Dopamine-β-Hydroxylase Activity and its Co-factors in Central and Peripheral Tissues and Serum of Malnourished Rats and Humans

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Introduction

Dopamine-β-hydroxylase (DBH) is the enzyme responsible for the biosyn-thesis of noradrenaline from dopamine, ultimately leading to the formation of adrenaline by the enzyme phenylethanolamine-N-methyl transferase in the mammalian tissues and serum. Noradrenaline and adrenaline are very important biochemically and pharmacologically. These monoamines act as intracellular messengers, such as neurotransmitters and hormones and involved in the regulations of neuronal functions, behaviour and emotion of higher animals. We measured the levels of DBH in central and peripheral tissues and serum of malnourished rats to know whether physical activity or emotion in malnutrition is affected by altered DBH activity. The enzyme activity was also measured in serum of malnourished human subjects. As DBH is a copper (Cu^{++}) and ascorbic acid (Vit-C) dependent enzyme, the micronutrients (Cu^{++} and ascorbic acid) were assayed as well.

Materials and Methods

Twenty healthy growing Long Evans rats, weighing in the range of 150-220 gms, were divided into two groups, namely control and malnourished. The rats of control group were given standard diet (containing 30% protein, 50% carbohydrate, 5% soyabean oil, 1% vitamin mixture and 5% salt mixture) while the rats of malnourished group were given 40% by weight of the control diet and water was given ad. Libitum. Five weeks after the experimental feeding, the rats of both groups were sacrificed by decapitation. Blood and tissues from central nervous system (cerebral cortex, brain stem, hypothalamus, caudate nucleas, colliculi, cerebellum), as well as peripheral tissues (liver, heart, kidney, small intestine, spleen, and adrenal gland) were collected and

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preserved at—20°C till the assays were performed. Human subjects were selected on the basis of their age, sex, height, weight, and mid upper arm circumference (MUAC).

DBH level was measured using the very simple and rapid one-step method of Kato *et al*, 1974[1]. The methods is sensitive enough to measure enzyme activity in as little as 2-20µl of serum or tissue homogenate. The principle of this assay is based on the enzymatic conversion of tyramine to octopamine, and the photometric assay of p-hydroxybenzyldehyde after the oxidation of the octopamine with periodate. Endogenous inhibitors that interfere with the assay of DBH in vitro are inactivated by adding excess N-ethylmaleimide[2].

Copper level was measured as described by Jackson, 1973[3] and ascorbic acid level was determined by the method of Lowry et al, 1945[4]. The K_m values for DBH in both human and rat serum were determined in control and malnourshed subjcets by Lineweaver-Burk[5].

Results

DBH activity increased in serum and in most of the tissues of rat during malnutrition (Table:1) A similar finding was also obtained in hyman serum assay (Table : 1) However, DBH activity was found unaltered in spleen and lower in caudate nucleus compared to the control group studied.

Among the malnoursihed rats, copper level was slightly increased in liver, adrenal gland, heart etc. but decreased in brain and in serum (Table:2). A lower serum copper level was also detected in malnourished human subjects (Table:2). Ascorbic acid level was decreased in both rat and human serum samples as well as in the adrenal gland of malnourished rats (Table: 3).

The Km values in control serum samples of human and rat were $5X10^{-4}M$ and $1.25X10^{-3}M$, respectively (table: 4), but the Km values decreased slightly in malnourished human and rat serum ($4x10^{-4}M$ and $1.17X10^{-3}M$, respectively) (Table: 4).

Discussion

Malnutrition is a major health problem for Bangladesh. According to UNICEF, 93% children under 5 years of our country are malnourished. The DBH activity in almost all the tissues was found to be increased during malnutrition. In malnourished-rat tissues, the activity was found to be 2-4 times higher than control. DBH activity in rat serum was fountd to be 1.2 times

Table 1. The DBH activity in the central and peripheral tissues and serum of rats and human.

Name of the tissues	No. of the sample	DBH activity (nmoles/min/g wet wt. tissues)	
		Control Mean+S.D.	Malnourished Mean+S.D.
1. Rat			
A .Peripheral tissues			
i. Liver	10	23.39±1.05	76.72±14.25
ii. Heart	10	10.58±1.10	11.85±0.05
iii. Kidney	10	6.94±0.20	10.27±2.50
iv. S. Intestine	10	19.88±4.20	34.47±8.62
v. Spleen	10	27.47±8.30	27.47±4.85
vi. A. Gland	10	31.82±2.0	46.91±5.0
B. Central tissues			
I. C. Cortex	10	23.43± 2.30	28.28±6.4
i. B. Stem	10	17.37± 0.40	24.24±2.0
iii. Hypothalamus	10	10.30±3.30	16.16±6.0
iv. C. Nucleus	10	36.36±0.40	32.54±5.1
v. Colliculi	10	14.54± 1.0	29.89±4.04
vi. Cerebellum	10	10.60±1.20	21.00±4.10
C. Serum*	10	0.54± 0.03	0.61±0.20
2. Human			
A. Serum*	10	38.56±2.40	51.60±5.0

**Serum DBH activity (nmoles/min/ml of serum)*

Table 2. Copper content of various tissues and serum of rats and human.

Name of the tissues	No. of samples	Amount of Copper (ug/g dry wt. tissues)	
		Control Mean+S.D.	Malnourished Mean+S.D.
1.Rat			
A. Peripheral tissuess			
i. Liver	10	525±20	1000±10
ii.	10	97±3	51±8
ii. Heart	10	97±3	109+5
iii. Kidney	10	57±5	51±8
iv. S. Intestine	10	120±6	100±10
v. Spleen	10	388±12	355±15
vi. A. Gland	10	2261±10	2500±20
B. Total brain	10	206±40	135±12
C. Serum*	10	0.87±0.3	0.75±0.25
2. Human			
A. Serum*	10	1.25±0.25	1.1±0.15

** Serum Cu^{++} conc. (µg/ml of serum)*

Table 3. Ascorbic acid content of serum and adrenal gland of rats and human

Name of the tissues	No. of samples	Amount of Ascorbic Acid (mg/g wet wt. tissue)	
		Control Mean±S.D.	Malnourished Mean±S.D.
1. Rat			
A. Serum*	10	1.48±0.04	0.90+0.40
B. A drenal gland	10	6.40 ±1.00	3.88±0.40
2. Human			
A. Serum*	10	2.08±0.20	1.40+0.20

** Conc. of ascorbic acid (mg/dl serum)*

Table 4. The Km values of DBH in rat and human serum

Name of the tissues	K_m values (M)	
	Control	Malnourished
Rat serum	$1.25X10^{-3}$	$1.17X10^{-3}$
Human serum	$5X10^{-4}$	$4X10^{-4}$

higher during malnutrition than that of control values whereas in the human, it was 1.25 times higher. DBH is an inhibitory enzyme so its increase in the tissues and serum resemble the lowering of the spontaneous activities.

DBH is a copper containing enzyme[6]. Copper level has slightly increased in malnutrition in liver, heart and adrenal gland and slightly decreased in the kidney, small intestine and spleen. The concentrations of copper in the brain and serum of rats were also decreased slightly. The copper content was decreased in human serum. Ascorbic acid is another co-factor for DBH and one μmole of ascorbate is oxidized per υmole of enzyme[7]. In malnutrition, ascorbic acid concentration was decreased in adrenal gland and serum of rats. The level of ascorbic acid also decreased in human serum.

Although ascorbic acid concentration has decreased, the DBH activity was increased. In a previous study, the enzyme activity was shown to remain unaffected by severe ascorbic acid deficiency[8]. Although DBH is a Cu^{++} and ascorbic acid dependent enzymes, we found higher DBH activity in malnutrition, this may be due to the fact that the still remaining conc. of cofactors were sufficient to binds the DBH enzyme for its catalytic activity or other changed constituents of serum may played some roles here.

The K_m value for DBH was found to be lower in the human serum than that for DBH in rat serum. In malnutrition, the Km values were decreased in the serum of both the species which indicated that the affinity of DBH for its substrate tyramine increased. This may be due to some modulators or affectors of

the enzyme present in malnourished samples.

Summary

DBH activity, in the various central and peripheral tissues and serum, was studied in malnourished rats. Human serum DBH activity was also assayed in malnutrition. It was found that the enzyme activity increases in all the tissues as well as in serum during malnutrition. As DBH is one of the key enzyme in the synthesis of catecholamines, such altered enzyme activity in malnutrition may affect regulation of neuronal functions, behaviour and emotion of higher animals.

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