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A STUDY OF THE POSSIBLE RELATIONSHIP OF SERUM DOPAMINE-BETA-HYDROXYLASE ACTIVITY WITH DIABETIC NEUROPATHY *

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SUMMARY

Serum DBH activity in a normal population of 58 individuals shows a gaussian distribution, on the opposite to what happens in a population of 100 diabetics. These two populations also differ statistically by the analysis of variance. However it was found that it is not possible to correlate serum DBH neither with insulin administration, type of diabetes, duration of disease, nor with the severity of the involvement of the peripheral nerves. It is hypothesized that the variation of DBH in diabetics may have a multifactorial cause, resulting from several metabolic and circulatory alterations that accompany the disease.

Dopamine-beta-hydroxylase (DBH) activity may be measured in serum or plasma, coming from noradrenergic nerves by exocytosis (Kopin et al 1976). This process of exocytosis is complex and is preceded by intraaxonal transport from the nerve cell to the synaptic ending. This axonal transport is energy dependent, possibly related to the Na^+, K^+ — ATPase, and is inhybited by ouabain and other compounds (Garcia et al 1974).

About 4 % of the normal population shows a very low activity of serum DBH. This character is inherited and the heterozygotes with subnormal serum activity amount to 32 % (Dunnette and Weinshilboum 1977). Besides inheritance there are other causes for low activity of serum DBH, such as primary orthostatic hypotension, in which low levels of the enzyme are found in plasma (Ziegler, Lake and Kopin 1977) and in sympathetic ganglia (Black and Pettito 1976).

It is possible to decrease serum DBH by chemical sympathectomy with drugs such as 6-hydroxydopamine (Weinshilboum and Axelrod 1971) or with DBH antibodies (Blessing et al 1977). In four patients with severe diabetic autonomic neuropathy a very low serum enzyme activity has been reported (Noth and Mulrow 1975).

It is the purpose of the present paper to describe our findings in the study of a large group of diabetics, in order to evaluate the importance of measurement of serum DBH in diabetics and to investigate if there is any correlation between these values and the degre of peripheral neuropathy.

MATERIAL AND METHODS

Serum DBH was assayed by the method of Nagatsu and Udenfried (Nagatsu and Udenfried 1972). The results are expressed as international units (micromoles/min/l of serum at 37°C).

A hundred diabetic patients from the outpatient department, were studied. None was hypertensive. Among them were cases of juvenile diabetes and late adult onset

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diabetes. Duration of disease was variable, from less than one year to more than 20 years. Approximately one half was taking insulin, whereas the others were controlled either by diet alone or by diet and oral medication. These cases are summarized in Table 1.

Table 1

Distribution of serum DBH activities according to therapy, type and duration of diabetes

	SERUM DBH — ACTIVITY u			
	N.°	low 0-30	average 30-60	high 60-200
WITH INSULIN	51	21	13	17
WITHOUT INSULIN	49	19	13	17
TOTAL	100	40	26	34
JUVENILE DIABETES	36	12	12	12
ADULT ONSET DIABETES	64	28	14	22
TOTAL	100	40	26	34
ONSET LESS 1 YEAR	18	5	8	5
» 1-5 YEARS	20	8	4	8
» 5-10 YEARS	16	9	4	3
» 10-15 YEARS	16	7	4	5
» 15-20 YEARS	14	4	2	8
» > 20 YEARS	16	7	4	5
TOTAL	100	40	26	34

In the second part of the study, 70 of these patients were submitted to neurological examination and classified in 3 groups, according to the degree of peripheral neuropathy:

Group I: without symptoms or signs of perypheral neuropathy (15 cases). Although this group is considered normal, we must recognize that 10 of the patients were submitted to an electromyographic study and 6 showed minimal abnormalities.

Group II: mild neuropathy loss of Achilles tendon reflexes with or without parestesias, and with or without complaints of autonomic nervous system involvment (31 cases).

Group III: moderate sensory-motor peripheral neuropathy, with or without complaints of ANS involvment (24 patients).

No severe neuropathy cases were included in this study. In Table 2 these elements of classification are summarized.

In 10 patients of Group I the following EMG studies were performed, with a three channel DISA machine: needle EMG of the anterior tibialis, extensor digitorum brevis and thenar eminence; motor conduction velocities (MCV) and distal latencies of deep peroneal and median nerves; sensory conduction velocities (SCV) of sural and median nerves with surface electrodes.

Table 2

Distribution of diabetics according to the severity of neurological lesions

Duration of Diabetes	Type of Diabetes	Sex	Neuropathy Groups		
			I	II	III
A-30	JOD-10	M	2	3	—
		F	3	1	1
	MOD-20	M	1	4	5
		F	6	—	4
B-28	JOD-6	M	—	2	1
		F	—	1	2
	MOD-12	M	1	3	5
		F	1	10	2
C-12	JOD-2	M	—	—	—
		F	—	2	—
	MOD-10	M	1	1	2
		F	—	4	2
Total = 70	JOD 19 / MOD 52	M 25 / F 45	15 (10-EMG)	31	24

Group A — less than 5 years; Group B — between 5 and 20 years; Group C — more than 20 years; JOD — juvenile onset diabetes; MOD — mature onset diabetes.

RESULTS

Study of a large diabetic population

The serum DBH activity of 100 diabetics in shown in Figure 1 and compared with the values found in the serum of 58 normal individuals.

There is a large population with extremely low values, and in eleven cases the activity was zero. A second group is irregularly distributed in the region of normal activity, and finally there are scattered cases of very high activity, up to 200U.

The comparison of the two populations (normals and diabetics) was made by the analysis of variance. The F test gave a result of F = 188, confirming that the two populations are statistically distinct both at the level of 1 % and at the level of 5 %:

Diabetics	Normals
$x_i = 5225.49$	$x_i = 2807.96$
$\bar{x}_1 = 52.49$	$\bar{x}_2 = 48.4131$
$s_1 = 1629.1289$	$s_2 = 868.7618$
$s_1 = 40.5658$	$s_2 = 29.7322$
$N_1 = 100$	$N_2 = 58$

$F = 188$

The test of normality for the normal population with a class amplitude of 10 gives $x^2 = 5.561$ and $X^2 = 11.1$ which accepts the hypotesis of the normality of data. For the population of diabetics the same test gives $x^2 = 32.58$ and $X^2 = 12.6$ which rejects the hypotesis of normality.

In Table 1 cases are divided according to therapy with and without insulin, to the type of diabetes and to the time of onset. The activity of serum DBH is divided in 3 groups low (0-30U) medium (30-60U) and elevated ($>$ 60U).

No statistically significant difference is found in any case.

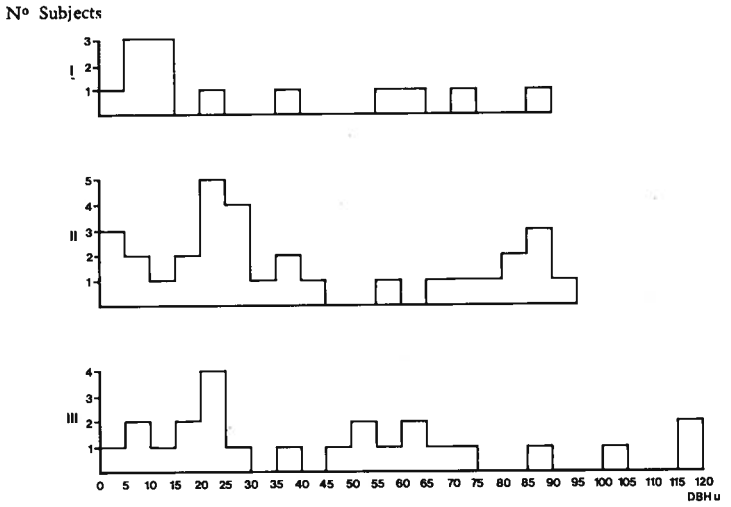

Fig. 1 — *Distribution of serum DBH activity in international units in a group of normales (dotted) and in a group of diabetics, showing the irregularity of the distribution of serum DBH in diabetics, in contrast to what happens in normals*

DBH and neuropathy

Table 3 shows the results of DBH in the seventy patients divided in 3 groups, as described. No significant difference was found between them. As can be seen in

Figure 2 a scattered distribution of DBH values in the 3 groups of diabetics, classified according to the degree of neuropathy was found.

Table 3

Statistical analysis of the diabetic population divided in 3 groups according to severity of the neurological findings

Group I	Group II	Group III
$\bar{x}_1 = 45.457$	$\bar{x}_2 = 51.277$	$\bar{x}_3 = 44.620$
$s_1 = 40.802$	$s_2 = 37.973$	$s_3 = 34.978$

T test: I/II	$t = -0.4628$
T test: I/III	$t = -0.1657$
T test: II/III	$t = 0.6870$

Group I — Clinically normal; Group II — Mild neuropathy; Group III — Moderate neuropathy.

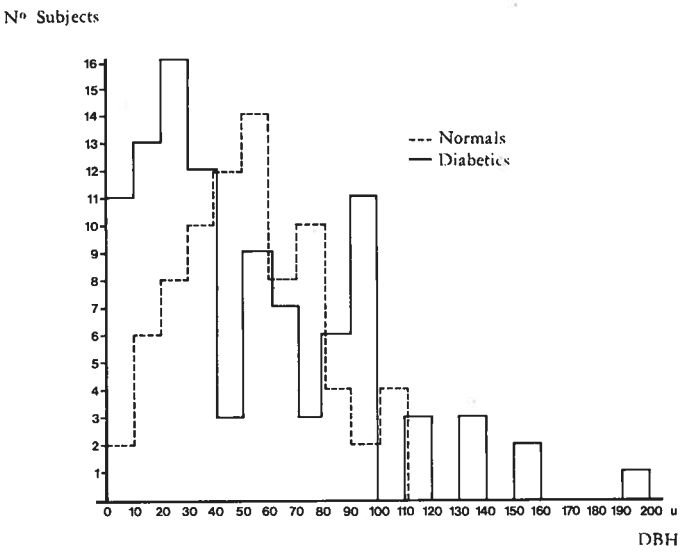

Fig. 2 — Distribution of serum DBH activity in diabetics *with clinically absent (I), mild (II) and moderate (III) neuropathy, showing that in all groups the results are scattered over a wide range*

Table 4 specifies the abnormalities in the EMG found in 6 of 10 diabetics with normal neurological examination. This results were not taken in account to subdivide the group 1 (with normal neurological examination). They only reinforce the idea generally accepted that most diabetics with absence of neurological symptoms and signs have subclinical polyneuropathy.

Table 4

Electroneurophysiological studies on diabetics with normal neurological examination

Normal	4
Slow M.C.V. of deep peroneal nerve (28 m/sec) and long distal latency (7.7 msec)	1
Normal M.C.V. of deep peroneal nerve($\geqslant$ 40 m/seg) and long distal latency ($>$ 5 msec)	5
Absent sensory potencial in distal segment of sural nerve	6
Slow S.C.V. on distal segment of median nerve ($<$ 50 m/sec) with low voltage spread potencials	4

DISCUSSION

In our studies, there is a positive finding in what concerns DBH activity: a large diabetic population has a different behaviour than a normal population.

Factors such as insulin therapy, juvenile or adult diabetes and time of onset do not to influence DBH activity.

The previous description of lowered DBH activity in severe diabetic neuropathy led us to make and attempt to correlate the severity of neurological disfunction with serum enzyme activity.

Also no correlation could be found, as can be seen at Figure 2, since there is a scattered distribution of values in all three groups of neuropathy. The possible explanation is that no cases with very severe diabetic neuropathy are included in this study.

The reasons why an index of autonomic nervous system activity shows such a wide degree of variation in diabetes must be multiple.

Among the factors that are responsible for an increased activity of serum DBH we count hypoglycemia (Okada et al 1975) and glucagon (Wada et al 1977).

On the opposite side, several factors may be responsible for its serum decreased activity namely hyperglycemia (Oberman and Herzberg 1978) lack of nutrients such as copper (Sourkes 1972) and possibly enzyme inhibitors or antibodies.

In the derranged metabolism of the diabetic there may be a possible change in concentration of metabolites or even an interference with the nerve blood supply due to diabetic vascular disease that may in some way interfere with synthesis or release of DBH from the noradrenergic nerves.

RESUMO

A actividade da DBH do soro numa população normal de 58 indivíduos tem uma distribuição gaussiana, ao contrário do que sucede numa população de 100 diabéticos. Estas duas populações também diferem estatisticamente pela análise da variância.

Contudo, verificou-se que não é possível correlacionar a actividade da DBH com a administração de insulina, tipo de diabetes, duração da doença, nem com a gravidade da afecção dos nervos periféricos.

Apresenta-se a hipótese de que a variação da DBH na diabetes possa ter uma causa multifactorial, em resultado de diversas alterações metabólicas e circulatórias que acompanham a doença.

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REFERENCES

BLACK IB, PETITO CK: Catecholamine enzymes in the degenerative neurological disease idiopathic orthostatic hypotension. *Science* 192: 910, 1976.

BLESSING WW, COSTA M, GEFFEN LB, RUSH RA, FINL G: Immune lesion of noradrenergic neurones in rat central nervous system produced by antibodies to DBH. *Nature* 267: 368, 1977.

DUNNETTE J, WEINSHILBOUM R: Inheritance of low immunoreactive human plasma DBH. *J Clin Invest* 60: 1080, 1977.

GARCIA AG, KIRPECAR SM, PRAT JC, WAKADE AR: Metabolic and ionic requirements for the axoplasmic transport of DBH. *J Physiol* 241: 809, 1974.

KOPIN IJ, KAUFMAN S, VIVEROS H, JACOBOWITZ D, LAKE R, ZIEGLER M, LOVENBERG W, GOODWIN F: DBH — basic and clinical studies. *Ann Intern Med* 85: 211, 1976.

NAGATSU T, UDENFRIEND S: Photometric assay of DBH activity in human blood. *Clin Chem* 18: 980, 1972.

NOTH RH, MULROW PJ: Serum DBH as an index of sympathetic nervous system activity in Man. *Circulation Res* 38: 2, 1975.

OBERMAN Z, HERZBERG M: Decrease in serum DBH activity during oral glucose tolerance test. *Israel J Med Sc* 14: 798, 1978.

OKADA F, YAMASHITA N, SUWA H, KUNITA H, HATA S: Elevation of plasma DBH activity during insulin induced hypoglycemia in man. *Experientia* 31: 76, 1975.

SOURKES T: Influence of specific nutrients on catecholamine synthesis and metabolism. *Pharmacol Rev* 24: 349, 1972.

WADA A, MIYASHITA T, IZUMI F, KASHIMOTO T, OKA M: Rapid changes and half life of DBH: effect of glucagon. *Biochem Pharmacol* 26: 986, 1977.

WEINSHILBOUM R, AXELROD J: Serum DBH: decrease after chemical sympathectomy. *Science* 173: 931, 1971.

ZIEGLER MG, LAKE CR, KOPIN IJ: The sympathetic nervous system defect in primary orthostatic hypotension. *New Engl J Med* 296: 293, 1977.

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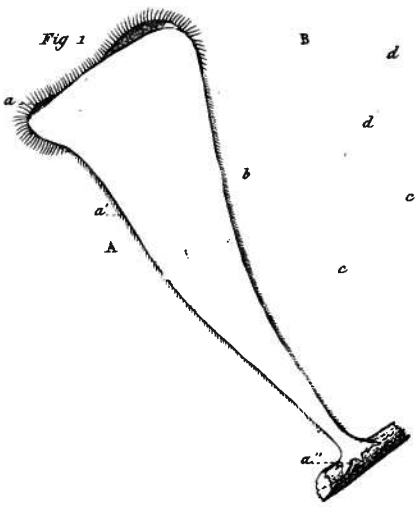

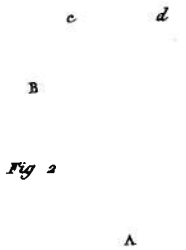

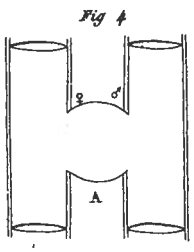

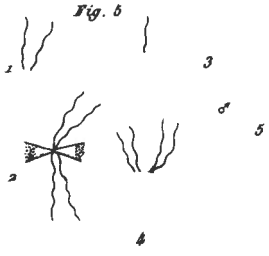

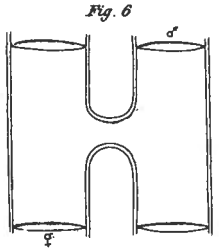

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