

A GENETIC STUDY OF PLASMA DOPAMINE BETA HYDROXYLASE ACTIVITY IN MAN

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Plasma dopamine beta hydroxylase (DBH) activity was determined in MZ twins and in siblings of both sexes. The enzyme activities were found to be virtually identical in MZ twins. DBH activity was less similar in same-sex sibs, but still significantly correlated. These results indicate that plasma DBH activity is genetically determined.

INTRODUCTION

Dopamine β hydroxylase (DBH), which catalyses the biosynthesis of norepinephrine, is released together with this neurotransmitter after stimulation of the adrenal medulla or of sympathetic nerves (Kaufman and Friedman 1965, Viveros et al. 1968, Gewirtz and Kopin 1970). It may thus be an indicator of peripheral sympathetic nervous activity (Axelrod 1972). Reduced blood levels of DBH have been found in paraplegic and quadriplegic subjects (Levitt et al., in press). Plasma DBH has also been studied in different genetic conditions. Patients with familial dysautonomia, Down's syndrome, and Parkinson's disease, have lower plasma DBH activity (Weinshilboum and Axelrod 1971a, Wetterberg et al. 1972a, Liberman et al. 1972), while increased activity was seen in patients with autosomal dominant torsion dystonia (Wooten et al. 1973) and in patients with Huntington's disease (Lieberman et al. 1972). No significant differences in DBH activity were found in patients with mental disorders (Wetterberg et al. 1972b, Shopsin et al. 1972, Dunner et al. 1973), as compared to normal controls.

In a given individual the level of plasma DBH activity is constant over long periods of time in spite of wide variations in levels of enzyme activity between individuals (Weinshilboum and Axelrod 1971b, Nagatsu and Udenfriend 1972, Horwitz et al. 1973). Exercise and cold exposure increase DBH activity but the rise is small compared to cardiovascular changes (Wetterberg et al. 1972b, Wooten and Cardon 1973, Horwitz et al. 1973, Frewin et al. 1974). Treatment with imipramine or lithium does not alter plasma DBH activity (Levitt et al., in prep.), nor does electroshock treatment (Wetterberg et al. 1972b).

Horwitz et al. (1973) reported significantly lower plasma DBH activity in black subjects as compared to whites and in black men versus black women, but a small difference between white men and white women. There is little if any increase in DBH activity after the age of five (Freedman et al. 1972, Wetterberg et al. 1972b, Weinshilboum et al. 1973, Horwitz et al. 1973).

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Recent family studies have shown high sibling-sibling correlations of DBH activity (Weinshilboum et al. 1973) and a high correlation in male MZ twins (Ross et al. 1973).

These observations suggest that plasma DBH activity is significantly dependent on genetic factors. This report describes a study on MZ twins and sibs to further characterize the extent to which genetic factors control plasma DBH activity.

METHOD

A sample of 11 pairs of twins (6 females and 5 males, aged between 29 and 65 years) was obtained by private referrals. The ascertainment was biased in that twins were more likely to be referred to us if they were of the same sex and resembled each other. The twins were all Caucasians, in good physical and mental health and not living together. Zygosity was determined by estimating similarity in physical traits, history of similar appearance to parents and teachers since early childhood, and serological determinations. Nine blood groups were investigated and the probability of zygosity was calculated according to the method of Smith and Penrose (1955). There were 9 pairs of MZ and 2 pairs of DZ twins. The probability of twins being MZ was 0.98 or over. The two DZ pairs of twins (one male, one female) both had at least three blood groups which were different. Because DZ twins are genetically like sibs, the two DZ pairs were pooled with the same-sex sibs.

The sample of sibs (individuals aged between 12 and 80 years) was obtained at the same time through family studies of patients with bipolar manic-depressive illness and unipolar depressive illness. The probands were consecutively admitted to an outpatient and inpatient clinic at the New York State Psychiatric Institute. Twenty-one sibships, including the two DZ pairs, were studied. A mixture of affectively ill and normal subjects could validly be used because affective disorders do not modify plasma DBH activity (Wetterberg et al. 1972*b*, Shopsin et al. 1972, Levitt et al., in prep.). In the current series of subjects, the mean DBH activity of the ill members of the sibships was less than 3 nM/ml/min below that of the well members, a small and insignificant difference.

Blood samples were collected by vein puncture in heparinized tubes on all subjects. Tubes were placed on ice and centrifuged in the cold to separate the plasma which was frozen until assayed. Enzyme measurements were performed blind to the status of the subjects.

DBH activity was assayed by a modification of the spectrophotometric method described by Nagatsu and Udenfriend (1972). Copper (1×10^{-6} M) was added to the incubation mixture and the Dowex 50 (H⁺) columns were eluted with 3 ml of 3N ammonium hydroxide which was used for the subsequent determination of p-hydroxybenzaldehyde. Duplicate 5 ml aliquots of plasma were assayed and the mean used in subsequent calculations. If the duplicates were more than 5% apart, the assay was repeated. Plasma activity was expressed as n-moles/min/ml plasma. DBH activity was measured at optimal assay conditions. The values are therefore higher than those reported by workers using the method of Goldstein et al. (1971) or Molinoff et al. (1971).

All correlations were calculated on the square roots of the serum DBH activity values in order to reduce the skewness in the original data (Weinshilboum et al. 1973).

RESULTS AND DISCUSSION

The plasma DBH values for the MZ twins are shown in Table 1, and those for the sibships in Table 2. No mean difference was found between males (mean = 59.7 nM/ml plasma/min, S.E. = 8.1) and females (mean = 57.7 nM/ml plasma/min, S.E. = 8.3), nor was any association found with age (product-moment correlation coefficient = -0.13, n.s.). These results confirm those of Horwitz et al. (1973) and Freedman et al. (1972).

Fig. 1 presents the scatter plot of pairs of square root values for the five pairs of female and four pairs of male MZ twins. The points cluster closely about the 45° line through the origin, indicating virtual identity between the values for MZ twins. For both the male and female pairs, the value of the intraclass correlation coefficient is 0.99 ($P < 0.001$) comparable

TABLE 1
PLASMA DBH ACTIVITY IN MZ TWINS

Twin pair	Sex	DBH Activity (nM/ml plasma/min)	
		Proband	Cotwin
1	M	73.0	68.0
2	M	62.8	62.3
3	M	17.3	16.9
4	M	36.2	33.6
5	F	103.5	92.1
6	F	60.9	62.3
7	F	28.9	29.9
8	F	101.1	99.2
9	F	101.2	93.8

TABLE 2
PLASMA DBH ACTIVITY IN SIBS

Sibship	DBH Activity (nM/ml plasma/min)			
	Males		Females	
1	21.8	17.6		
2	44.7	0.6		
3	40.3	25.4	30.8	22.7
4	65.8	85.7		
5	56.1			85.0
6	42.7	96.8		48.3
7	75.2			14.6
8	38.4			32.1
9	99.3			12.9
10	25.9			29.8
11	65.0	89.0		
12	61.8	34.8		49.7
13	0.85			105.3
14				65.5
15	109.3			54.7
16	112.4			41.0
17	50.1	92.6		
18	34.7	33.5	31.9	
19				1.6
20	185.3	135.0		10.4
21				123.0
				133.3

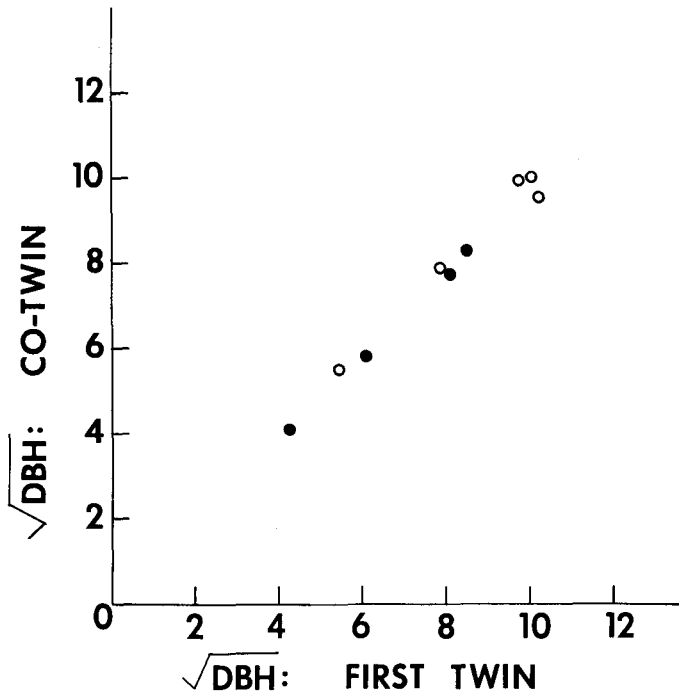


Fig. 1. Square roots of plasma DBH activities for pairs of male (filled circles) and female (empty circles) MZ twins. Selection of first twin and cotwin was at random.

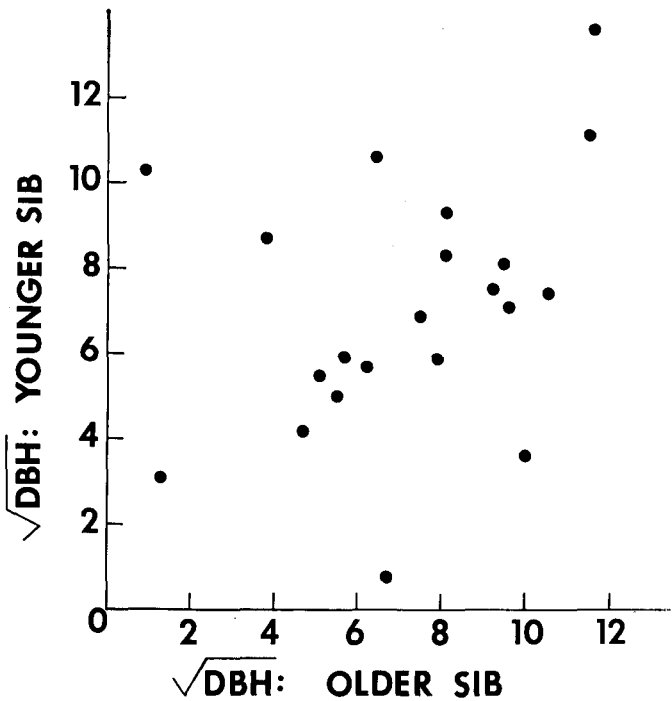


Fig. 2. Square roots of plasma DBH activities for 21 pairs of siblings.

to the value (0.96) found by Ross et al. (1973) for male MZ twins. The heritability estimates, obtained by comparing the within-twin-pair variances with the within-like-sexed sibship variances, are above 0.90 for both males and females.

Fig. 2 presents the scatter plot of pairs of square root values in the 21 sets of siblings. When a sibship consisted of more than two siblings, the values for the two who were closest in age were selected for plotting. The resulting value of the product moment correlation coefficient, $r = 0.33$, is not significantly different from zero. This overall value masks marked variability in the correlation when sex is controlled. In five pairs of sisters, the correlation is $r = 0.98$ ($P < 0.01$). In ten pairs of brothers, it is $r = 0.73$ ($P < 0.05$), a value equal to the one ($r = 0.75$) reported by Ross et al. (1973) for male DZ twins.

In 11 brother-sister pairs, however, it is $r = -0.42$ (n.s.). One of the brother-sister pairs (sibship 13) had the most discrepant values of the square root of serum DBH activity, 0.9 for the male and 10.3 for his sister. Even when this pair is excluded, the correlation rises to only $r = 0.08$ (n.s.). Whether or not this pair is included in the analysis, the correlation between a brother and sister is small, whereas that for the like-sexed sibs is high. Weinshilboum et al. (1973) reported no differences in correlations by sex. They do not present correlations specific to sex, so we cannot compare our values with theirs.

Our results confirm and extend those of Weinshilboum et al. (1973) and of Ross et al. (1973). We find high concordance for DBH activity in both male and female MZ twins. Correlations in DZ twins (Ross et al. 1973) and same-sex sibs are of a lower order of magnitude than those found in MZ twins, but are still significantly greater than zero. These results, together with those of others who have shown only small effects of environmental factors on DBH activity, indicate that the activity of this enzyme in plasma is genetically determined. Thus, plasma DBH activity may be useful as a marker in studies of human genetics. Pedigree analyses are being conducted to elucidate the mode of transmission of this enzyme.

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RIASSUNTO

Studio Genetico dell'Attività della Dopamina Plasmatica Beta-Idrossilasi nell'Uomo

L'attività della dopamina beta-idrossilasi (DBH) è stata determinata in gemelli e gemelle MZ e in fratelli e sorelle. Le attività dell'enzima sono risultate praticamente identiche nei gemelli e meno simili fra fratelli e sorelle dello stesso sesso, per quanto ancora significativamente correlate. Tali risultati indicano che l'attività della DBH è geneticamente determinata.

RÉSUMÉ

Etude Génétique de l'Activité de la Dopamine Plasmatique Béta-Hydroxylase chez l'Homme

L'activité de la dopamine beta-hydroxylase (DBH) a été relevée chez des jumeaux et jumelles MZ et chez des frères et soeurs. Les activités de l'enzyme se sont révélées pratiquement identiques chez les jumeaux et moins semblables entre frères et soeurs du même sexe, bien qu'elles soient encore significativement corrélées. Ces résultats indiquent que l'activité de la DBH est déterminée génétiquement.

ZUSAMMENFASSUNG

Erbforschung über die Aktivität der Dopamin-beta-Hydroxylase im menschlichen Plasma

Bei EZ beider Geschlechter sowie bei Brüdern und Schwestern wurde die Aktivität der Dopamin-beta-Hydroxylase (DBH) festgestellt. Es ergab sich, dass die Aktivität dieses Enzyms bei den Zwillingen praktisch identisch ist. Bei gleichgeschlechtlichen Geschwistern war sie zwar weniger ähnlich, jedoch bestand noch immer eine wesentliche Korrelation. Diese Ergebnisse zeigen, dass die DBH-Aktivität erbbedingt ist.

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