Monoamine Oxidase Activity in Diabetes

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SUMMARY

Monoamine oxidase (MAO) activities were measured in platelets from insulin-dependent and non-insulin-dependent diabetic subjects and in platelets from nondiabetic controls. Circulating levels of glycosylated hemoglobin (HbA_1) were determined simultaneously. Mean MAO activities were not significantly different in any of these groups. MAO activity did not relate to the age of the individual, but mean values for females were higher than mean values for males in healthy controls and in insulin-dependent diabetics. In this study mean HbA_1 levels were higher in female than in male diabetics. There was no relationship between MAO activity and HbA_1 level when results for males and females were analyzed separately. DIABETES 32:130–133, February 1983.

The mitochondria of blood platelets contain monoamine oxidase (MAO, amine reductase [deaminating] [containing flavin] E.C. 1.4.3.4), the activity of which appears to differ in a variety of human disease states.[1,2] A state in which MAO activity is reported to be low is insulin-dependent diabetes.[2] The purpose of the present investigation was to compare MAO activities in platelets from insulin-dependent and non-insulin-dependent diabetic subjects with those in platelets from nondiabetic subjects, and to determine whether a relation exists between MAO activity and the degree of diabetic control. The latter was judged by the measurement of circulating HbA_1.

MATERIALS AND METHODS

Patients and volunteers. A group of diabetics being treated with insulin was selected at random from a medical outpatient clinic. A group of diabetics receiving dietary advice and oral hypoglycemic drugs was similarly selected. Nondiabetic subjects from hospital staff served as an age- and sex-matched control group. Only the group of non-insulin-dependent diabetics was studied in the fasting state. Some details of the patients and volunteers are given in Table 1.

Preparation of blood samples. Blood was taken from each subject by clean venipuncture and aliquots were dispensed into tubes that contained an anticoagulant. One aliquot (9 ml) was collected in a tube that contained 3.8% (wt/vol) trisodium citrate dihydrate (1 ml) and another aliquot (4 ml) was collected into a tube that contained EDTA. In the case of the maturity-onset diabetics, another blood sample (3 ml) was collected in a tube that contained fluoride oxalate.

Preparation of platelet-rich plasma (PRP). The blood that had been collected into citrate was centrifuged at 300 × *g* for 10 min, and the upper turbid layer of PRP was removed. Platelet-poor plasma (PPP) was then prepared by centrifuging the residual blood at 2000 × *g* for 10 min. The number of platelets in the sample of PRP was determined using a Coulter counter; the count was adjusted to 3×10^8 platelets/ml by diluting the PRP with PPP, and the samples were then recounted and stored at −20°C.

Platelet MAO activities. MAO activities were measured in the samples of PRP within 2 wk of their preparation, and the assays were undertaken in triplicate. This was achieved using a radiochemical method in which an aliquot of the PRP (300 μl) was incubated with β-phenyl[1-^{14}C]ethylamine (final concentration, 3 μM; specific activity, 5 mCi/mmol) in 10 mM sodium pyrophosphate buffer, pH 7.2, in a final assay volume of 500 μl at 30°C for 15 min.[3] The product was then extracted into 1% wt/vol 2,5-diphenyloxazole in 1:1 (vol/vol) ethylacetate:toluene and quantitated using a scintillation counter. It was established that the reaction rate was unaffected by either freezing and thawing the PRP or by storage for up to at least 2 wk. The activity was not enhanced by subjecting the PRP to sonication. MAO activity was always expressed as nmol substrate oxidized per hour per 10^9 platelets. When samples of PPP were treated in a similar manner no MAO activity was detected.

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TABLE 1
Data of diabetics and controls

Group	Number in group	Age (± SEM)	Age range
Insulin-dependent diabetics	26	21.3 ± 1.1	14–41
Males	9	21.7 ± 1.9	14–34
Females	17	21.1 ± 1.4	15–41
Controls	24	23.5 ± 1.3	17–46
Males	9	22.1 ± 1.8	17–34
Females	15	24.3 ± 1.7	17–46
Non-insulin-dependent diabetics	18	56.5 ± 1.6	46–70
Males	10	57.6 ± 2.2	47–70
Females	8	55.2 ± 2.4	46–62
Controls	18	55.8 ± 1.6	42–69
Males	10	57.6 ± 2.1	46–69
Females	8	53.5 ± 2.4	42–64

Glycosylated hemoglobin levels. The blood that had been collected into EDTA was assayed for HbA_1 using the kit supplied by Bio-Rad (Richmond, California). The results were always expressed as a percentage of the total hemoglobin.

Blood glucose concentrations. The blood that had been collected into fluoride oxalate was analyzed for glucose using a Technicon Autoanalyzer II (Technicon Corp., Tarrytown, New York) and a modification of Trinder's method.[4]

Statistical analyses. Both Student's *t* test (paired and unpaired) and regression analysis were used. Results were considered to be significantly different if $P < 0.05$.

RESULTS

Platelet MAO activities. The Km of platelet MAO for phenylethylamine was identical (4.0 μM) in healthy controls and in diabetics, and, above 3.5 μM phenylethylamine, the enzyme in all groups was subject to substrate inhibition (results not shown). The enzyme was therefore assayed using 3.0 μM phenylethylamine and, with this concentration of substrate, the assay was linear for at least 15 min.

The mean values for the groups of insulin-dependent and non-insulin-dependent diabetics (Table 2) were not significantly different from those for the groups of matched controls, whether results for males and females were analyzed separately or together. There was no relationship between MAO activity and the age of the individuals in any of the groups. When all the results were analyzed together, values for females were higher than values for males and the difference was highly significant ($P < 0.001$). Indeed, females had significantly higher MAO values than males in the insulin-dependent diabetics ($P < 0.02$), in their control group ($P < 0.01$), and in the control group for the non-insulin-dependent diabetics ($P < 0.05$). The difference between males and females was not statistically significant in the non-insulin-dependent diabetic group ($P < 0.10$), but the trend was the same.

Glycosylated hemoglobin levels. HbA_1 was high in both groups of diabetics (Table 2). In this study it was higher in diabetic females than in diabetic males and this led to a spurious correlation between HbA_1 and MAO activity (Figure 1). There was no correlation between these two parameters when results for males and females were analyzed separately.

Blood glucose concentrations. In the group in which the fasting glucose concentration was measured, its level correlated significantly ($P < 0.01$) with the level of HbA_1. In ad-

TABLE 2
Platelet MAO activities, glycosylated hemoglobin levels, and blood glucose concentrations of diabetics and controls

Group	MAO activity (nmol/h^{-1} 10^{-9} platelets ± SEM)	HbA_1 (% ± SEM)	Glucose (mM ± SEM)
Insulin-dependent diabetics	27.9 ± 1.5	11.6 ± 0.6 (N = 25)*	—
Males	23.7 ± 1.6	9.9 ± 0.5	—
Females	30.1 ± 2.0	12.6 ± 0.9 (N = 16)*	—
Controls	29.8 ± 1.7	6.6 ± 0.3 (N = 14)*	—
Males	24.6 ± 1.0	6.0 ± 0.2 (N = 8)*	—
Females	32.9 ± 2.2	7.5 ± 0.3 (N = 6)*	—
Non-insulin-dependent diabetics	29.1 ± 2.3	11.6 ± 0.8	13.3 ± 1.2
Males	25.2 ± 2.8	9.6 ± 0.6 (N = 8)*	10.4 ± 3.3
Females	33.9 ± 3.1	13.6 ± 1.0	17.0 ± 1.4
Controls	27.7 ± 1.8	—	—
Males	24.4 ± 2.3	—	—
Females	31.7 ± 2.2	—	—

*Figures in parentheses = number of subjects for whom data obtained if different from number in Table 1.

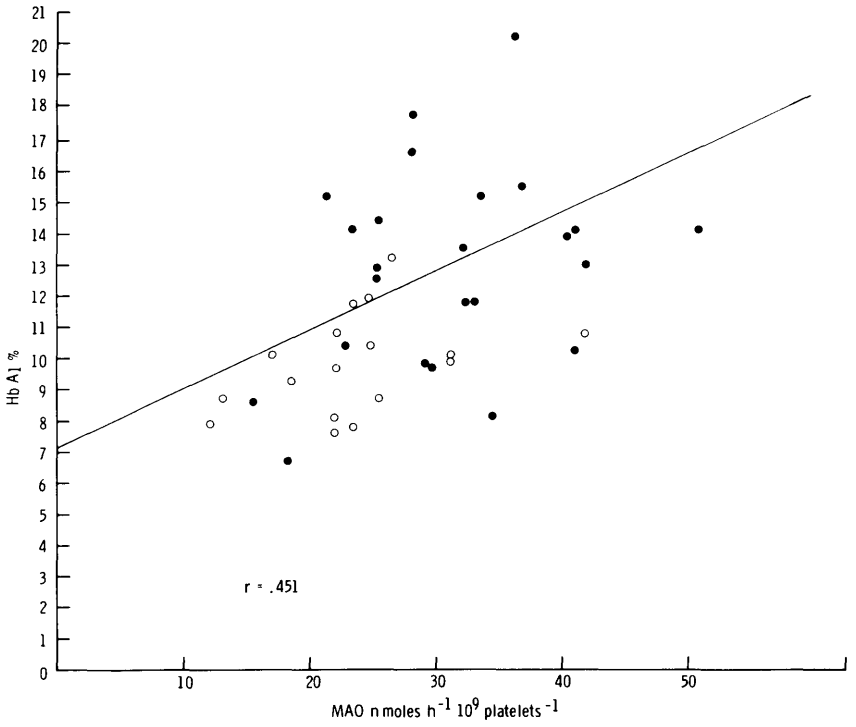

FIGURE 1. Correlation between MAO activity and HbA_1 levels. ○ = **males;** ● = **females.**

dition, the values for this group (10.4–13.3 mM, Table 2) were much higher than the normal range in this laboratory for a fasting group of individuals (3.0–5.5 mM).

DISCUSSION

Unlike Mosnaim et al.,[2] we did not find that platelet MAO activity in insulin-dependent diabetics is significantly lower than in nondiabetic subjects. However, in agreement with these authors, we could not detect a difference in MAO activity in non-insulin-dependent diabetics. We have confirmed that MAO activity in females is significantly higher than in males;[5,6] like Bond and Cundall[7] and Murphy et al.,[6] we did not observe a relation between MAO activity and the age of the individuals who took part in this study. Robinson et al.,[5] on the other hand, found an increase in MAO activity above the age of 65, but not below this age. In our study only two subjects were over 65; hence, an increase with age would not be expected. A relationship between MAO activity and HbA_1 levels was observed in the diabetics, but only because HbA_1 levels, like MAO activity, were higher in females than in males. When results for males and females were analyzed separately, no relationship between these parameters was evident.

The study in which low MAO activity was observed in insulin-dependent diabetics[2] differed from our own in three major respects. First, we measured MAO activity in PRP rather than in platelets that had been separated from plasma by centrifugation, washed, and then resuspended and homogenized. The use of PRP rather than washed platelets has been advocated by Murphy et al.,[6] who obtained results that were similar using both preparations. Second, we used β-phenylethylamine as substrate rather than tyramine and derivatives of benzylamine. Yu and Boulton[8] have shown that plasma can amplify MAO activity when substrates such as benzylamine are used for the assay, but that plasma has little effect when β-phenylethylamine is used as substrate. It is also more appropriate to use β-phenylethylamine rather than benzylamine to measure MAO activity in PRP because it is not a substrate for plasma benzylamine oxidase.[9] Third, we analyzed a greater number of insulin-dependent diabetics and the variation in values for MAO activity was small, and similar to that in controls.

The low MAO activity observed by Mosnaim et al.[2] in platelets from insulin-dependent diabetics might have resulted from differential loss of MAO from platelets during the centrifugation and washing procedure. We think it unlikely that use of different substrates in the two studies could itself account for the different results that were obtained, and we are satisfied that our assay gives a reliable estimate of MAO activity in the platelets of both diabetics and healthy controls.

Clearly, more experiments need to be carried out before a role, if any, can be attributed to platelet MAO in insulin-dependent diabetics.

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REFERENCES

[1] Sandler, M., Reveley, M. A., and Glover, V.: Human platelet monoamine oxidase activity in health and disease: a review. J. Clin. Pathol. *34*:292–302, 1981.

[2] Mosnaim, A. D., Wolf, M. E., Huprikar, S., Singh, S. P., and Zeller, E. A.: Reduced monoamine oxidase activity in blood platelets from insulin-dependent diabetic subjects. *Diabetes 28*:455–56, 1979.

[3] Russell, S. M., Davey, J., and Mayer, R. J.: The vectorial orientation of human monoamine oxidase in the mitochondrial outer membrane. Biochem. J. *181*:7–14, 1979.

[4] Varley, H., Gowenlock, A. H., and Bell, M.: Practical Clinical Biochemistry. Vol. I. London, Heinemann, 1980 (5th Edition), p. 405.

[5] Robinson, D. S., Davis, J. M., Nies, A., Ravaris, C. L., and Sylwester, D.: Relation of sex and aging to monoamine oxidase activity of human brain, plasma and platelets. Arch. Gen. Psychiatry *24*:536–39, 1971.

[6] Murphy, D. L., Wright, C., Bucksbaum, M., Nicols, A., Costa, J. D., and Wyatt, R. J.: Platelet and plasma amine oxidase activity in 680 normals: sex and age differences and stability over time. Biochem. Med. *16*:254–65, 1976.

[7] Bond, P. A., and Cundall, R. L.: Properties of monoamine oxidase (MAO) in human blood platelets, plasma, lymphocytes and granulocytes. Clin. Chim. Acta *80*:317–26, 1977.

[8] Yu, P. H., and Boulton, A. A.: Activation of platelet monoamine oxidase by plasma in the human. Life Sci. *25*:31–36, 1979.

[9] Lewinsohn, R., Glover, V., and Sandler, M.: Development of benzylamine oxidase and monoamine oxidase A and B in man. Biochem. Pharmacol. *29*:1221–30, 1980.