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Low platelet monoamine oxidase activity in headache: no correlation with phenolsulphotransferase, succinate dehydrogenase, platelet preparation method or smoking

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SUMMARY Platelet monoamine oxidase activity in male migrainous and cluster headache patients was significantly lower than in male controls, confirming our previous study. The activity range showed a normal distribution and low mean values could not be attributed to a subgroup with particularly low activity. When Corash's platelet preparation method was used, with its high platelet yield, specific enzyme activities of a similar order were obtained. Thus, the low values encountered were not due to abnormal recovery within the platelet population. Two other enzyme activities, phenolsulphotransferase M and succinate dehydrogenase, were also measured in the same platelet samples. Although low succinate dehydrogenase activity was identified in the headache groups, it appeared to represent a separate phenomenon and there was no significant correlation between activity of either enzyme and that of monoamine oxidase. This shows that the low activity of platelet monoamine oxidase in headache is not related to a generalised platelet enzyme deficit. It was also shown that the low monoamine oxidase activity in the headache patients could not be attributed to smoking.

Platelet monoamine oxidase (MAO) activity in headache sufferers has been measured extensively, and several studies have reported lower mean values in migrainous and cluster headache patients.[1–6] The finding is most apparent in males, and low activity which appears to be a permanent trait[5] is not drug-induced. In this study we sought to establish whether the platelet deficit is specific for this enzyme by measuring phenolsulphotransferase M (PST M) and succinate dehydrogenase (SDH) in the same platelet samples. PST M is a cytoplasmic enzyme which acts on many of the same substrates as MAO, including tyramine and dopamine, and catalyses their conjugation with sulphate.[7] SDH, like MAO, is a membrane-bound mitochondrial enzyme. Because Corash[8] has drawn attention to the fact that most platelet preparation methods only harvest a moderate proportion of the platelet population, we have compared enzyme activities using his method of maximal platelet recovery with our own routine method.

Several recent studies show that there is an increased incidence of cigarette smoking among subjects with low platelet MAO activity.[9–13] We have therefore also monitored the incidence of smoking in groups of male migraine and cluster headache patients and controls and compared it with their platelet MAO activity.

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Patients and methods

Blood samples were collected from male patients presenting at the Princess Margaret Migraine Clinic at Charing Cross Hospital and from unaffected male volunteers from

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the hospital staff as controls. Both groups of samples were processed in parallel. The criteria of Vahlquist[14] were used to diagnose common and classical migraine and a diagnosis of cluster headache was made using conventional criteria.[14 15] Patients in whom an unequivocal diagnosis could not be made were excluded from the study. All patients were free from headache at the time blood was drawn. Patients were asked whether they smoked and, if so, how many cigarettes per day or how much tobacco they consumed. Tobacco consumption was assessed in terms of an equivalent number of cigarettes. Although there was a considerable overlap between the patients and controls used for this part of the study and the rest, some new ones were included, and some for whom it was not possible retrospectively to establish smoking behaviour were excluded.

Venous blood (10 ml) was collected into 5% EDTA, 0·5 ml, in Universal containers. To harvest the platelet population, the sample was treated as described previously,[5] with the exception that platelets were stored in 1 ml of 0·3 M sucrose rather than 0·15 M NaCl.

A further group of platelet samples was obtained from patients whose platelet MAO activity level was already known. Venous blood, anticoagulated with EDTA, was drawn from each of two preparation techniques, 10 ml as described earlier[5] and 10 ml for the method of Corash,[8] with the exception that 0·5 ml of 5% EDTA solution was used instead of 0·1 ml of 10% EDTA. To assess recovery, platelet counts were made in whole blood and from the red cell layer following separation of the platelet rich plasma preparation.

MAO activity was assayed blind and in duplicate as described previously.[16]

Protein was measured by the method of Lowry *et al.*[17]

Platelet PST activity was estimated by a modification of the radio-enzymatic method of Foldes and Meek[18] using radioactive (^{35}S)-3′-phospho-adenosine-5-phosphosulphate (PAPS) as sulphate donor. The incubation mixture consisted of 100 μl potassium phosphate buffer (10 mM, pH 7·4), 10 μl of platelet suspension and 20 μl tyramine solution (1 mM) giving a final tyramine concentration of 133 μM in the incubation mixture. Water (20 μl) replaced tyramine in the blanks. All platelet suspensions were assayed, in duplicate, using a blank for each patient sample. The reaction was initiated by adding 20 μl of 4·9 μM solution of ^{35}S-PAPS and non-radioactive PAPS in water (final concentration in incubation mixture 0·66 μM) to successive tubes at 10 second intervals. After a 10 minute incubation at 37°C the reaction was stopped at 10 second intervals by adding barium acetate (200 μl, 0·1 M) and transferring from the water bath to ice. Unreacted PAPS was removed by two successive precipitations with barium hydroxide (200 μl, 0·1 M), the second being carried out on the supernatant obtained from centrifuging the first precipitate. Following centrifugation, the final supernatant was removed to a counting vial insert and mixed with 2·5 ml Instagel to form a gel. Radioactivity in the reaction product was measured in a liquid scintillation counter. Results were expressed as nmol product formed/mg protein/10 min incubation.

Succinate dehydrogenase was assayed using a modification of the method of Pennington.[19] The incubation mixture consisted of 100 μl water, 25 μl sucrose (0·5 M), 12·5 μl sodium phosphate buffer (1·0 M, pH 7·4), 25 μl enzyme and 25 μl sodium succinate (0·5 M). Sodium succinate solution was replaced by 25 μl water in the blanks. To initiate the reaction 62·5 μl of 0·4% solution of p-iodonitrotetrazolium violet (2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyltetrazolium chloride) was added to successive tubes at 10 s intervals. After a 20 min incubation at 37°C, the reaction was stopped at 10 s intervals by adding trichloroacetic acid (250 μl of 10% solution) and transferring from the water bath to a bed of ice. The formazan salt formed in the reaction, which has a rose pink colour, was then extracted by adding 1 ml ethyl acetate to each tube and vortex mixing for 20 s. This was followed by centrifugation at 1400 *g* for 5 min to sediment the precipated protein. The supernatant was aspirated into clean tubes and the depth of the rose pink colour read at 490 nm in a spectrophotometer and water as blank was used to set the zero. All assays were carried out in duplicate.

Student's two-tailed *t* test was used to assess significance unless otherwise stated.

Results

Male migrainous and cluster headache groups both had significantly lower MAO activity than male controls ($p < 0·01$ for each) (fig).

Table 1 shows mean activities for PST M and SDH in control subjects in migrainous and cluster headache groups, and in patients grouped according to low MAO activity. PST M activity was similar in all groups. Mean SDH activity was significantly reduced below control values in both patient groups but levels were no lower in the low MAO groups. The lack of positive correlation of either PST M or SDH with MAO activity is shown more clearly in table 2: the only significant correlation is a *negative* one between MAO and PST M in the low MAO

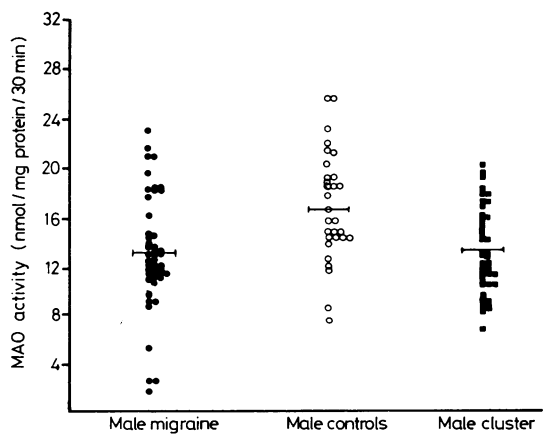

Fig *Platelet MAO activity levels in headache patients and controls (nmoles tyramine oxidised/mg protein/30 min).*

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Table 1 *Platelet enzyme activity levels in male headache patients and male control subjects*

	n	MAO Mean	± SD§	n	PST M Mean	± SD‖	n	SDH Mean	±SD¶
Mean controls	30	16·9	4·5	23	0·331	0·125	11	0·039	0·014
Mean migraine	48	13·1	4·7‡	45	0·370	0·098	19	0·027*	0·009
Mean cluster	41	13·5	3·7†	32	0·360	0·100	11	0·028*	0·003
Low MAO group more than 1 SD below control	22	9·3‡	3·04	22	0·322	0·087	19	0·027*	0·009
Mean lowest MAO 2 SD below male control mean	4	3·1‡	1·6	4	0·390	0·087	4	0·031	0·020

*p < 0·02 less than controls
†p < 0·01 less than controls
‡p < 0·001 less than controls
§nmoles tyramine oxidised/mg protein/30 min
‖nmoles tyramine conjugated/mg protein/10 min
¶arbitrary units (see Methods)

groups (more than 1 SD below male control, p < 0·02).

The use of the Corash method of platelet preparation (table 3) did yield significantly more platelets than our routine method, 89% rather than 62%, but this made virtually no difference to any of the final specific activities observed for either MAO or PST M.

The incidence of smoking was considerably higher in the cluster headache group (95%) compared with the migrainous group (26·1%) or the controls (23%) (both p < 0·001, χ^2 test).

Table 4 shows mean platelet MAO activity values in the different groups classified according to their smoking behaviour. Both migraine and cluster headache groups had significantly low mean platelet MAO activity compared with controls (both p < 0·001). Migrainous non-smokers also had a significantly lower activity than the control group and very similar but slightly higher activity values than migrainous smokers. There were only two cluster headache non-smokers but they had even lower activity values than the cluster headache smokers. In both migraine and cluster headache groups, those subjects smoking less than 20 cigarettes a day had lower activity values than those smoking more but the differences were slight and not significant. Only seven controls were smokers and they, in fact, had slightly higher platelet activity than the control group as a whole. Migraine smokers and cluster headache smokers had significantly lower platelet activity than these control group smokers.

Five of the subjects tested gave up smoking while under scrutiny and it was possible to re-assay their platelets after at least 3 months of abstinence. The results are shown in table 5. In each case, activity remained remarkably constant.

Table 2 *Correlation coefficients of MAO with SDH and PST in male headache patients and controls*

	MAO/SDH n	Correlation coefficient	MAO/PST n	Correlation coefficient
All patients and controls	41	0·099	100	0·124
Controls	11	−0·083	23	0·082
All headache	30	−0·248	77	0·186
Migraine	19	−0·248	45	0·145
Cluster	11	−0·273	32	0·271
Low MAO more than 1 SD below controls	19	−0·248	22	−0·495*

*p < 0·02

Discussion

This study confirms earlier reports of an increased incidence of low platelet MAO activity in male migrainous and cluster headache patients, using larger patient and control groups than before. Once again, a small number of patients with particularly low activity was observed (fig). In order to establish whether this subgroup was responsible for the low activity of the group as a whole, we compared mean activity of this population minus the four patients with values 2 SD less than control mean with the controls; mean activity of the remaining patients was still significantly lower than the controls (p < 0·01). In addition, the range of activity values in the whole migrainous group (n = 48) showed a normal distribution. Consecutive elimination of the four lowest activities produced increasing skewness which was significantly different from a normal distribution at n = 44. The cluster headache group also showed a normal distribution of MAO activity. It thus seems likely that the low platelet MAO activity in headache patients is not due to the presence of a high aberrant subgroup, but rather reflects some more minor abnormality of the group as a whole. Studies in which ^{3}H-pargyline was used to titrate the

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Table 3 *Specific activities of MAO and PST M in platelets prepared by standard method and Corash method*

Patient	*Platelet recovery*	*MAO†*	*PST M‡*	*Platelet recovery*	*MAO†*	*PST M*
1	64·0	1·7	0·262	93·0	2·4	0·371
2	58·0	6·4	0·346	94·0	6·6	0·338
3	57·0	11·1	0·342	90·5	12·8	0·379
4	65·8	12·1	0·227	83·2	10·9	0·263
5	65·4	23·7	0·347	89·6	22·3	0·365
6	65·8	18·2	0·605	93·5	18·9	0·459
7	68·5	20·1	0·392	92·6	21·7	0·386
8	47·3	19·8	0·506	75·2	19·5	0·448
9	62·0	19·6	0·297	87·5	17·1	0·249
10	69·8	19·3	0·510	87·6	15·7	0·424
Mean	62·4	15·2	0·387	88·7*	14·9	0·365
± SE	2·23	2·35	0·13	1·94	2·2	0·12

*different from platelet recovery with the standard method, $p < 0.001$
†nmoles tyramine oxidised/mg protein/30 min
‡nmoles tyramine conjugated/mg protein/10 min

number of MAO molecules present in platelets from male migrainous and cluster headache patients showed that low activity is, in fact, due to a reduced number of molecules present, all with normal turnover number.[16] This is also evidence mitigating against the concept of an abnormal structural gene causing an abnormal enzyme in some patients. It is possible that some factor is present, to a variable degree, in all patients which inhibits the production of MAO molecules.

One obvious possibility to be considered is that low platelet MAO in headache patients reflects a generalised platelet abnormality.[20] No changes in mean platelet size can be observed in headache patients.[21] However, certain platelet subgroups have different biochemical properties, including specific MAO activities and platelets from migrainous patients show abnormal aggregation properties.[20 22 23] It seemed possible that we were harvesting a different platelet population in our headache patients from those in controls. However, using the Corash[8] method, which yielded 89% of the platelet population compared with our standard method with a recovery of only 63%, the final specific activities were remarkably similar, even in headache patients with unusually low MAO activity (table 3).

In the numerous studies of platelet MAO activity in a variety of clinical conditions, there have been few investigations of other enzymes in the same platelets. Murphy *et al*[24] examined SDH in a study of schizophrenics with low platelet MAO activity and failed to find any concomitant reduction. Such a deficit might have been predicted in the presence of a generalised platelet abnormality. We have shown here, however, that there is no evidence for any generalised enzyme deficiency in headache patients with the lowest MAO activity. Neither PST M nor SDH activities were unusually low in these platelets. Indeed, the only significant correlation observed was a negative one between PST M and MAO in the low MAO group, suggesting the possibility of a control mechanism where a deficiency of one enzyme is compensated for by an increased production of the other.

Table 4 *Platelet MAO activity values and smoking in male migraine and cluster headache patients*

Group	*n*	*Mean platelet MAO activity‖ ±SD*
All controls	30	16·6 ± 4·5
Smoking controls	7	18·4 ± 5·7
All migraine	53	13·7 ± 5·2*
Migraine nonsmokers	39	13·9 ± 5·6‡
Migraine smokers	14	13·4 ± 4·1§‡
Migraine, > 20 cigarettes/day	3	12·7 ± 1·7
< 20 cigarettes/day	11	13·5 ± 4·6
< 5 cigarettes/day	5	13·8 ± 2·5
All cluster headache	36	13·0 ± 3·8*
Cluster headache nonsmokers	2	12·8 ± 0·1
Cluster headache smokers		
> 20 cigarettes/day	19	12·8 ± 4·0*§
< 20 cigarettes/day	15	13·3 ± 4·0†§

*$p < 0.01$ less than all controls
†$p < 0.02$ less than all controls
‡$p < 0.05$ less than all controls
§$p < 0.05$ less than smoking controls
‖nanomoles tyramine oxidised/30 min/mg protein

Table 5 *Platelet MAO activity (nanomoles tyramine oxidised/30 min/mg protein) in the same individual while smoking and after 3 months' abstinence*

Smoking rate (number of cigarettes/day)	*1st (smoking)*	*2nd (non-smoking)*
1. 10–20	22·6	19·6
2. 15–20	6·4	6·3
3. 15–20	10·5	9·9
4. 15–20	18·7	18·0
5. > 20	22·6	19·6
Mean ± SE	15·2 ± 3·3	14·1 ± 2·9

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Although there was no link with MAO, pointing to the absence of a generalised mitochondrial outer membrane deficit, both the migraine and cluster headache groups had significantly low SDH activity (table 1). It would be of interest to confirm this finding in a larger population.

This study also shows that low platelet MAO activity in migrainous patients cannot be attributed to their smoking because low values were also observed in those who did not indulge in this habit. What is more, both cluster headache and migrainous smokers had significantly lower platelet activity than smoking control subjects. Thus, the association between low activity and such headache syndromes can be independent of and stronger than that with smoking.

The high incidence of smoking in cluster headache patients confirms previous reports. These patients also drink more than average[25] and may fit the extravert[25] smoking,[9 11 12] drinking,[27] sensation seeking[28 29] personality pattern, alleged to be associated with low MAO activity. Whether drinking and smoking actually initiate headache attacks in migrainous subjects with low MAO activity so that a proportion thereby abstain and, indeed, whether giving up smoking will induce lasting remission in patients with cluster headache are questions that require further investigation.

The control subjects in this study who smoked did not show activity any lower than non-smoking controls but their numbers were small. Cumulative evidence from other studies[9 11 12] points to a strong association between low MAO activity and smoking but they provide no evidence as to whether or not smoking actually causes a reduction in platelet activity. The evidence from our five subjects who have given up smoking, strongly suggests that it does not. If low mean platelet MAO activity is a characteristic of smokers in general, it seems more likely to reflect a predisposition to smoking than a consequence.

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