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Abstract

Rats were fed a choline-free low protein diet for 12 or 26 weeks. In the 12-week group, the brain tyrosine concentration did not change. Dopamine levels were low in both the cerebral cortex and striatum. Norepinephrine level was low in the diencephalon. In the 26-week group, the tyrosine concentration was high in the brain. However, the dopamine and norepinephrine levels did not change in the cerebral cortex, striatum and hypothalamus. Furthermore, in another group of rats which were intraperitoneally injected with tyrosine, the brain tyrosine concentration was high, whereas the dopamine and norepinephrine levels in the hypothalamus were not significantly different from control levels.

KEYWORDS: brain tyrosine, catecholamine, malnutrition, liver injury

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—— BRIEF NOTE ——

RELATIONSHIP OF TYROSINE CONCENTRATION TO CATECHOLAMINE LEVELS IN RAT BRAIN

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Abstract. Rats were fed a choline-free low protein diet for 12 or 26 weeks. In the 12-week group, the brain tyrosine concentration did not change. Dopamine levels were low in both the cerebral cortex and striatum. Norepinephrine level was low in the diencephalon. In the 26-week group, the tyrosine concentration was high in the brain. However, the dopamine and norepinephrine levels did not change in the cerebral cortex, striatum and hypothalamus. Furthermore, in another group of rats which were intraperitoneally injected with tyrosine, the brain tyrosine concentration was high, whereas the dopamine and norepinephrine levels in the hypothalamus were not significantly different from control levels.

Key words : brain tyrosine, catecholamine, malnutrition. liver injury.

Catecholamine metabolism in catecholaminergic neurons depends partly on the availability of tyrosine (1, 2). Intraperitoneal administration of l-tyrosine increases the tyrosine concentration in the brain (3).

Liver injury causes major changes in the patient's plasma tyrosine level (4). Jellinger *et al.* (5) reported that brain dopamine showed a mild decrease in non-comatous patients with liver cirrhosis. On the contrary, Cuilleret *et al.* (6) reported that brain dopamine and norepinephrine levels were not low in cirrhotic patients with hepatic encephalophathy. Therefore, reports conflict as to the changes in the catecholamine concentrations in the brain of patients with hepatic failure.

In this study, we investigated the effect of altered brain tyrosine concentrations due to liver injury on the catecholaminergic nervous system in rat brain, because these neurons participate in many key physiologic events.

Materials and Methods. Male Wistar rats were maintained at 24 ℃ and 55 % humidity with a 12 h light and dark cycle (lights on 01:00 to 13:00). Rats were fed *ad libitum* for 12 weeks (the first group; initial body weight of 125 g) or 26 weeks (the second group; initial body weight of 95 g) a choline-free diet which has already been described (7). Animals of the control group were fed *ad libitum* a commercial stock diet (Oriental Yeast Co. Ltd., Japan).

Rats (body weight of 200 g) in the third group were intraperitoneally given

l-tyrosine (100 mg/ml/kg body weight) suspended in 0.1% Triton X-100 solution. The control rats were given only 0.1 % Triton X-100 solution. These rats were sacrificed one hour after the injection.

The animals were sacrificed between 08 : 00 and 10 : 00 by the near-freezing method (8). Brains were removed and dissected following a modification of the procedure described by Schubert and Sedvall (9). Brain tissues were kept frozen at -70 ℃ until assayed. Blood samples were taken from the neck, and the plasma was collected by centrifugation and stored at -20 ℃.

The dopamine (DA) and norepinephrine (NE) contents were measured by radioenzymatic assay (10) or by high performance liquid chromatography using a highly sensitive electrochemical detector.

Measurement of catecholamine contents by high-performance liquid chromatography with electrochemical detection (HPLC-ECD, Irika, Japan) was performed according to the following procedure. The brain tissues were weighed and homogenized (Kinematica, Switzerland) in 9 vol (w/v) of cold 0.9 % potassium chloride. Extraction of catecholamine was performed using the protocol of Maruyama *et al.* (11). Catecholamines were separated by chromatography on a reverse phase HPLC column (Lichrosorb RP-18 (5 μm), 4 mm $\times$ 250 mm, Cica-Merck), and were measured electrochemically using an amperometric detector with an Ag/AgCl electrode. The mobile phase was composed of 3.0 % acetonitrile, 97 % 0.1 M potassium phosphate buffer (pH 3.2), 0.1 mM EDTA-Na and 0.6g/L sodium heptansulfonate. The detector potential was set at +0.80 V versus the Ag/AgCl reference electrode. The mobile phase was pumped at a flow rate of 1.0 ml/min.

Tyrosine concentrations in the plasma and brain tissue were assayed fluorometrically as described by Waalkes and Udenfriend (12). Triglycerides in the liver were determined according to the method of Sardesai and Manning (13). Protein content was determined by the procedure of Lowry *et al.* (14) using human serum albumin as the standard.

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

Results and Discussion. In the first and second experimental diet groups, the body weight gain of animals was much less than that of animals given the control diet, whereas both the brain and liver wet weights of experimental diet rats were not significantly different from those of control diet rats.

Formaldehyde paraolast-embedded sections were stained with hematoxylin-eosin, Sudan III or Azan and examined light microscopically. Rats of the first group revealed large fatty cysts in the liver. Fibrosis (nine-twelfths) or cirrhosis (three-twelfths) was recognized in the liver from rats of the second group. Although the triglyceride level in the livers of the first control group was 13.7 $\pm$ 2.5 mg/g, fatty degeneration was hardly recognized in livers of this group.

The tyrosine concentration in the plasma of the first group (54.0 $\pm$ 14.8

TABLE 1. CONTENTS OF CATECHOLAMINE IN THE BRAIN OF RATS FED A MALNUTRITIONAL DIET FOR 12 WEEKS

	Dopamine		Norepinephrine	
	Control diet (8)	Experimental diet (7)	Control diet (8)	Experimental diet (7)
Cerebral cortex	0.25 ± 0.08	0.15 ± 0.06*	0.20 ± 0.09	0.13 ± 0.05
Striatum	4.98 ± 1.09	3.18 ± 0.86**	0.23 ± 0.09	0.25 ± 0.13
Diencephalon	0.14 ± 0.04	0.10 ± 0.04	0.60 ± 0.12	0.44 ± 0.14*

Values are presented as means (μg/g tissue) ± 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 2. CONTENTS OF CATECHOLAMINE IN THE BRAIN OF RATS FED A MALNUTRITIONAL DIET FOR 26 WEEKS

	Dopamine		Norepinephrine	
	Control diet (6)	Experimental diet (12)	Control diet (6)	Experimental diet (12)
Cerebral cortex	2.8 ± 1.2	2.1 ± 1.0	2.1 ± 0.28	2.1 ± 0.37
Striatum	42.3 ± 13.7	45.3 ± 13.3	0.74 ± 0.30	1.2 ± 0.55
Hippocampus	0.11 ± 0.04	0.17 ± 0.07	2.3 ± 0.59	2.1 ± 0.24
Thalamus	0.25 ± 0.06	0.32 ± 0.09	2.6 ± 0.41	2.9 ± 0.57
Hypothalamus	2.5 ± 0.35	2.3 ± 0.70	9.0 ± 1.7	7.8 ± 1.1

Values are presented as means (ng/mg protein) ± S.D.
The numbers in parentheses are the numbers of animals.

μmol/l vs. 80.2 ± 9.7 μmol/l, $P < 0.01$) was less than in the control group, whereas that in the cerebral cortex was the same in both groups. On the other hand, DA and NE concentrations in the brain were significantly low (Table 1). These data are generally in agreement with our previous study (7). No change, however, was found in the activities of enzymes involved in the synthesis and degradation of catecholamines; the activity (nmol/mg protein/30 min) of tyrosine hydroxylase (12.5 ± 7.9 vs. 12.7 ± 4.1) and the activity (nmol/mg protein/20 min) of phenylethylamine-monoamine oxidase (8.82 ± 1.26 vs. 9.03 ± 1.43) in the striatum were not significantly different from each control.

In the second group, the plasma (226 ± 105 μmol/l vs. 93.4 ± 4.8 μmol/l, $P < 0.01$) and striatum (325 ± 180 nmol/g vs. 131.7 ± 11.8 nmol/g, $P < 0.01$) tyrosine concentrations were higher than in the control groups. DA and NE levels, however, were not significantly different from the controls in all brain regions examined (Table 2). To our knowledge, no similar results have so far been reported. Zieve and Olsen (15) observed normal brain concentrations of DA and NE in the acute experimental hepatic coma rat. On the contrary, another paper (16)

TABLE 3. PLASMA AND CEREBRAL CORTEX TYROSINE CONCENTRATIONS AND HYPOTHALAMUS CATECHOLAMINE LEVELS OF THE RATS AFTER TYROSINE ADMINISTRATION

	Tyrosine		Dopamine	Norepinephrine
	Plasma (μmol/l)	Cerebral cortex (nmol/g)	Hypothalamus (ng/mg protein)	(ng/mg protein)
Control (6)	62.1 ± 10.8	82.9 ± 10.8	2.17 ± 0.29	5.44 ± 1.00
Tyrosine (6)	116.7 ± 23.1*	163.1 ± 48.2*	1.95 ± 0.50	5.48 ± 0.62

Values are 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.

reported that brain DA and NE levels decreased in the pig with acute experimental hepatic coma.

In rats of the third tyrosine-loaded group, the brain tyrosine concentration was significantly higher than in control animals. However, DA and NE levels in the hypothalamus were not different (Table 3). This result confirms partly the data of Oishi and Wurtman (17). They recognized that tyrosine administration did not cause significant changes in brain levels of DA and NE, or their metabolites DOPAC, HVA and MHPG-SO_4.

The present investigation has shown that brain DA and NE levels in the brain were not significantly different from control levels in rats whose brain tyrosine concentration was elevated due to possible liver injury.

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