Platelet monoamine oxidase activity in Huntington's chorea

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SUMMARY The possibility that genetically determined abnormalities in the monoamine oxidase of certain central nervous system aminergic neurones may play a part in the pathology of Huntington's chorea was investigated using human platelet monoamine oxidase. Significantly elevated monoamine oxidase activity was found in male patients compared to control subjects suggesting this may be a screening test for this disorder. Low monoamine oxidase activity was associated with a better clinical response to drugs.

Huntington's chorea is an autosomal dominant degenerative central nervous disorder producing chorea and dementia. The disorder has been considered the "opposite" motor lesion to Parkinson's disease. The principal neuropathology involves degeneration of the basal ganglia, particularly the caudate nucleus, affecting the small interneurons, Golgi type 2 (Bruyn, 1968).

The neurochemical changes involve findings in three neurotransmitter systems: amine, gamma aminobutyric acid (GABA), and cholinergic.

Attempts to find reproducible abnormalities of amines, particularly dopamine and 5-hydroxytryptamine (5-HT) have failed (Bernheimer and Hornykiewicz, 1973; Chase, 1973; Bird and Iversen, 1974). Evidence from drug effects is more suggestive. Dopamine competitive inhibitors, such as haloperidol, and dopamine depleters, such as reserpine and tetrabenazine, improve choreatic movements temporarily, and this suggests that there is relative overactivity of the dopaminergic system.

Decreased levels of GABA and its biosynthetic enzyme, glutamine acid decarboxylase (GAD) have been reported (Bird *et al.*, 1973; Iversen *et al.*, 1974; Urquhart *et al.*, 1975). Degeneration of the small cholinergic interneurons of the corpus striatum and low levels of choline acetyltransferase have been found in choreatic brains at post-

mortem (Bird and Iversen, 1974; McGeer and McGeer, 1976).

The human platelet provides a ready source of monoamine oxidase in humans. Alterations in the activity of this intraneuronal enzyme can affect free intracellular amine levels and uptake from the intrasynaptic cleft (Hughes, 1972). Platelet monoamine oxidase activity may be altered in diseases affecting the central nervous system, for example, Parkinson's disease (Zeller *et al.*, 1976), migraine (Sicuteri *et al.*, 1972; Glover *et al.*, 1977a) affective disorders (Murphy and Weiss, 1972; Landowski *et al.*, 1975), and schizophrenia (Wyatt and Murphy, 1976). These conditions have amine disorders as a common suggested feature.

No studies of platelet monoamine oxidase activity in Huntington's disease have been reported. Platelet monoamine oxidase is a type B monoamine oxidase (Collins and Sandler, 1971) of which dopamine is a substrate (Glover *et al.*, 1977b). Its activity is under significant genetic control (Nies *et al.*, 1974). Increased dopamine uptake has been found in human platelets in Huntington's chorea (Aminoff *et al.*, 1974; McLean and Nihei, 1977).

As Huntington's chorea is determined genetically and aminergic neurone dysfunction is suggested as part of the pathology, a study of platelet monoamine oxidase activity in this disorder seemed indicated.

Patients and methods

Since 1972, a Huntington's chorea clinic has been

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conducted in the Department of Psychiatry, University of Melbourne. Fifteen consecutive patients attending the clinic, and on medication, were used in this study. The diagnosis was established by the clinical picture, the evolution of the disease under observation, and the family history.

A global assessment was made of the patients' condition by EC at the time of blood sampling. The samples were assayed by JM who was unaware of the clinical assessment. Each patient was sampled on two occasions but some samples were discarded because of contamination of the platelet plug with red cells. On the second run samples, patients were coded differently and the order changed.

A group of normal control subjects was drawn from members of staff who were drug-free, without physical and psychiatric disorders, and had no family history of neurological or psychiatric disorders. These were assayed in the same batches as the patients.

The method of sampling and assay is detailed elsewhere (Mann, 1977). Ten millilitres of blood was withdrawn into a plastic tube containing EDTA and immediately transferred to the laboratory. At 4°C a platelet plug was separated by differential centrifugation, washed in 5 ml of normal saline and suspended in 1 ml of normal saline. Storage at -20°C preserved monoamine oxidase activity for at least three weeks. The radiochemical assay was modified from Jarrott (1974); 50 µl of platelet suspension was incubated

at 30°C with 20 µl of ¹⁴C-benzylamine (2 mmol, 1 µCi/µl) or ¹⁴C-tyramine (2 mmol, 1 µCi/µl) and 30 µl of distilled water. Blanks had 10 µl of 3 M HCl (used to stop the reaction) added just before the substrate. Products were extracted into butylacetate and counted in a Packard Tricarb liquid scintillation spectrometer. Reaction rates were linear with respect to incubation time and enzyme concentration.

All samples were assayed in triplicate with the two substrates. All runs included internal blanks for increased reliability. The coefficient of variance (CV) for a single sample assayed 10 times in one batch was 1.1% and the interbatch CV was 5%.

Protein concentration was estimated according to the method of Lowry *et al.* (1951).

Results

The clinical and biochemical details are shown in Table 1 for the experimental group.

Results for the control group studied are shown in Table 2.

In the control group, females had higher mean platelet monoamine oxidase activity than males ($P < 0.01$). This created the need for same sex contrasts in establishing real differences in monoamine oxidase activity. The normal control group showed no significant correlation between age and platelet monoamine oxidase activity with either substrate ($P > 0.05$).

Table 1 Platelet monoamine oxidase activity in Huntington's chorea: sex, age, illness duration, medication, response, platelet monoamine oxidase activity on two occasions

Patient	Sex	Age (yr)	Illness duration (yr)	Medication daily dosage (mg)	Global response	Platelet monoamine oxidase activity* (nmol/mg/hr)		Clinical change	Platelet monoamine oxidase activity† (nmol/mg/hr)	
						Benzylamine	Tyramine		Benzylamine	Tyramine
1	M	69	12	HPL 3	good	46.8	39.1	stable	46.0	37.2
2	F	51	13	HPL 3	fair	41.7	37.1	stable	—	34.2
3	F	53	5	PZ 8	good	53.5	46.0	improved	21.0	17.8
4	M	34	2	LC 1250	good	38.8	31.0	stable	—	—
5	F	56	8	HPL 10	poor	35.1	28.1	deteriorated	35.3	27.5
6	F	47	17	HPL 3	good	31.8	23.3	stable	—	—
7	F	60	13	HPL 9	fair	34.7	25.6	stable	—	—
8	M	42	10	TB 50	poor	41.0	34.1	improved‡	—	21.2
9	M	36	5	HPL 3	excellent	20.9	18.7	stable	18.3	16.0
10	M	63	11	HPL 3	good	52.5	46.6	deteriorated	53.4	50.8
11	F	39	7	HPL 12	fair	39.9	34.2	deteriorated	37.8	30.6
12	F	69	2	HPL 1.5	good	34.6	28.1	stable	—	29.3
13	F	31	5	LC 1000	good	45.3	39.7	stable	—	—
14	F	68	16	HPL 7.5	good	38.4	33.2	deteriorated	44.4	46.2
15	F	50	4	HPL 1.5	excellent	20.4	17.0	stable	25.7	20.9
mean		51.2	8.7			38.4	32.1			
SD		12.9				9.5	8.9			

*First sampling

†Second sampling one to three months later

‡Haloperidol 6 mg added.

Global response refers to behavioural changes and choreatic movements, not intellectual function.

HPL = haloperidol; PZ = pimozide; LC = lithium carbonate; TB = tetrabenazine.

Table 2 Normal control subjects: numbers, age, and platelet monoamine oxidase activity (means and SD)

Number in group		Age (yr)		Platelet monoamine oxidase activity (nmol/mg/hr)			
		Mean	SD	Benzylamine		Tyramine	
				Mean	SD	Mean	SD
Total	59	28.3	12.5	32.8	9.0	27.9	9.0
Male	31	25.3	7.2	30.5	9.4	25.6	9.1
Female	28	30.0	14.2	35.2	7.9	31.3	8.1

Both whole group and same sex contrasts were made of mean platelet monoamine oxidase activity as measured by the two substrates. A two-tailed *t* test indicated that the experimental group as a whole had a significantly higher mean platelet monoamine oxidase activity than normal controls using benzylamine as a substrate ($P=0.04$). Same sex contrasts showed this was true only for male Huntington's chorea patients. This group had higher monoamine oxidase activity than male controls with benzylamine ($P=0.036$) and tyramine ($P=0.057$).

The two patients (numbers 9 and 15) whose treatment response was assessed as excellent had the lowest monoamine oxidase activity. Both had almost identical activity (nmol/mg/hr), respectively 20.9 and 20.4 for benzylamine and 18.7 and 17.0 for tyramine. No other patient had a monoamine oxidase activity below 30 nmol/mg/hr with benzylamine. Similar results were obtained with these two patients on the second sampling.

At the second sampling, two patients had improved significantly clinically (numbers 3 and 8). Patient 8 had had haloperidol 6 mg daily added to his treatment. Both these patients showed a significant fall in platelet monoamine oxidase activity. Patient 3 had gone from 53.5 to 21.0 (benzylamine) and 46.0 to 17.8 (tyramine), and patient 8 from 34.1 to 21.2 (tyramine). These values were comparable to the patients rated as excellent.

Four patients had deteriorated and there was no consistent significant change in monoamine oxidase activity. A change of greater than 17% was considered significant, as this was the variance in controls over one month.

A paired *t* test applied to the whole Huntington's group failed to show any significant difference between the first and second samples ($P>0.70$ for benzylamine, $P>0.60$ for tyramine).

Discussion

Significantly higher platelet monoamine oxidase activity was found in male Huntington's patients compared with control subjects. Although mean

female platelet monoamine oxidase values were higher than normal female control subjects, they were not significantly different. There are no other reports in the literature of studies of platelet monoamine oxidase activity in this condition with which to contrast these results. Low monoamine oxidase activity has been reported in Parkinson's disease (Zeller *et al.*, 1976).

Markedly lower values on both sampled occasions were found in the two patients assessed to be in the best clinical condition. Two further patients improved between the first and second assessments, and their monoamine oxidase activity fell to levels comparable with those of the first two patients. The coding system, passage of time, and altered order between the two runs makes chance an unlikely explanation of these results.

The biochemical implications of these findings are not clear. The expectation was to find low platelet monoamine oxidase activity and improvement to be associated with a rise in this activity. If low monoamine oxidase activity represented the primary pathology in dopaminergic neurones, it could have led to excess transmitter and activity. The reverse was found. Differences in platelet monoamine oxidase activity could be reflections of the genetic manifestations of the disorder, a systemic effect of an unknown agent, or the medication. Neuroleptic agents have been shown not to alter platelet monoamine oxidase activity (Wyatt and Murphy, 1976). Lithium carbonate has been demonstrated to raise monoamine oxidase activity (Bockar *et al.*, 1974). Exclusion of the two patients on lithium did not alter the significance of the findings at the 0.05 level.

The change in platelet monoamine oxidase activity downwards corresponded with clinical improvement. Perhaps this biochemical change represents a useful therapeutic endpoint of treatment—that is, medication should be adjusted until it is achieved, because it is the point at which maximum response is to be expected. Further longitudinal study of this group of patients may provide support or otherwise for this line of reasoning.

If these differences in platelet monoamine oxidase activity precede clinical onset on this disease, they would be a valuable screening test, particularly in males. Family studies may clarify this possibility.

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