Influence of malnutrition on brain catecholamine metabolism in young rats

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Abstract

The present investigation deals with brain catecholamine metabolism in rats with low plasma tyrosine concentration. Young rats were fed *ad libitum* low casein diet for 4 weeks (group I) or 12 weeks (group II).

The tyrosine concentrations in the plasma of groups I and II were lowered whereas those in the cerebral cortex were not changed. In group I, dopamine (DA) concentrations were decreased in the both cerebral cortex and diencephalon. Norepinephrine (NE) concentrations were also decreased in the cerebral cortex, striatum and diencephalon. In group II, DA concentrations were decreased in the cerebral cortex and striatum, and NE concentration was also decreased in the diencephalon. Tyrosine hydroxylase (TH) activity was elevated in the striatum in group I, while monoamine oxidase (MAO) activity was decreased. In group II, however, TH and MAO activities did not differ from those in control group.

These data suggest that catecholaminergic neurons in the brain may be variously affected with low plasma tyrosine concentration in malnutritional rats.

Introduction

Plasma tyrosine concentrations fluctuate during the day in response to food consumption (Fernstrom *et al.*, 1979; Melamed *et al.*, 1980); these changes affect brain tyrosine concentration (Gibson and Wurtman, 1977; Fernstrom and Faller, 1978). Treatments that increase or decrease brain tyrosine concentrations in rats cause parallel changes in the rates at which their brains after the administration of an aromatic 1-amino acid decarboxylase inhibitor accumulate DOPA (Wurtman *et al.*, 1974; Gibson and Wurtman, 1977).

Fatty liver arises from malnutrition, and chronic fatty liver is apt to shift to liver chirrhosis. The plasma tyrosine concentration is decreased with a low protein diet but elevated in hepatic encephalopathy (Rosen *et al.*, 1977). We investigated the effect of altered plasma tyrosine concentration due to malnutrition on catecholaminergic nervous system in rat brain, because these neurons participate in many key physiologic event. The present study describes the change of catecholamine metabolism in the brain possibly due to lowered plasma tyrosine concentrations following malnutrition.

Materials and Methods

Animals

Four- to five-week-old male Wistar rats weighing 90-125g were used. The animals were maintained at 24°C and 55% of humidity with a 12h light and dark cycle (lights on 0100 to 1300 h). Rats were fed *ad libitum*

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for 4 weeks (group I: initial body weight of 90g) or 12 weeks (group II: initial body weight of 125g) a low protein diet shown on Table I. Control groups were fed *ad libitum* a commercial stock diet (Oriental Yeast Co., Ltd., Japan). Low protein diet contained 0.17% tyrosine derived from casein, whereas commercial stock diet contained 0.57% tyrosine.

The animals were decapitated between 0800 and 1000 h of the lighted period. After decapitation the head was immersed into liquid nitrogen for about 5 seconds. The brain was removed and dissected rapidly into the following three regions (Schubert and Sedvall, 1972); whole cerebral cortex, striatum and diencephalon. The tissues were weighed and homogenized with a polytron homogenizer (Kinematica, Switzerland) in 30 vol (w/v) of cold 0.1N perchloric acid. The homogenate was kept in an ice bath for 15 min and then centrifuged at 4°C for 40 min at 30,000 x g. Supernatants and tissues of brain regions were kept frozen at -70°C until assayed. Blood samples were taken from the decapitated neck, and the plasma was collected by centrifugation and stored at -20°C.

Assays

The concentrations of dopamine (DA) and norepinephrine (NE) were determined in the supernatant of homogenate by a radioenzymatic assay according to a slightly modified method of Sole and Hussain (1977) using catechol-0-methyltransferase. Thin layer chromatography was performed for separation and purification of the synthesized products according to the method of Peuler and Johnson (1977) using a solvent system; t-amylalchol:benzene:40% methylamine (6:2:3). Coefficients of intraassay variation in the radioenzymatic method were 5.6% for DA and 7.6% for NE.

The activity of tyrosine hydroxylase [TH, tyrosine 3-monooxygenase (E.C.1.14.16.2, 1-tyrosine, tetrahydropteridine: oxidoreductase)] was assayed according to Coyle (1972). Monoamine oxidase [MAO, amine oxidase (Flavin containing) (E.C.1.4.3.4, amine: oxygen oxidoreductase (deaminating)] in the striatum was measured by a radioisotope method using two substrates, i.e., phenylethylamine [PEA, the final concentration at about 0.01mM (Wurtman *et al.*,1963)] and 5-hydroxytryptamine [5-HT; the final concentration at about 1mM (McCaman *et al.*,1965)]. Tyrosine concentrations in the plasma and cerebral cortex were estimated by the method of Udenfriend and Cooper (1952). Total lipid, triglycerides and cholesterol in the liver were determined according to the methods of Folch *et al.* (1957), Sardesai and Manning (1968), and Zak *et al.* (1954), respectively. Protein content was determined by the procedure of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Data were shown as mean ± S.D. Results were statistically analyzed using a Student's t-test.

Results

Body weights and brain wet weights

The effects of experimental low casein diet on both body weight and brain wet weight are represented in Table 2. In group I, body weight ($P<0.01$) and brain wet weight ($P<0.01$) gains of animals fed the experimental diet were much less than those of the group given the control diet. In group II, body weight gain of animals fed the experimental diet was

much less than that of the group given the control diet, whereas the brain weight of experimental diet rats was not significantly different from that of control diet rats.

Determination of fatty liver

Total lipid, triglycerides and total cholesterol values in the livers of group I and II were significantly elevated as compared with the control groups (Table 3). Formaldehyde paraolast-embeded sections were stained with hematoxylin-eosin or sudan III, were examined light microscopically, and revealed large fatty cysts in the livers of groups I and II.

Tyrosine concentrations in plasma and brain

The tyrosine concentrations in the plasma of groups I ($P<0.01$) and II ($P<0.01$) were lowered as compared with the control groups whereas those in the cerebral cortex were not changed (Table 4).

Catecholamine metabolism in the brain

Tables 5 and 6 show the catecholamine concentrations in various brain regions examined in both groups. In group I, DA concentrations were decreased in the cerebral cortex ($P<0.01$) and diencephalon ($P<0.01$). NE concentrations were decreased in all three regions, i.e., cerebral cortex ($P<0.01$), striatum ($P<0.01$) and diencephalon ($P<0.01$). In group II, DA concentrations were decreased in the cerebral cortex ($P<0.02$) and striatum ($P<0.01$). NE concentration was decreased in the diencephalon ($P<0.05$)

The TH activity was elevated in the striatum in group I as compared with control group, while the enzyme activity in group II did not differ from control group. MAO activities were determined in the striatum of malnutritional rats. The activities of PEA-MAO and 5-HT-MAO were decreased as compared with control group, while the enzyme activity in group II did not differ from control group (Table 7).

Table 1. Composition of the experimental diet (%)

Lard	38.0	Vitamin mix.**	1.0
Sucrose	45.375	1 - cystine	0.625
Casein	8.0	Cellulose	3.0
Mineral mix.*	4.0		

* Mineral mixture (%): $CaHPO_4 \cdot 2H_2O$, 14.56; KH_2PO_4, 25.72; $NaH_2PO_4 \cdot H_2O$, 9.35; NaCl, 4.66; Ca-lactate, 35.09; Fe-citrate, 3.18; $MgSO_4$, 7.17; $ZnSO_4 \cdot 4\text{-}6H_2O$, 0.12; $CuSO_4 \cdot 5H_2O$, 0.03; KI, 0.01

** Vitamine mixture (choline free/100g): vitamin A acetate, 50,000IU; vitamin D_3, 10,000IU; thiamin HCl, 120mg; riboflavin, 400mg; pyridoxine HCl, 80mg, vitamin B_{12}, 0.05mg; ascorbic acid, 3,000mg; vitamin E acetate, 500mg; vitamin K_3, 520mg; d-biotin, 2mg; folic acid, 20mg; calcium pantothenate, 500mg; p-aminobenzoic acid, 500mg; nicotinic acid, 600mg; inositol, 600mg; cellulose powder.

Table 2. Body, liver and brain weights of animals fed experimental diet

	Initial body weight	Body weight	Liver weight	Brain weight
Control (7)	91 ± 7	316 ± 17	13.5 ± 0.7	1.97 ± 0.07
Group I (14)	92 ± 8	127 ± 16*	6.3 ± 1.6*	1.74 ± 0.06*
Control (9)	128 ± 5	404 ± 43	12.2 ± 1.7	1.95 ± 0.12
Group II (7)	126 ± 10	249 ± 36*	11.9 ± 2.4	1.87 ± 0.12

Values presented as means (g) ± S.D. *P<0.001 as compared with control group (Student's t-test). The numbers in parentheses are the numbers of animals.

Table 3. Effects of the experimental diet on lipids levels in rat liver

	Total lipid	Triglycerides	Total cholesterol
Control (7)	33.7 ± 3.6	10.0 ± 1.1	3.9 ± 0.4
Group I (7)	212.5 ± 75.7**	149.0 ± 50.5**	5.6 ± 1.5*
Control (9)	40.9 ± 2.9	13.7 ± 2.5	3.6 ± 0.4
Group II (7)	134.3 ± 38.9**	90.6 ± 11.1**	6.1 ± 1.5**

Values presented as means (mg/g) ± S.D. *P<0.02; **P<0.001 as compared with control group (Student's t-test). The numbers in parentheses are the numbers of animals.

Table 4. Effect of the experimental diet on the tyrosine concentrations in rat plasma and brain

	Control (8)	Group I (8)	Control (9)	Group II (7)
Plasma (μmoles/l)	60.3 ± 4.2	29.9 ± 12.8*	80.2 ± 9.7	54.0 ± 14.8*
Cerebral cortex (nmoles/g)	77.0 ± 9.5	88.9 ± 14.1	81.0 ± 17.3	93.0 ± 11.3

Values presented as means ± S.D. *P<0.01 as compared with control group (Student's t-test). The numbers in parentheses are the numbers of animals.

Table 5. Effect of the experimental diet on the dopamine concentrations in rat brain

	Control (7)	Group I (7)	Control (8)	Group II (7)
Cerebral cortex	0.78 ± 0.26	0.26 ± 0.10**	0.25 ± 0.08	0.15 ± 0.06*
Striatum	6.51 ± 1.74	5.90 ± 1.67	4.98 ± 1.09	3.18 ± 0.86**
Diencephalon	0.21 ± 0.06	0.11 ± 0.04**	0.14 ± 0.04	0.10 ± 0.04

Values presented as means (μg/g) ± S.D. *P<0.02, **P<0.01 as compared with control group (Student's t-test). The numbers in parentheses are the numbers of animals.

Table 6. Effect of the experimental diet on the norepinephrine concentrations in rat brain

	Control (7)	Group I (7)	Control (8)	Group II (7)
Cerebral cortex	0.42 ± 0.06	0.14 ± 0.01**	0.20 ± 0.09	0.13 ± 0.05
Striatum	0.65 ± 0.19	0.33 ± 0.13**	0.23 ± 0.09	0.25 ± 0.13
Diencephalon	0.43 ± 0.20	0.16 ± 0.04**	0.60 ± 0.12	0.44 ± 0.14*

Values presented as means (μg/g) ± S.D. *P<0.05; P<0.01 as compared with control group (Student's t-test). The numbers in parentheses are the numbers of animals.

Table 7. Effect of the experimental diet on MAO and TH activities in the rat striatum

	Control	Group I	Control	Group II
	nmoles/mg protein/20 min.			
PEA-MAO	9.30 ± 0.67 (6)	7.26 ± 1.28**(7)	9.03 ± 1.43 (9)	8.82 ± 1.26 (7)
5-HT-MAO	26.60 ± 2.05 (7)	20.15 ± 3.41**(7)	36.10 ± 7.9 (9)	37.0 ± 7.4 (7)
	nmoles/mg protein/30 min.			
Tyrosine hydroxy-lase	10.9 ± 4.9 (6)	17.7 ± 2.7* (6)	12.7 ± 4.1 (9)	12.5 ± 7.9 (5)

Values presented as means ± S.D. *P<0.05; **P<0.01 as compared with control group (Student's t-test). The numbers in parentheses are the numbers of animals.

Discussion

Undernutrition in early life produces neurological and neurochemical alterations (Dobbing 1971; Merat and Dickerson 1973; Shoemaker and Wurtman 1971, 1973; Winick and Rosso 1973; West and Kemper 1976). A nutritional deprivation during the early life of the animals leads to irreversible alterations in the brain, while a comparable stress during the adult life has no such permanent effect (Dobbing 1971; Guroff 1980). After giving weanling rats a low protein diet for 56 days, plasma tyrosine concentration was reduced while brain tyrosine concentration was not changed. Prolonged feeding of the low protein diet for 176 days resulted in low concentrations of tyrosine in forebrain and brainstem (Pao and Dickerson 1975). Only a few studies have been made concerning the effects of malnutrition on brain growth and development after weaning. In our study, tyrosine concentrations in the plasma were decreased in group I and II, whereas tyrosine concentrations in the cerebral cortex were not significantly changed. The reason of this discrepancy remains to be investigated furthermore.

Shoemaker and Wurtman (1971; 1973) reported that changes in the levels and metabolism of biogenic amines in rat brain had resulted from malnutrition in early life. Using another method of nutritional deprivation, Marichich *et al.* (1979) and Detering *et al.* (1980,b) reported that an impaired metabolism of catecholamine was detectable in the developing brain of early life as a consequence of malnutrition. In our study, brain and body weights revealed a significant reduction in group I (Table 2). Therefore, it is considered that the function and development of catecholaminergic neurons are reduced in the rat brains of group I. The brain growth spurt is entirely postnatal (Dobbing 1971), and occurs between birth and the 28th day of neonatal life (Detering *et al.*, 1980,a). Another effect of malnutrition on the developing brain is the retardation of the accumulation of myelin (Krigman and Hogan 1976). The relative lack of this substance in the undernourished rat's brain contributes substantially to its poor development. In our study, DA and NE concentrations of all three brain regions were decreased or tended to decrease in group I (Table 5 and 6). Furthermore, TH activity in the striatum in group I was significantly increased while MAO activity was significantly decreased. Other investigaters have found that malnutrition increased the specific activity of TH (Shoemaker and Wurtman 1971; Marichich *et al.*, 1979; Detering *et al.*, 1980,a). In postnatal rats exposured to malnutrition, the activities of TH and dihydroxyphenylalanine decarboxylase in the brain were increased whereas those of dopamine β-hydroxylase and MAO were decreased (Detering *et al.*, 1980,a).

Pao and Dickerson (1975) and Stern *et al.* (1974) reported that administration of a low protein diet for 140-176 days to weanling rats retarded the growth of the brain. In group II of our study, body weight gain was significantly decreased following the malnutritional diet, whereas brain weight was not changed. The effect of undernutrition on the developing rat brain depends to some extent on the timing, duration, and severity of the deprivation. Thus, it is considered that the malnutrition in the group II may hardly influence development of catecholaminergic

neurons in rat brain.

Fatty liver and decreased blood tyrosine concentration following malnutrition were recognized in groups I and II. Since the brain tyrosine concentration was not changed, catecholamine metabolism in the brain may not be directly related to the fatty liver.

Since tyrosine concentration as well as, TH and MAO activities in group II did not differ from the control, decreased concentration of brain dopamine might result from increased release of dopamine into synaptic cleft or from inhibited re-uptake of dopamine from the synaptic membrane which is possibly damaged by malnutrition.

The present investigation has proved that lowered function of catecholaminergic neurons in young rats may be due to malnutrition. However, it should be considered that malnutrition may disturb the development of brain particularly in younger animals such as rats in our group I.

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