protein per hour.

Results

The mean $\pm$ SD platelet MAO activity of the 17 patients (32 ± 1.2 units) was significantly raised compared with that of the control group (15 ± 3.8 units), $P < 0.001$ (two-tailed Student's *t* test). Ten of the 17 patients with megaloblastic anaemia had levels above the upper limit (26 units) of the normal range (Figure). Two patients were retested after two months of vitamin B₁₂ therapy, and in each case platelet activity had fallen to normal, from 57 to 22

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Platelet monoamine oxidase (MAO) activity in patients with megaloblastic anaemia and in controls.

units in one and from 22 to 9 units in the other. The Table compares platelet MAO activity with other measurements in the same patient. There was significant correlation with both the degree of abnormality of the dU suppression test ($P < 0.025$) and marrow morphology ($P < 0.05$), both by one-tailed Student's t tests. There was no relationship between platelet MAO activity and initial haemoglobin concentration or platelet count. There was no difference between the patients with anaemia due to vitamin B₁₂ deficiency or folate deficiency, nor in the patients with vitamin B₁₂ or folate deficiency considered separately was there a correlation of platelet MAO activity with serum vitamin B₁₂ or folate level (Table).

Platelet MAO activity compared with other indices of megaloblastic anaemia

Platelet MAO activity (units)	dU suppression	Marrow morphology	Vitamin B ₁₂ (normal 160-925 pg/ml)	Serum folate (normal 3-21 ng/ml)
64	—	+++	310	1.0
57	—	+++	0	5.8
56	—	+	10	>28
43	—	++	0	17.6
41	30	+++	20	4.0
40	34	+	20	26.8
39	15	+++	170	0.8
30	28	++	30	6.0
30	16	+	110	2.1
30	—	+	220	3.0
24	—	++	80	13.0
22	19	++	20	7.0
22	21	+	20	12.0
21	—	+	30	12.8
16	15	+	120	0.6
14	10	+	60	7.2
9	—	+	210	2.8
P < 0.025		P < 0.05	NS	NS

Discussion

Platelet MAO activity is to some extent under genetic control, as shown by twin studies.⁹ However,

other factors, including adrenaline injection,¹⁰ exercise,¹¹ glucose ingestion,¹² and unknown small molecule endogenous activators¹³ or inhibitors,¹⁴ are able to alter platelet MAO activity.

Our results show that platelet MAO activity can be greatly increased in megaloblastic anaemia, and that the increase is related to the severity of megaloblastic change as assessed by bone marrow morphology or dU suppression test. Serum levels of either vitamin B₁₂ or folate are not reliable guides to the degree of megaloblastic changes as very low concentrations of either vitamin may be associated with either severe or mild changes. There is an overlap in activity between the patient and control group, especially when there is a mild degree of megaloblastic change and the measurement of platelet MAO activity cannot replace marrow aspiration in the diagnosis of megaloblastic anaemia. However, the ease of measuring platelet MAO activity and its correlation with the severity of marrow megaloblastic change suggest that, in certain circumstances, especially when marrow aspiration is impracticable, it may be a useful diagnostic aid. Studies of activity in other haematological conditions, in which there may be macrocytosis without megaloblastic change, for example, in some alcoholics, liver disease, and myxoedema, are needed before a firm opinion can be given of the specificity of the raised platelet MAO noted here to megaloblastic anaemia due to folate or vitamin B₁₂ deficiency.

One explanation of the increased activity is that it is associated with an altered population of platelets in megaloblastic anaemia. In autoimmune thrombocytopenic purpura, in which there is a young circulating platelet population, Friedhoff *et al.*¹⁵ have shown that the platelet MAO specific activity is much reduced. Megaloblastic anaemia is characterised by an increase in serum lactate dehydrogenase activity, stemming from both intramedullary lysis of cells and from the fact that megaloblasts have a higher than normal activity of the enzyme. This may reflect an increase in cell cycle time. As thrombopoiesis is affected in a similar manner to erythropoiesis, it is reasonable to suppose that an increase in platelet MAO content may similarly occur through prolonged maturation associated with increased RNA and protein synthesis.

However, the observation of Murphy *et al.*¹⁶ that substantial differences in platelet MAO activity are present between some individuals in all platelet density fractions shows that individual variation in expression of the enzyme as well as differences in platelet subpopulations are important in determining platelet MAO levels. It is possible that the metabolic changes induced by vitamin B₁₂ and folate deficiency, which are most marked in haemopoietic

cells, include a generalised increase in MAO activity throughout the body. Psychiatric symptoms can occur in vitamin B₁₂ and folate deficiency, and in one survey¹⁷ of 101 patients presenting with a depressive illness, 24% were found to have subnormal folate levels. A recent study has linked amine metabolism with folate deficiency¹⁸ by showing that folate-deficient rats have a decrease in brain 5-hydroxytryptamine concentration. A marked increase of MAO activity in the brain might reduce the concentration of noradrenaline or 5-hydroxytryptamine in the synaptic cleft and thus, in accordance with the somewhat simplistic amine hypothesis,¹⁹ contribute to the generation of depressive symptoms.

The mechanisms controlling platelet MAO activity are obviously complex. It is now important to determine whether the changes described here are merely secondary to maturational deficit or truly reflect alterations in central MAO metabolism.

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