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PLATELET MONOAMINE OXIDASE ACTIVITY IN DIABETICS*

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SUMMARY

Platelet monoamine oxidase (MAO) activity was determined, using benzylamine as substract. Results are expressed as nM benzaldehyde/mg protein/hour, at 37°. A total of 79 diabetics (40 M and 39 F) ranging in age from 3 to 70 years was studied. A normal control group included 39 persons (17 M and 22 F) ranging in age from 16 to 60 years. Platelet MAO activity shows a slight variation in relation to age (not significant). But, in each age group, diabetics have an increased activity in comparison with normals. MAO activity of the plasma shows no significant difference in relation to age or sex, but the values for diabetics are approximately twice the normal. The activity of MAO in platelets shows a significant increase in activity in diabetics of either sex, in comparison to normal individuals. Besides, women show a significantly increased activity. No relationship was found between platelet MAO activity of young vs old diabetics, juvenile onset; insulin vs non insulin treated, duration of the disease, or presence vs absence of retinopathy.

Monoamine oxidase (monoamine: O_2 oxireductase (MAO) E.C. 1.4.3.4) has, as a main function, the oxidation of amines, according to the following reaction:[1]

$$R\ CH_2\ NH_2 + H_2O + O_2 \rightarrow R\ CHO + H_2O_2 + NH_2$$

It has been thought for a long time that this enzyme existed only in tissues innervated by the sympathetic system, but today it is known to exist in several tissues, and in almost all types of cells.[2]

Fundamentally there are two types of MAO: the lipossoluble enzyme of the membrane and the hydrossoluble enzyme of connective tissue and plasma.[3] MAO connected to the membrane exists in the mitochondria and microsomes and possesses FAD.[4] The mitochondrial enzyme has been the most widely studied and, in the particular case of Man, platelet MAO, since it is the only easily accessible.

Mitochondrial MAO is localized in the external membrane of the mitochondria, it is transvectorial, that is crosses the external membrane from its external to its internal face,[5] and is bound to phospholipids, mainly to cardiolipin.[6]

According to the response to inhibitors, it is divided in type A and type B. Enzyme A is inhibited by Clorgiline and enzyme B by Deprenil.[3] It seems that the difference between both types of enzyme results from their content in phospholipids.[6,7]

Platelet MAO is mostly of type B: it deaminates dopamine, tiramine, phenylethylamine, but has maximal activity towards benzylamine (20 times superior to dopamine).[8]

Platelet MAO has been studied in schizophrenia. Most of the Authors find low values for the enzyme activity in this situation,[9,10,11] but there are discordant opinions.[12]

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It is known that the platelet enzyme is under genetic control and that its low activity is considered as susceptibility to schizophrenia.[13,14]

Plasma MAO is devoid of FAD, and has pyridoxal phosphate instead.[15] It also has copper and its plasma activity is proportional to plasma copper concentration.[16]

Its best substract is also benzylamine, and is denominated benzylamine oxidase by some Authors.[17] Although it possesses the same activity towards substrats of the mitochondrial type B, it does not respond to its inhibitors,[18] but to KCN and carbonil reagents.[15] Isoniazid is one of its main inhibitors.[15]

Plasma MAO activity is increased in several pathological situations, such as diabetes,[19,20] heart failure, thyrotoxicosis, and in hepatic pathology accompanied by fibrosis.[21,22] A decrease in activity was described in tumors and in burns.[23]

In normal individuals no correlation was found between plasma and platelet MAO, using benzylamine as a substrate for both,[24] but Yu found a potentiation of platelet MAO activity by plasma, and suggested the presence of activators.[25]

The administration of oral glucose to volunteers decreases significantly platelet MAO activity with different substrates at 3 h, with normalization at 24 h, but incubation of platelets with glucose has no effect on MAO activity.[26] Also insulin and glucagon do not affect MAO activity.[26]

Mosnaim et al studied platelet MAO activity in a group of juvenile diabetics, 5 men and 4 women, and found a decreased activity in comparison with individuals of the same age and sex.[27]

In a previous work we studied plasma MAO activity in diabetics, confirming the results of other Authors, and demonstrating a positive correlation between plasma MAO and glycemia, in patients whose diabetes had more than 10 years of duration.[20]

In the present report we describe the study of plasma and platelet MAO activity in a group of diabetics of both sexes and various ages.

MATERIAL AND METHODS

The patients were attending the Diabetes Clinic of Hospital de Santa Maria (M = 40, F = 39). Their ages varied between 3 and 70 years. As normal controls a group of 17 males and 22 females was used. They ranged in age between 16 and 60 years.

Patients were divided according to the type of diabetes (adult onset or juvenile onset) to the time of duration (less than two years or more than 10 years of disease) to the type of treatment (with or without insulin) to age (less than 30 years, more than 30 years old) and to the presence of retinopathy (with or without retinopathy).

Glycemia was determined by the glucose oxidase method, using a kit from *Sygma*. Plasma MAO was determined by the method of McEwen and Cohen (1963),[28] based on the enzymatic oxidation of benzylamine, with formation of benzaldehyde, followed by extraction with ciclohexane. The absorbancy of the extract was measured against ciclohexane at 242 nm.

The results are expressed in McEwen units.

For platelet MAO activity determination, platelets were isolated by a modification of the technique of Bond and Candall:[30] 9 ml of blood was drawn with a plastic syringe into a tube of propylpoliethylene siliconated, containing 1 ml cytrate at 3,8%. The tube was inverted carefully and centrifuged at 4°C, at 200 g/min. Plasma rich in platelets was removed and centrifugation at 3000 g at 4°C for 10 min.

Plasma was utilized for determination of glycemia and MAO. Sedimented platelets were washed and centrifuged twice in ice cold saline. Saline was decanted and 1 ml, sacharose 0.3 m was added to the platelets. They were shaken and centrifuged at 3000 g for 10 min. Sacharose was decanted and again 1 ml sacharose was pipetted, followed by freezing at —20°C. A few hours later the solution was defrosted and shaken vigorously in a vortex. It was frozen again until used, usually next day. There was no loss of activity for two weeks.

Platelet MAO activity was determined by the technique of Tabor and Rosenthal[39] (oxidation of benzylamine, followed by extraction with ciclohexane and absorbancy determination at 242 nm)[28] using 0.3 ml platelets and an assay in duplicate and a blank, benzylamine is added after deproteinization with perchloric acid. The results are expressed in nanomoles of benzaldehyde/g protein/hour.

Proteins were determined by the method of Lowry.[31]

RESULTS

Table 1 shows the data obtained for blood glucose and for plasma MAO activity. There is no difference between sexes, but in each sex the activity of MAO in diabetics is almost double of the normal plasma activity.

Table 1

Glycemia

GLYCEMIA	MEN			WOMEN		
	X	S	N	X	S	N
Diabetics	219,3	101,07	40	225,5	101,38	39
Normals	70,18	12,29	14	75,71	16,69	21
Diabetics/Normal	t = 5,403	n = 52	p<0,001	t = 6,609	n = 58	p<0,001

MAO plasma

PLASMA MAO	MEN			WOMEN		
	X	S	N	X	S	N
Diabetics	11,7	15,18	40	13,0	12,08	39
Normals	23,2	6,28	17	25,9	7,13	22
Diabetics/Normal	t = 5,138	n = 55	p<0,001	t = 5,969	n = 59	p<0,001

Above: glycemias at the moment blood was drawn for study; below: plasma MAO activity in normals and diabetics. Notice that there is no difference between sexes, but diabetics have much higher values in either group.

Table 2 shows the results obtained for platelet MAO activity. A significant difference is found between sexes, both in normals and in diabetics.

Table 2

Platelet MAO in diabetics and normal individuals by sexes

GROUPS	MEN			WOMEN		
	X	S	N	X	S	N
Diabetics	108,2	61,54	36	162,4	99,11	37
Normals	57,4	25,19	14	100,6	32,29	18
Normal/Diabetics	t = 2,930	n = 48	p<0,01	t = 2,532	n = 53	p<0,02
Normal (M/F)	t = 3,978	n = 30	p<0,001			
Diabetics (M/F)	t = 2,759	n = 71	p<0,01			

Platelet MAO activity in normals and in diabetics, according to sex. Notice that platelet MAO activity is almost double in females, in groups.

Besides, in each sex, MAO activity is significantly elevated in diabetic patients.

In Table 3 diabetics are separated according to age, type of onset of disease, treatment, duration of disease, and presence or absence of retinopathy. In no case a significant difference is found.

Table 3

Platelet MAO according to age and sex

GROUPS		MEN			WOMEN		
		X	S	N	X	S	N
Diabetics	< 30 Years	117,5	76,21	11	146,2	95,30	6
	> 30 Years	99,3	55,07	25	168,5	101,44	30
Diabetics < 30 · > 30 Years		t = 0,785	n = 34	p: ns	t = 0,482	n = 34	p: ns
Normal	< 30 Years	59,5	21,24	7	95,9	36,45	6
	> 30 Years	55,3	30,76	7	98,5	41,12	12
Normal < 30 > 30 Years		t = 0,275	n = 12	p: ns	t = 0,124	n = 16	p: ns

Platelet MAO according to types of diabetes

GROUPS	MEN			WOMEN		
	X	S	N	X	S	N
Adult	105,74	57,12	24	166,46	105,26	23
Juvenile	106,9	72,74	13	146,25	76,89	14
Adult/Juvenile	t = 0,052	n = 35	p: ns	t = 0,540	n = 45	p: ns
With Insulin	119,8	68,43	20	161,8	92,26	28
Without Insulin	95,51	50,20	16	156,2	105,1	20
With Ins./Without Ins.	t = 1,154	n = 34	p: ns	t = 0,191	n = 46	p: ns
Duration < 2 Years	113,9	69,12	16	141,6	58,91	17
Duration > 10 Years	113,9	77,49	23	165,2	112,12	28
< 2 Years/> 10 Years	t = 0,008	n = 37	p: ns	t = 0,762	n = 43	p: ns
With Retinopathy	115,8	63,56	22	181,4	105,16	16
Without Retinopathy	94,7	55,76	18	136,2	68,08	28
With Retinopathy/*	t = 1,075	n = 38	p: ns	t = 1,688	n = 42	p: ns

* Without Retinopathy

Platelet MAO activity according to age (above) to type of diabetes, tratment with or without insulin, duration of disease and to the presence or absence of retinopathy. No significant diference was found in any case.

Figure 1 shows the distribution of platelet MAO activity according to age, showing a tendency for more elevated values in both extremes of age in diabetics.

Figure 2 shows the mean and its standard deviation for normal (N) and diabetic (D) men and women, and for diabetics in the presence (Ret) and in the absence (No) of retinopathy. It shows a tendency for the cases with retinopathy to have increased platelet MAO activity, although these results are not statistically significant.

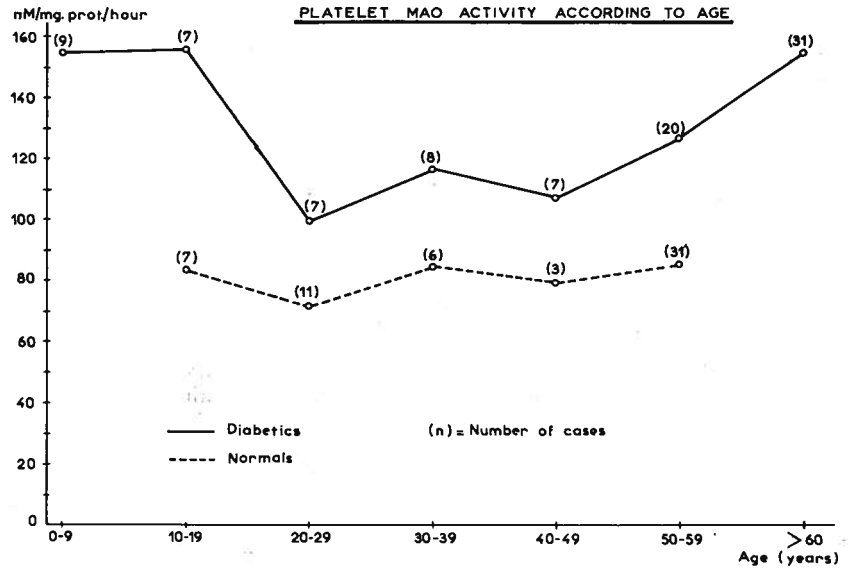

Fig. 1 — *Comparison of platelet MAO activity in normals and in diabetic patients, according to age. MAO activity determined as nM benzaldehyde formed/mg protein/hour.*

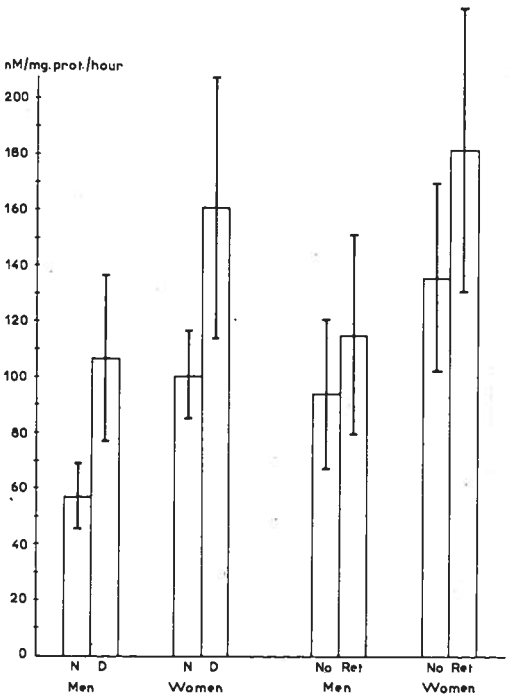

Fig. 2 — *Platelet MAO activity in normal, and in diabetic men and women (left). In the right, diabetic men and women, with and without retinopathy. Notice that, although the difference is not significant, there is a tendency for platelet MAO activity to be increased in persons of both sexes in the presence of retinopathy.*

DISCUSSION

MAO is important in carbohydrate metabolism although not completely understood. Recently Ismahan demonstrated a direct correlation between MAO activity in tissues and their glycogen content, after inhibition by Deprenil.[52] Also small concentrations of MAO inhibitors, depress insulin secretion, and in elevated concentrations, potentiate insulin secretion induced by glucose.[53] Treatment with MAO inhibitors in depressive patients with diabetes has to be careful due to potentiation of hypoglycemia induced by insulin or by sulphonylureas.[54, 55] Obese hyperglycemic rats possess an hepatic MAO activity superior to the control group.[56]

As platelets are rich in serotonin, it was thought at first that the main purpose of MAO consisted in serotonin deamination, but serotonin is not a substrate for type B enzyme.

Collins and Sandler think that the platelet enzyme is important in deaminating amines ingested with food. Platelet MAO is decreased in migraine[57] accompanied possibly by increased tendency for aggregation, although this is in doubt.[58]

Robinson finds brain, platelet and plasma MAO activity increased with age, and thinks that the increase in depression with age, as well as the appearance of parkinsonism, may be related with increase of MAO with age.[59]

Sex differences have been described before, women having more elevated values than men, especially after menopause. In iron deficiency anemia platelet MAO is depressed. The enzyme does not possess iron, but this is necessary for the covalent union of FAD to the enzyme.[60]

There is a direct correlation between MAO activity in platelets and the aggregation induced by adrenalin.[11] Niamid, a MAO inhibitor, diminishes platelet aggregation.[12]

The origin of plasma MAO is unknown but there are reasons to believe that it comes from connective tissue,[15, 11] where it could be necessary to stabilize collagen and elastin.[15, 16]

In our study we confirmed previous reports of elevation of plasma MAO activity in diabetics and of platelet MAO activity in the feminine sex.

Our studies differ from those of Mosnaim et al, since we found a definite difference between platelet MAO activity in diabetics when compared to normal persons. It must be understood, however, that these Authors studied a very small series (5 M and 4 F) in comparison to our own, and besides they use different substrates for MAO determination. Since benzylamine is considered the best substrate for platelet MAO activity determination we believe that our results deserve more confidence.

It is also interesting to notice that, although the presence or absence of retinopathy does not bring a statistically significant difference, there is a tendency for more elevated activity in the first cases, as shown in Fig 2. This fact may be important, since platelet aggregation is increased in diabetics and may be a factor in the evaluation of retinopathy. Unfortunately a large degree of individual variation increases the standard deviation of our cases and makes it impossible to demonstrate mathematically this difference.

RESUMO

Doseou-se a monoaminoxidase (MAO) das plaquetas, usando como substrato a benzilamina. Os resultados são expressos em nM benzaldeído/mg proteína/hora, a 37°.

Estudaram-se 79 diabéticos (40 M, 39 F) de idades compreendidas entre os 3 e os 70 anos. Um grupo controle normal incluiu 39 indivíduos (17 M, 22 F) com idades variando entre os 16 e os 70 anos.

A actividade da MAO das plaquetas mostra uma ligeira variação com a idade (não significativa). Porém, em cada grupo etário, os diabéticos têm actividade aumentada em comparação com indivíduos normais.

Pelo contrário a actividade da MAO na plasma não mostra variações dependentes da idade ou sexo, mas os valores para os doentes diabéticos são aproximadamente o dobro dos encontrados em indivíduos normais.

A actividade da MAO das plaquetas revela um aumento significativo de actividade em diabéticos de qualquer sexo. No entanto, as mulheres têm uma actividade mais elevada que os homens.

Não se encontrou qualquer relação entre a actividade da MAO plaquetária e a idade dos doentes, tempo de início, duração da doença, tratamento com insulina e presença de retinopatia.

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