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RELATIONSHIP BETWEEN THE ACTIVITY OF SERUM DOPAMINE-β-HYDROXYLASE AND THE ENZYMES RELATED TO HEPATIC FUNCTION

(dopamine-β-hydroxylase/hepatic disorder/human)

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Serum dopamine-β-hydroxylase (DBH) activities in 94 male and 93 female normal subjects and of 36 subjects with chronic hepatic disorders were measured. The activities in normal subjects were distributed widely and were not normal in shape but skewed. There was no significant difference in serum DBH activity according to age or sex. There was no difference in serum DBH activity between normal subjects and hepatic disorders. In hepatic disorders there were significant positive correlations between DBH and GOT or AlP, suggesting a possible correlation between enhancement of serum DBH activity and development of hepatic disorders.

Dopamine-β-hydroxylase (DBH) (E.C. 1.14.17.1), the enzyme that converts dopamine (DA) to noradrenaline (NA), is localized to granules in chromaffin cells and in noradrenergic nerve endings, and it is released along with catecholamines (CA) from the granules into the blood via exocytosis (1). Plasma and/or urine levels of CA and their metabolites have been measured, and those values are considered as the indexes of sympathetic functions. However, there are many problems concerning these measurements such as the need for a large amount of blood for serum CA and the unreliability of urinary CA or CA metabolites as

the indexes. There is, however, an idea to use serum DBH as an index of sympathetic functions (2), because DBH is released with NA and a small amount of blood is enough for the measurement of DBH activity. Serum DBH does not change with acute stimulation (3) or circadian rhythm (4), however, it is well known that serum DBH changes with chronic disorders (5-8). There have been a lot of reports on the relationships between serum DBH and hypertension (5), cadiac disorders (6), psychiatric diseases (7) and thyroid disorders (8, 9) and in these reports the changes in sympathetic functions have been discussed. There have been a few reports (13) about the relationship between serum DBH and hepatic disorders, but the relationship is considered not to have been established yet.

In severe hepatic disorders, amino acids such as tyrosine and phenylalanine increase (10, 11) and these amino acids are converted by decarboxylase to tyramine, which is a substrate of DBH and is converted to octopamine. Octopamine was considered as a false transmitter and it has suggested that central nervous system (CNS) symptoms in severe hepatic disorders appear through such a mechanism (12). Thus, it is important to examine the relationship between serum DBH activity and hepatic disorders.

In the present study, serum DBH activity in patients with chronic liver disorders patients and normal human samples were examined.

SUBJECTS AND METHODS

Subjects

Thirty six patients (22 male and 14 female), who were admitted to Shimane medical university hospital and visited the out-patient department, and who ranged in age from 7 to 69 were studied. The hepatic disorders consisted of 9 cases of liver cirrhosis and 27 of chronic hepatitis. The diagnosis of hepatic disorders was done by the enzymological and imaging methods and biopsy. As the control, 94 normal male and 93 normal female volunteers were examined.

Methods

Blood collection was done via the antecubital vein in the early morning. After centrifugation at 3000 rpm for 5 min, sera were stored at -20°C until assay. For DBH activity

determination, the method of Nagatsu and Udenfriend (14), which was based on the enzymatic conversion of tyramine to octopamine, was applied with a slight modification. The incubation mixture contained 40 µl of serum as enzyme, 10 µmol of sodium fumarate, 10 µmol of ascorbic acid, 1500 units of catalase (Sigma), 20 µmol of tyramine hydrochloride (Sigma), 3 µmol of pargyrine hydrochloride and 30 µmol of N-ethylmaleimide and 200 µl of 1 M sodium acetate buffer, pH 5.0, in a final volume of 1 ml. As a blank, serum boiled at 95°C for 5 min was used. After 20 min of incubation at 37°C, the reaction was stopped by adding 200 µl of 3 M trichloroacetic acid and the incubation mixture was centrifuged at 2000 rpm for 10 min. The supernatant fluid was transferred to the column of Dowex 50Wx4 (H^+, 200-400 mesh), of which the diameter and height were 5 and 10 mm, respectively. The column was washed with distilled water and the formed octopamine was eluted with 2 ml of 4N ammonium solution. Octopamine in the eluate was converted to p-hydroxybenzaldehyde by adding 200 µl of 2 % $NaIO_4$ solution. Excess periodate was reduced by adding 200 µl of 10 % $Na_2S_2O_5$ solution. Absorbance at 330nm was measured by spectrophotometer (Hitachi 139). As a standard, 20 µmol of octopamine (Sigma) in distilled water was applied to the column. Serum DBH activity was expressed as the amount of converted octopamine per litre serum per min at 37°C (international unit: I.U.).

Serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic tansaminase (GPT), alkaline phosphatase (AlP), pseudo-cholineesterase (ChE) and lactate dehydrogenase (LDH) which were considered as the indexes for hepatic functions, were measured by an automated analyser (Hitachi 726).

Statistics

Comparison of serum DBH activity of the two groups was assessed by Mann-Whitney's U-test. Relationships between serum DBH and serum enzymes were tested by Kendall's rank test.

RESULTS

Human Serum DBH Activity in Normal Subjects

The distribution of human serum DBH activity in normal subjects is shown in Fig.1. Serum DBH activities were not significantly different between males and females. The mode was

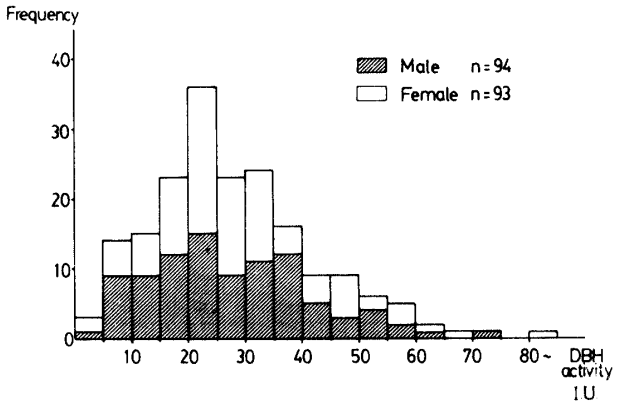

Fig.1. The distribution of serum dopamine-β-hydroxylase activity in normal subjects.

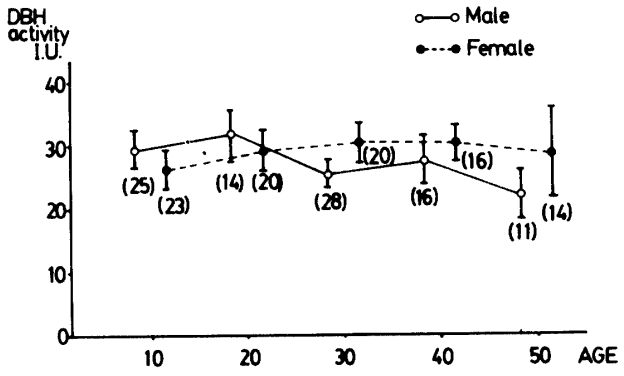

Fig.2. The changes of serum dopamine-β-hydroxylase activity with aging. The vertical bar shows the standard error. Numbers of subjects are given in parenthesis.

in a range from 20.0 to 24.9 I.U. in male, female or total subjects. The distribution of serum DBH activity spread widely from 3.9 to 129.0 and was not normal in shape but was skewed. Neither significant difference in serum DBH activity among subjects of different ages nor significant male-female difference in each age group were observed (Fig. 2).

Table I THE CORRELATION BETWEEN DBH AND SERUM ENZYMES

		GOT	GPT	AlP	ChE	LDH
Chronic hepatitis (n=27)		92.4±13.2*	97.4±17.4	71.3± 6.2	1382.5±84.0	340.7±19.9
	DBH			26.7±2.8		
	τ	0.10	0.35	0.22	-0.10	-0.08
	p	N.S.	N.S.	0.025	N.S.	N.S.
Liver cirrhosis (n=9)		59.1± 9.5	46.2± 4.7	81.9±17.6	729.6±81.3	362.1±37.2
	DBH			26.3±5.6		
	τ	0.69	0.50	0.44	0.17	-0.03
	p	0.025	N.S.(0.08)	N.S.	N.S.	N.S.
Total (n=36)		82.9±10.3	83.2±13.1	72.9± 6.4	1249.3±81.6	363.0±20.2
	DBH			26.4±2.5		
	τ	0.24	0.16	0.25	-0.14	-0.03
	p	0.025	N.S.(0.08)	0.025	N.S.	N.S.

* Mean ± Standard error (I.U.)
τ; correlation coefficient
N.S.; not significant

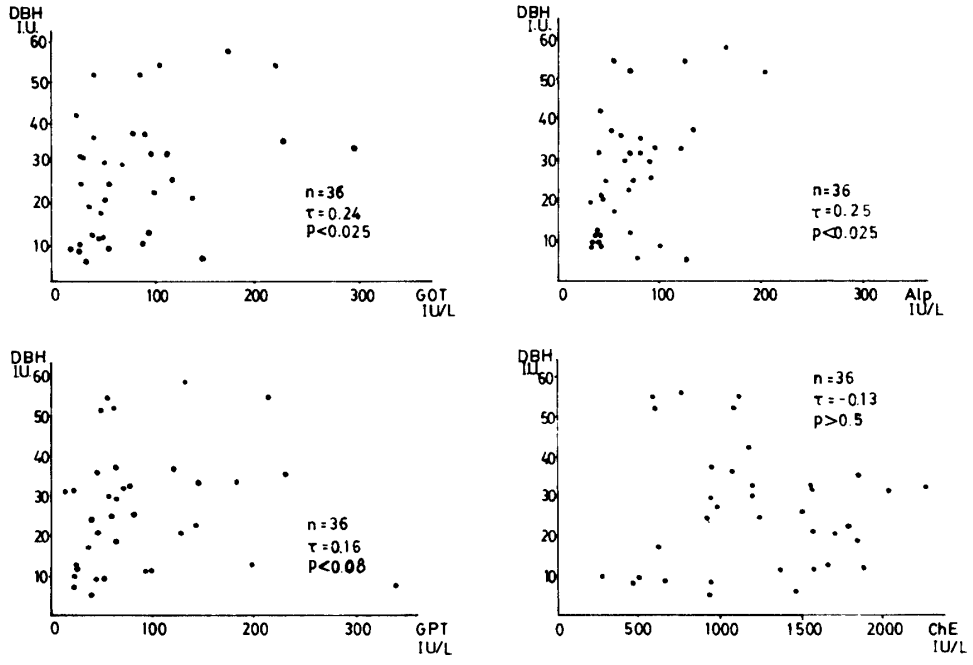

Fig.3. Correlations between serum dopamine-β-hydroxylase and serum enzymes activities in chronic hepatic disorders.

Serum DBH Activity in Hepatic Disorders

There was no significant difference between serum DBH activity of normal subjects and hepatic disorder patients. No significant difference was observed between those of liver cirrhosis and chronic hepatitis patients either.

Relationships between Serum DBH and Serum Enzymes in Hepatic Disorders

The mean ± SE of serum GOT, GPT, AlP, ChE and LDH activity in two kinds of hepatic disorders as well as serum DBH activity were shown in Table I. In chronic hepatitis, positive relationship existed between AlP and DBH ($p<0.025$). Positive correlation was also observed between GOT and DBH in liver cirrhosis ($p<0.025$). In total, significant positive correlation between DBH and GOT, and between DBH and AlP was obtained ($p<0.025$, in both cases)(Fig. 3). No significant difference was observed between DBH and GPT, ChE or LDH activity.

DISCUSSION

There was a large individual variation on serum DBH activity in normal subjects, ranging from 3.9 to 129.0 I.U. There was no significant difference between male and female or among tested age-groups. These results agreed with those of the previous reports in American (15, 16) and Japanese (14) subjects. It was reported that serum DBH activity increased markedly until 1 year old and became a plateau after that age, then it decreased gradually after 50 years old (16, 17). In our results, there was a tendency for it to decrease after 50 years old; however, there was no significant difference.

The large individual variation on serun DBH activity in human subjects has been explained mainly by genetic factors (18); however, several minor factors such as drugs (19), hormones (9, 20) or chronic disorders (5-9) have also been reported.

Hara et al. (13) have observed an increase of serum DBH activity in chronic hepatic disorders compared with normal subjects. In normal subjects, our results agreed with their results, however, their results in hepatic disorders (43.1-62.5 I.U.) were larger than ours. Serum DBH activity should not be consudered as a reliable index of chronic hepatic disorders, because of its large individual variation.

The present results indicate a significant positive correlation between serum DBH activity and serum GOT or AlP in hepatic disorder. In addition, there was a tendency towards a positive correlation between GPT and DBH activity. However, no significant correlation between DBH and any other index in hepatic disorders such as LDH and ChE was observed in our research. A positive correlation of DBH and the serum enzymes observed by Hara et al. (13) was, however, partially supported by our results, at least in hepatic disorders. In addition, a correlation between serum DBH activity and the serum enzymes in the same patient with a hepatic disorder was observed (unpublished observation). On the other hand, serum DBH activity was reported to be stable in the same normal subject (5). These results suggested a possible correlation between enhancement of serum DBH activity and the development of hepatic disorders.

The following two possibilities are considered as the mechanisms of that relationship: (i) Induction, activation or enhancement of the release of the enzyme in sympathetic nerve endings induced by an increase of the substrate of the enzyme in peripheral tissues, (ii) malfunction of inactivation mechanism of the enzyme in the liver with the development of hepatic disorders.

Maghani et al. (21) reported the enhancement of blood and urinary octopamine contents in cases of severe hepatic disorders. If the enhancement of serum DBH in hepatic disorders occurred through the first mechanism, it seems to fit with their results, and CNS symptom in severe hepatic disorders seem to be correlated, at least partially, with the enhancement of DBH.

On the contrary, there is information suggesting the second mechanism. Rush et al. (22) examined the distribution in various tissues of rats after intravenous injection of ^{125}I labelled DBH and found that the radioactivity was concentrated in the liver. Their results may suggest that the liver is an organ inactivating serum DBH. Further experiments are necessary to clarify the mechanism of the possible correlation of serum DBH activity with serum enzymes in hepatic disorders.

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