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Abstract

The present study investigated the brain catecholamine metabolism of rats with liver injury induced either by malnutrition or with CCl₄. In the malnutrition group, the plasma tyrosine concentration was low, while it showed a tendency to be high in the cerebral cortex. Dopamine concentrations were low in both the cerebral cortex and diencephalon. Norepinephrine concentrations were low in the cerebral cortex, striatum and diencephalon. Tyrosine hydroxylase activity was elevated while monoamine oxidase activity was decreased in the striatum. In the CCl₄ group, tyrosine concentrations in the plasma and cerebral cortex did not change. The dopamine concentration in the cerebral cortex increased five days after, and the norepinephrine concentration in the diencephalon increased 24 h after the last administration of CCl₄. These data suggest that catecholaminergic neurons in the brain may be substantially affected by liver injury. It may be considered that malnutrition disturbs brain development, particularly in young rats.

KEYWORDS: brain, catecholamine, malnutrition, carbon tetrachloride, liver injury

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INFLUENCE OF LIVER INJURY ON THE CATECHOLAMINE METABOLISM IN RAT BRAIN

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Abstract. The present study investigated the brain catecholamine metabolism of rats with liver injury induced either by malnutrition or with CCl_4 . In the malnutrition group, the plasma tyrosine concentration was low, while it showed a tendency to be high in the cerebral cortex. Dopamine concentrations were low in both the cerebral cortex and diencephalon. Norepinephrine concentrations were low in the cerebral cortex, striatum and diencephalon. Tyrosine hydroxylase activity was elevated while monoamine oxidase activity was decreased in the striatum. In the CCl_4 group, tyrosine concentrations in the plasma and cerebral cortex did not change. The dopamine concentration in the cerebral cortex increased five days after, and the norepinephrine concentration in the diencephalon increased 24 h after the last administration of CCl_4 . These data suggest that catecholaminergic neurons in the brain may be substantially affected by liver injury. It may be considered that malnutrition disturbs brain development, particularly in young rats.

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The plasma tyrosine concentration fluctuates during the day in response to food consumption (1, 2), and affects the brain tyrosine concentration (3, 4). The brain tyrosine level influences the rate at which neurons synthesize catecholamine neurotransmitters such as dopamine (DA) and norepinephrine (NE) (5).

Fatty liver arises from malnutrition, and chronic fatty liver is apt to lead to liver cirrhosis. The plasma tyrosine concentration decreases with a low protein diet, but is elevated in hepatic encephalopathy (6). Therefore, plasma tyrosine alteration due to fatty liver might influence catecholaminergic activity in the brain.

In this study, an attempt was undertaken to examine whether or not liver injury affects catecholaminergic neurons in the central nervous system.

MATERIALS AND METHODS

Animals. Male Wistar rats were maintained at 24 °C and 55 % humidity with a 12 h light and dark cycle (lights on 01 : 00 to 13 : 00). Animals of the first group (Group I) weighed 90 g when the experiment began. Rats were fed *ad libitum* for 4 weeks with the diet shown in Table 1, and then decapitated. Animals of the second group (Group II) weighed approximately 150 g at the start of the experiment. They received one i.p. injection of CCl_4 (0.1 ml/150 g body weight) a day for 2 days. Rats were sacrificed by decapitation 24 h and

TABLE 1. COMPOSITION OF THE EXPERIMENTAL DIET (%)

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

*Mineral mixture (%): $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 14.56; KH_2PO_4 , 25.72; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 9.35; NaCl , 4.66; Ca-lactate, 35.09; Fe-citrate, 3.18; MgSO_4 , 7.17; $\text{ZnSO}_4 \cdot 4\text{-}6\text{H}_2\text{O}$, 0.12; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.03; KI, 0.01.

**Vitamin mixture (choline free/100 g): vitamin A acetate, 50,000 IU; vitamin D₃, 10,000 IU; thiamin HCl, 120 mg; riboflavin, 400 mg; pyridoxine HCl, 80 mg; vitamin B₁₂, 0.05 mg; ascorbic acid, 3,000 mg; vitamin E acetate, 500 mg; vitamin K₃, 520 mg; d-biotin, 2 mg; folic acid, 20 mg; calcium pantothenate, 500 mg; p-aminobenzoic acid, 500 mg; nicotinic acid, 600 mg; inositol, 600 mg; cellulose powder.

five days after the last injection of CCl_4 . Control groups and Group II were fed *ad libitum* with oriental MF (Oriental Yeast Co. Ltd., Japan).

The animals were decapitated between 08:00 and 10:00. After decapitation the head was immersed into liquid nitrogen for about five seconds. The brain was removed and dissected rapidly into the following three regions: whole cerebral cortex, striatum and diencephalon (7). The tissues were weighed and homogenized in a polytron homogenizer (Kinematica, Switzerland) with 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 xg. Supernatants were kept frozen at -80 °C until assayed. Blood samples were taken from the neck, and the plasma was collected by centrifugation and stored at -20 °C.

Assay. The concentrations of dopamine (DA) and norepinephrine (NE) were determined in the supernatant of the homogenate by a radioenzymatic assay according to a slightly modified method of Sole and Hussain using catechol-O-methyltransferase [COMT, catechol methyltransferase (E.C.2.1.1.6, S-adenosyl-1-methionine; catechol-O-methyltransferase)] (8). Thin layer chromatography was performed for separation and purification of the synthesized products according to the method of Peuler and Johnson using t-amylalcohol: benzene: 40% methylamine (6:2:3) as the solvent system (9). Coefficients of intraassay variation in the radioenzymatic assay 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 (10). The activity of 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, phenylethylamine (PEA, the final concentration at about 0.01 mM) and 5-hydroxytryptamine (5-HT: the final concentration at about 1 mM) (11, 12). Tyrosine concentrations in the plasma and cerebral cortex were estimated by the method of Udenfriend and Cooper (13). The method of Folch *et al.* was used to extract lipids from liver (14). Aliquots of lipid extracts were used to assay for triglycerides (15) and cholesterol (16). Total lipid was determined gravimetrically. Protein content was determined by the procedure of Lowry *et al.* using human serum albumin as a standard (17).

Data are shown as the mean \pm S.D. Results were statistically analyzed using Student's t-test.

RESULTS

Table 2 shows the lipid contents in the liver of rats which were fed a malnutritional diet or administered CCl₄. For histological examination, formaldehyde paraolast-embedded sections were stained with hematoxylin-eosin or Sudan III and examined light microscopically. Group I rats had large fatty cycts in their liver, and fatty degeneration was recognized in the liver of Group II rats.

TABLE 2. CONTENTS OF TOTAL LIPID, TRIGLYCERIDES AND TOTAL CHOLESTEROL IN THE LIVER OF RATS

	Total lipid	Triglycerides	Total cholesterol
Group I			
Control (8)	33.7 ± 4.4	10.0 ± 1.1	3.9 ± 0.4
Malnutrition (7)	212.5 ± 75.7**	149.0 ± 50.5**	5.6 ± 1.5*
Group II			
Control (8)	42.6 ± 2.8	7.8 ± 1.6	3.5 ± 0.5
After administration of CCl ₄			
24 hours (7)	50.8 ± 4.8**	13.1 ± 3.1**	4.8 ± 0.8**
5 days (7)	38.7 ± 5.4	7.9 ± 1.9	4.1 ± 0.6*

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

TABLE 3. THE WEIGHTS OF BODY, BRAIN AND LIVER FROM RATS FED THE MALNUTRITIONAL DIET

	Body weight	Brain weight	Liver weight
Control (7)	316 ± 17	1.97 ± 0.07	13.52 ± 0.72
Group I (14)	127 ± 16*	1.74 ± 0.06*	6.31 ± 1.63*

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

TABLE 4. CONTENTS OF TYROSINE IN THE PLASMA AND BRAIN OF RATS

	Group I		Group II		
	Control	Malnutrition	Control	24 hours	5 days
Plasma (μmol/l)	60.3±4.9 (6)	29.9±12.8*(10)	49.8±20.3 (7)	49.4±29.6 (5)	57.2±15.5 (5)
Cerebral cortex (nmol/g)	77.0±9.5 (8)	88.9±14.1 (8)	69.1±18.6 (6)	91.4±32.2 (6)	72.5±20.7 (6)

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

Table 3 shows the body, brain and liver weight in Group I. The plasma tyrosine concentration in Group I was low, while the cerebral cortex showed a flange. The entire capsular attachment needed to be detached, and the cup's anatomical setting on the defected acetabulum was impossible. Fichler (1) developed

	Group I		Group II		
	Control	Malnutrition	Control	24 hours	5 days
Plasma (μmol/l)	60.3±4.9 (6)	29.9±12.8*(10)	49.8±20.3 (7)	49.4±29.6 (5)	57.2±15.5 (5)

Table 5 shows the catecholamine concentration in each region of the brain of Group I. DA concentrations were low in the cerebral cortex and diencephalon. NE concentrations were low in all three regions. In Group II, the NE concentration in the diencephalon was increased 24 h after ($P < 0.01$), and the DA concentration in the cerebral cortex was increased 5 days after the last injection of CCl_4 ($P < 0.05$). However, the catecholamine concentrations were not significantly different from the controls in all the other regions.

TABLE 5. CONTENTS OF CATECHOLAMINE IN THE BRAIN FROM RATS FED THE MALNUTRITIONAL DIET

	Dopamine		Norepinephrine	
	Control (8)	Malnutrition (8)	Control (7)	Malnutrition (7)
Cerebral cortex	0.78 ± 0.26	$0.26 \pm 0.10^*$	0.42 ± 0.06	$0.14 \pm 0.01^*$
Striatum	6.51 ± 1.74	5.90 ± 1.67	0.65 ± 0.19	$0.33 \pm 0.13^*$
Diencephalon	0.21 ± 0.06	$0.11 \pm 0.04^*$	0.43 ± 0.20	$0.16 \pm 0.04^*$

Values are presented as means ($\mu\text{g/g}$) \pm S.D. * $P < 0.01$ as compared with control group (Student's t-test). The numbers in parentheses are the numbers of animals.

TH activity (nmol/mg protein/30 min) in the striatum of Group I (17.7 ± 2.7 vs. 10.9 ± 4.9 , $P < 0.05$) and of Group II 24 h after the CCl_4 administration (8.40 ± 1.00 vs. 6.77 ± 1.03 , $P < 0.05$) increased above the control group levels. The activities (nmol/mg protein/20 min) of PEA-MAO (7.26 ± 1.28 vs. 9.30 ± 0.67 , $P < 0.01$) and of 5-HT-MAO (20.15 ± 3.14 vs. 26.10 ± 2.05 , $P < 0.01$) in the striatum of malnourished rats were lower than in the control group.

DISCUSSION

Undernutrition in early life produces neurological and neurochemical alterations (18, 19, 20, 21, 22, 23). A nutritional deprivation during the early life of animals leads to irreversible alterations in the brain, while a comparable stress during adult life has no such permanent effect (18, 24). The effect of undernutrition on the developing rat brain depends to some extent on the timing, duration and severity of the deprivation (24). However, only few studies have been made concerning the effects of malnutrition on brain growth and development after weaning. Since brain and body weights were significantly lower in group I rats (Table 2), we suspected that malnutrition might have influenced the development of their brains.

In our study, tyrosine concentrations in the plasma were low in Group I, whereas those in the cerebral cortex were not significantly different from controls. Similarly Pao and Dickerson (25) reported that the plasma tyrosine concentration was reduced in weanling rats after giving them a low protein diet for 56 days, while the brain tyrosine concentration was not changed. However, prolonged

feeding of the low protein diet for 176 days resulted in low concentrations of tyrosine in the forebrain and brainstem.

In our study, DA and NE concentrations of all three brain regions were decreased or tended to decrease in Group I. Furthermore, TH activity in the striatum from group I was significantly increased while MAO activity was significantly decreased in the same region. Shoemaker and Wurtman (20, 21) reported that changes in the levels and metabolism of biogenic amines in rat brain resulted from malnutrition in early life. Using another method of nutritional deprivation, Marichich *et al.* (26) and Detering *et al.* (27) reported that impaired metabolism of catecholamine was detectable in the developing brain during early life as a consequence of malnutrition. Furthermore, other investigators have found that malnutrition increased the specific activity of TH (20, 26, 28). In postnatal rats subjected to malnutrition, the activities of TH and dihydroxyphenylalanine decarboxylase in the brain were increased, whereas those of dopamine β -hydroxylase and MAO were decreased (28). Therefore, it is considered that the function and development of catecholaminergic neurons are disturbed in the brains of Group I rats. In our study, fatty liver and decreased blood tyrosine concentration following malnutrition were recognized in Group I. Since the brain tyrosine concentration was not changed, catecholamine metabolism in the brain was not directly related to the fatty liver.

In our study, catecholamine concentrations in the brains of Group II rats were different from those in control rat brains. Although Lahl (29) reported that acute administration of CCl_4 directly influenced the fine structure of the rat brain, it remains to be clarified whether or not CCl_4 affects catecholaminergic neurons in the brain.

The present investigation has not proved that function of catecholaminergic neurons in rats may be directly affected by liver injury. However, malnutrition may disturb the development of the brain, particularly in young rats, and consequently give rise to impaired function of catecholamine neurons.

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