Neurochemical Research, Vol. 5, No. 1, 1980

EFFECT OF FOOD RESTRICTION ON SEROTONIN METABOLISM IN RAT BRAIN

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Accepted June 5, 1979

The brain concentration of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) increased in rats maintained on restricted volume of low-protein or normal-protein diet, whereas these two agents decreased in rats fed low-protein diet ad libitum. In these two food-restricted groups brain 5-HT and 5-HIAA concentrations were not correlated with brain tryptophan hydroxylase activity, but the concentrations correlated closely with cerebral tryptophan concentrations. The cerebral tryptophan concentration in the two food-restricted groups was not consistent with the total or free tryptophan concentration in plasma. In these restricted rats cerebral tryptophan concentration was elevated, and, unlike the plasma tryptophan, it showed no diurnal variation. These results suggested that tryptophan uptake into the brain from plasma was enhanced by limiting food volume intake. Tryptophan uptake was increased by glucagon injection without changing the plasma tryptophan level, but injection of hydrocortisone or insulin had little or no effect on tryptophan concentration in either the plasma or brain. D-Glucose injection elevated plasma tryptophan concentration but decreased brain tryptophan concentration.

INTRODUCTION

Rats fed a low-protein diet ad libitum show a decrease in brain serotonin (5-HT) concentration (1–3). The mechanism of this decrease has been well investigated (1). The investigators concluded that low brain concentration of 5-HT reflects a low concentration of cerebral tryptophan (precursor of 5-HT), since a low-protein diet results in a low level of plasma tryptophan and insulin.

In our previous study (4) a high level of brain 5-HT concentration was found in rats fed a restricted volume of low- or normal-protein diet from

0364-3190/80/0100-0069$03.00/0

weaning to 8 months of age. The difference between our results and those of others (1–3) might be due to the restriction of the food volume.

In the present study we examined the effect of restricting the food volume on brain 5-HT metabolism with rats fed a low- or normal-protein diet and examined the effect of injection of certain hormones on cerebral and plasma tryptophan levels.

EXPERIMENTAL PROCEDURE

Animals. Pregnant Wistar albino rats were purchased from an animal dealer (Japan Laboratory Animals Inc.) on the first day of gestation. Prior to delivery the dams were fed a normal diet containing 25% casein. After delivery, the rats were divided into four groups as described below:

1. *Normal-protein diet group (control rats).* Pups were nursed by dams fed the normal diet and weaned at the 22nd day of age. They were fed the normal diet ad libitum until 4 months of age.
2. *Restricted normal-protein diet group (C-80 rats).* Pups were nursed by dams fed the normal diet. After weaning, they were fed the normal diet ad libitum until 3 months of age. Thereafter, the normal diet volume was restricted, based on the body weight of rats, being maintained at about 80% of the control rat weight. Rats were fed once daily between 4:00 and 6:00 P.M.
3. *Low-protein diet group (deprived rats).* Pups were nursed by dams fed a low-protein diet containing 12% casein. After weaning, they were fed the low-protein diet ad libitum until 4 months of age.
4. *Restricted low-protein diet group (D-80 rats).* Pups were nursed by dams fed the low-protein diet. After weaning, they were fed the low-protein diet ad libitum until 3 months of age. Thereafter, the low-protein diet volume was restricted and the body weight was maintained at about 80% of the deprived rat weight. Rats were fed once daily between 4:00 and 6:00 P.M.

In all groups, there were eight pups to each dam. The total calories of both the low- and normal-protein diet (Oriental East Co. Ltd.) corresponded to 3.5 kcal/g. The diet compositions are shown in Table I.

Chemical Determinations. Biochemical analyses were performed when the rats reached 4 months of age. At 10:00 A.M. the rats were anesthetized with ether and blood samples were withdrawn from the abdominal artery. The brain was rapidly removed and separated into the pallium cerebri and brain stem in a cold room.

5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the pallium cerebri and brain stem were measured by the fluorometric method of Fischer et al. (5). The fluorescence of 5-HT and 5-HIAA was measured at 300 nm (excitation) and 535 nm (emission) using spectrophotofluorometry (Hitachi-204).

Tryptophan concentration in the brain stem and plasma was measured according to the norharman method of Denckla and Dewey (6). Free tryptophan in plasma was measured by application of 1 ml of plasma to an ultracentriflo cone (CF-50A, Amicon Corp., Lexington, Massachusetts) and centrifugation at 800*g* for 30 min at room temperature. The total effluent was used (7).

The activity of tryptophan hydroxylase (E.C. 1.99.1.4) in the brain stem was measured by the modified method of Gal and Patterson (8). The brain stem was homogenized in 4 vol

TABLE I
CHARACTERISTICS OF THE TWO DIETS USED IN STUDY

Composition (%)	Normal-protein diet	Low-protein diet
Casein	25	12
Cornstarch	38	51
Sugar	5	5
Vegetable oil	6	6
Salt mixture	6	6
Vitamin mixture	2	2
Calories (kcal/g)	3.52	3.45

of tris-acetate buffer (pH 7.6) containing 0.1 mM dithiothreitol (Nakarai Chem.) and 0.1% Triton X-100 (New England Nuclear) and centrifuged at 30,000*g* for 20 min. The supernatant (100 μl) was incubated at 37.6°C for 20 min with the assay medium in a final volume of 1 ml, consisting of 50 mM tris-acetate buffer (pH 7.6), 0.1 mM NSD-1055 (Calbiochem), 0.1 mM dithiothreitol, 10 μg catalase (Sigma), 0.088 mM L-tryptophan (Nakarai Chem.), and 0.16 mM 6-methyltetrahydropteridine (Calbiochem). The reaction was stopped with 0.1 ml of 70% perchloric acid. After centrifugation at 800*g* for 10 min, 0.5 ml of the deproteinized supernatant was reacted with 0.1 ml of 1% cysteine (Nakarai Chem.) and 1.65 ml of *o*-phthalaldehyde reagent [1 mg *o*-phthalaldehyde (Nakarai Chem.) in 82.5 ml of 10 N HCl] for 15 min in boiling water. After cooling, fluorescence was measured at 360 nm (excitation) and 470 nm (emission).

Plasma glucose concentration was measured by the method of Hultman (9), and protein concentration was assayed by the method of Lowry et al. (10).

Administration of Hormones and D-*Glucose*. Previously untreated control rats were given a single injection of hormone or D-glucose. Hydrocortisone acetate (10 mg/kg, Nakarai Chem.) or bovine glucagon (10 mg/kg, Calbiochem.) was injected intramuscularly 1 hr before the rats were killed. Porcine insulin (5 units/kg, Novo Inudstry) was injected intraperitoneally at 30 min, 1 hr, and 2 hr before the rats were killed. D-Glucose (150 mg/kg, Nakarai Chem.) was injected intravenously 30 min before they were killed.

RESULTS

Brain 5-HT and 5-HIAA Concentrations

Table II shows the concentrations of 5-HT and 5-HIAA in the pallium cerebri and brain stem in the four animal groups. In control rats the mean concentration of 5-HT and 5-HIAA in the pallium cerebri was 0.70 and 0.39 (μg/g wet wt), respectively. Higher concentration was found in the brain stem. In both of these brain regions 5-HT and 5-HIAA were significantly lower in deprived rats than in control rats. In contrast, 5-hy-

TABLE II
CONCENTRATIONS OF 5-HT AND 5-HIAA IN PALLIUM CEREBRI AND BRAIN STEM[a]

Group		Pallium cerebri		Brain stem	
		5-HT	5-HIAA	5-HT	5-HIAA
Control rats	(6)	0.70 ± 0.02	0.39 ± 0.02	1.06 ± 0.03	0.63 ± 0.01
C-80 rats	(7)	1.05 ± 0.04***	0.57 ± 0.01***	1.38 ± 0.04***	0.83 ± 0.03***
Deprived rats	(4)	0.57 ± 0.03**	0.29 ± 0.02*	0.88 ± 0.05*	0.49 ± 0.03***
D-80 rats	(5)	1.04 ± 0.04***	0.52 ± 0.03**	1.37 ± 0.04***	0.74 ± 0.01***

[a] Values are expressed in μg/g wet wt as mean ± SEM with the number of rats shown in parentheses. Values with asterisks are significantly different from controls by Student's *t* test with two-tailed distributions.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

TABLE III
TRYPTOPHAN (TRP) HYDROXYLASE ACTIVITY AND TRYPTOPHAN CONCENTRATION IN THE BRAIN STEM[a]

Group	TRP hydroxylase activity (nmol/mg protein/hr)	TRP concentration (nmol/g wet wt)
Control rats	1.71 ± 0.05 (6)	11.4 ± 0.03 (12)
C-80 rats	1.70 ± 0.05 (8)	13.6 ± 0.2*** (11)
Deprived rats	1.42 ± 0.07** (4)	8.5 ± 0.6*** (5)
D-80 rats	1.35 ± 0.03** (4)	14.0 ± 0.3*** (4)

[a] Values are expressed as mean ± SEM with the number of rats shown in parentheses. Values with asterisks are significantly different from controls by Student's *t* test with two-tailed distributions.
** $P < 0.01$.
*** $P < 0.001$.

droxyindoles were remarkably higher in both brain regions of the two restricted groups (C-80 and D-80 rats).

Brain Stem Tryptophan Concentration and Tryptophan Hydroxylase Activity

In control rats the mean hydroxylase activity was 1.71 nmol/mg protein/hr (Table III). The C-80 rats showed practically the same value as the control, but the deprived and D-80 rats showed significantly lower values.

In the two restricted groups (C-80 and D-80 rats) the tryptophan hydroxylase activities were not directly correlated with brain 5-HT and 5-HIAA concentrations.

The mean tryptophan concentration of the control was 11.4 nmol/g wet wt (Table III). The tryptophan concentration in the brain stem of deprived rats was significantly lower than that in the control rats. In the two restricted groups (C-80 and D-80 rats) the concentration was significantly higher than in the control. These alterations in tryptophan concentration in the brain stem were positively correlated with brain 5-HT and 5-HIAA levels.

Total and Free Tryptophan Concentrations in Plasma

The mean total and free tryptophan concentration in plasma of control rats was 125 and 5.45 (nmol/ml), respectively (Table IV). The free tryptophan concentration was 4.36% of the total tryptophan concentration. Total tryptophan concentration in plasma of the deprived and D-80 rats was significantly lower, and it was reduced slightly in C-80 rats but not significantly. In all animal groups free tryptophan levels were parallel to total tryptophan levels, and the ratio of free tryptophan to total tryptophan varied little between the groups. Total and free tryptophan levels in plasma did not correspond to tryptophan, 5-HT, and 5-HIAA levels in the brain.

TABLE IV
TOTAL AND FREE TRYPTOPHAN CONCENTRATIONS IN PLASMA[a]

Group	Total tryptophan (nmol/ml)	Free tryptophan (nmol/ml)
Control rats	125 ± 3 (11)	5.45 ± 0.33 (3)
C-80 rats	113 ± 2 (11)	5.58 ± 0.16 (3)
Deprived rats	84 ± 4*** (8)	3.66 ± 0.57* (3)
D-80 rats	101 ± 7** (4)	3.02 ± 0.21*** (4)

[a] Values are expressed as mean ± SEM with the number of rats shown in parentheses. Values with asterisks are significantly different from controls by Student's *t* test with two-tailed distributions.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Diurnal Rhythm of Tryptophan in Brain Stem and Plasma

Figure 1 shows the diurnal rhythms of total tryptophan concentrations in plasma and brain stem in control and C-80 rats. In control rats, plasma tryptophan concentration showed a peak at 2:00 A.M.; however, brain stem concentration was lowest at this time. In C-80 rats, tryptophan concentration in plasma showed a peak at 6:00 P.M., just after food intake, and decreased gradually until 2 A.M. Tryptophan concentration in the brain stem of C-80 rats remained high throughout the diurnal period. In both groups of rats the brain tryptophan level did not correlate with the plasma tryptophan level.

Effect of Hormones and D-*Glucose on Tryptophan Level*

Figure 2 shows the effect of hormones and D-glucose on tryptophan level in the brain stem and plasma. At 1 hr after hydrocortisone injection, no change in tryptophan level was observed in either the brain stem or plasma. However, at 1 hr after glucagon injection, tryptophan concentration in the brain stem increased by about 60% in the control, but no effect was observed on plasma tryptophan level. At 30 min after injection of D-glucose, plasma tryptophan concentration increased significantly, while cerebral tryptophan concentration decreased. Figure 3 shows the

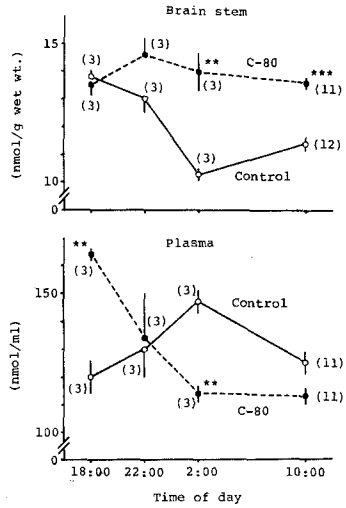

FIG. 1. Diurnal rhythms of tryptophan concentration in the brain stem and plasma. Each point represents the mean value, and vertical lines represent SEM with the number of rats in parentheses. The values with asterisks are significantly different from controls at each time by Student's t test with two-tailed distributions: ** $P < 0.01$, *** $P < 0.001$.

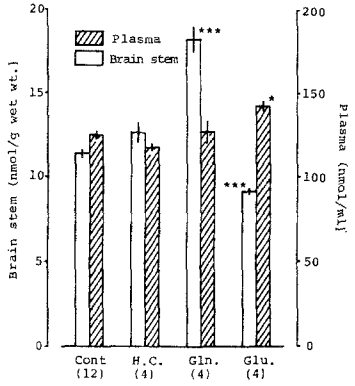

FIG 2. Effect of hormones and D-glucose on tryptophan concentration in the brain stem and plasma. 10 mg/kg of hydrocortisone acetate (H.C.) or 10 mg/kg of glucagon (Gln.) was injected into rats intramuscularly 1 hr before they were killed. 150 mg/kg of D-glucose (Glu.) was injected intravenously 30 min before the rats were killed. Each column represents the mean value, and vertical lines represent SEM with the number of rats in parentheses. The values with asterisks are significantly different from controls by Student's t test with two-tailed distributions: * $P < 0.05$, *** $P < 0.001$.

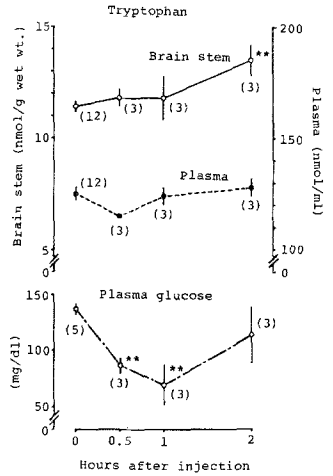

FIG. 3. Effect of insulin injection on tryptophan concentration in the brain stem and plasma and on plasma glucose concentration. Insulin (5 units/kg) was injected into rats intraperitoneally at 30 min, 1 hr, and 2 hr before they were killed. Each point represents the mean value, and vertical lines represent SEM with the number of rats in parentheses. The values with asterisks are significantly different from the values at 0 time by Student's t test with two-tailed distributions: ** $P < 0.01$.

concentrations of tryptophan in plasma and brain stem and the blood glucose level after injection of insulin. Blood glucose level declined clearly at 30 min and at 1 hr after the injection, but rebounded to the normal range at 2 hr after the injection. Plasma tryptophan level remained constant after insulin treatment. Cerebral tryptophan level varied little at 30 min and 1 hr after insulin injection, but at 2 hr after injection the concentration increased significantly over 0 time.

DISCUSSION

The present study demonstrates that brain 5-HT and 5-HIAA levels were highly influenced by restricting the volume of normal- and low-protein diet fed to rats. Brain 5-HT and 5-HIAA concentrations were significantly lower in rats fed the low-protein diet ad libitum, as already indicated by others (1–3). In contrast, the concentrations were much higher in food-restricted animals (C-80 and D-80 rats).

Brain tryptophan hydroxylase is thought to be a rate-limiting enzyme for 5-HT synthesis (11,12) and a factor in determination of brain 5-HT and 5-HIAA levels. However, in the present study, the activities of tryptophan hydroxylase in the four animal groups did not correspond to brain 5-HT and 5-HIAA levels. In contrast, the concentration of cerebral tryptophan was positively correlated with brain 5-HT and 5-HIAA concentrations. In fact, the K_m value of tryptophan hydroxylase for its substrate is much higher (13) than the concentration of tryptophan normally present in the rat brain (14). Thus it is likely that the brain 5-HT and 5-HIAA levels depend more closely on brain tryptophan levels than on tryptophan hydroxylase activity.

The complexity of the relationship between food intake and brain tryptophan metabolism has been reported by Fernstrom and Wurtman (15): high-carbohydrate diet increases both plasma and cerebral tryptophan levels, and cerebral tryptophan level is reflected by plasma tryptophan level (16). Tryptophan in plasma is present in two forms, free and bound (17,18), and it is thought that only free tryptophan can enter into brain tissue (19,20). However, according to our findings, alterations in cerebral tryptophan level were reflected in neither changes in total nor free tryptophan in plasma. It was found in food-restricted rats (C-80 and D-80 rats) that the concentrations of cerebral and plasma tryptophan were inversed. In C-80 and D-80 rats, cerebral tryptophan remained at a higher level, although total and free tryptophan in plasma decreased or tended to decrease. It is therefore postulated that the uptake of tryptophan into brain from plasma could be enhanced by some aspect of food restriction. Such

a possibility is supported by the diurnal study. In food-restricted rats (C-80 rats), cerebral tryptophan remained high, whereas plasma tryptophan gradually decreased from 6:00 P.M. to 2:00 A.M. This suggests that the higher level of cerebral tryptophan in food-restricted rats was caused by acceleration of its uptake into brain from plasma. Perez-Cruet et al. (21) and Curzon et al. (22) also suggested that the possibility that tryptophan uptake into brain was enhanced in rats fasted for 24 hr.

The acceleration of tryptophan uptake into the brain could be regulated by hormones related to blood glucose level or stress. Perez-Cruet et al. (21) and Curzon et al. (22) ruled out the possibility that the increase in brain tryptophan concentration after fasting for 24 hr was due to secretion of corticosterone or pituitary hormones caused by stress. Our results also indicated that hydrocortisone injection had no effect on tryptophan concentration in either the brain or plasma. On the other hand, glucagon injection caused a remarkable increase of cerebral tryptophan without changing plasma tryptophan level. Mans et al. (23) recently reported a high cerebral tryptophan level in a rat with acute hepatic failure, while the plasma tryptophan level was lower, resulting in a decrease in the ratio of insulin to glucagon. These findings strongly suggest that acceleration of tryptophan uptake into the brain in food-restricted rats was regulated by glucagon, which might be secreted under the food-restricted conditions.

A number of reports (7,24–28) have shown that changes in cerebral tryptophan level could be attributed to changes in plasma neutral amino acids that compete with tryptophan transport into the brain. It is believed that the neutral amino acid levels in plasma are regulated by plasma insulin. However, in the present study, at 30 min and 1 hr after insulin injection, when blood glucose level was lowest, no change in cerebral tryptophan level was observed. But 2 hr after insulin injection, when blood glucose level tended to be restored, cerebral tryptophan level increased significantly. Thus it appears that insulin itself has little or no effect on tryptophan uptake into the brain and that the cerebral tryptophan increase at 2 hr after insulin injection might be caused by glucagon secretion or some other hormones, as a secondary effect of insulin injection.

In control rats, diurnal fluctuations of tryptophan level in brain and plasma were observed. The levels in brain and plasma were inversely influenced by food intake. Thus the cerebral and plasma tryptophan levels are apparently controlled by different mechanisms. After injection of D-glucose, cerebral tryptophan level decreased significantly while plasma level increased. Therefore, the increase in plasma tryptophan following food intake could be attributed to increased blood glucose. The increased amount of plasma tryptophan might be mobilized from amino acid pools,

such as in the muscle and liver. And the decrease of cerebral tryptophan level after injection of D-glucose might be mediated by the decreased glucagon level caused by increased blood glucose.

From these results the increase in brain 5-HT and 5-HIAA concentrations in food-restricted rats (C-80 and D-80 rats) is probably caused by elevated cerebral tryptophan level resulting from enhanced uptake into the brain from plasma. This enhancement of tryptophan uptake into brain might be caused by hormones related to blood glucose level, such as glucagon.

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