Effect of a Histidine-Excess Diet on a Tetrahydrofolylpolyglutamate Pattern in Rat Liver

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Summary The effect of a histidine-excess diet on the hepatic folylpolyglutamate pattern in rat was studied. Rats were fed ad libitum 9.7% casein basal diets with 0.6% methionine (controls) or the basal diets with 3.5% histidine. The average daily weight gain and the food intake in histidine-supplemented rats (His-rats) did not significantly differ from controls. The liver weight in His-rats, however, was 50% higher than controls. Hepatic methyltetrahydropteroylpentaglutamate (CH3H4PteGlu5), and tetrahydropteroylmonoglutamate concentrations in His-rats was 5.7- and 2-fold higher than controls, respectively. The tetrahydropteroylpentaglutamate (H4PteGlu5) concentration in the His-rats was 74% lower than controls. Considering the homeostasis of folate cofactors in tissues, these results suggest that the hepatic regeneration systems for H4PteGlu5 in His-rats might be repressed and an apparent methylfolate trap might be attained rather on a pteroylpentaglutamate level than a monoglutamate level, and that the activity for catabolizing the excess histidine might exceed the regenerating activity for folate cofactors.

Key Words excess-histidine, liver, tetrahydrofolylpolyglutamate, methyltetrahydrofolylpolyglutamate, methyl trap

The growth retardation, liver enlargement, and stimulation of cholesterolgenesis has been observed in rats fed excess-histidine diets (1). There are a few reports on the effect of an excess-histidine diet on folate metabolism.

An in vivo catabolism of histidine through the one-carbon pool is mediated by folate-dependent enzymes (2) and modulated by methionine metabolism (3–5). It is now accepted that folylpolyglutamates rather than the monoglutamate are the acceptors and donors of one-carbon units in amino acid and nucleotide metabolism.

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in animal tissues, whereas the monoglutamate is merely a transport form (6, 7). Brody et al. (8) showed a specific pattern of the folylpolyglutamates which mediated one-carbon metabolism in nitrous oxide-exposed rats fed a 0.2% methionine diet. In the practically B12-deficient rats, the activity of hepatic methionine synthase, i.e., one of the enzymes which mediate the regeneration of folate cofactors, has been depressed, and 5-methyltetrahydropteroylpentaglutamate (CH₃-H₄PteGlu₅) and tetrahydropteroylhexaglutamate (H₄PteGlu₆) have consequently been increased, while both tetrahydropteroylpentaglutamate (H₄PteGlu₅) and CH₃-H₄PteGlu₆ are in a smaller portion (8).

Mackenzie and Baugh (9) showed that an enzyme for histidine oxidation preferred H₄PteGlu₅ as substrate and is regulated with the polyglutamate chain length by a metabolic channeling system.

Fell and Steele (3) showed that the histidine catabolism was depressed and the urinary excretion of formiminoglutamate was increased in rats fed 10% casein diets with 0.6% methionine and 1% histidine.

This evidence suggests that histidine-excess diets might suppress the folate-dependent one-carbon metabolism in rat, and affect the hepatic folylpolyglutamate pattern, especially tetrahydro and its 5-methyl forms.

In order to test this assumption, we have studied the hepatic folylpolyglutamate pattern in rats fed ad libitum 10% casein diets with 0.6% methionine and 3.5% histidine. We found a decrease and a concomitant accumulation of specific folylpolyglutamates.

**MATERIALS AND METHODS**

*Chemicals.* Folylpolyglutamates (PteGluₙ, n = 2–6) were kindly provided by Professor C. L. Krumdieck. Tetrahydrofolylpolyglutamates (H₄PteGluₙ) and their 5-methyl derivatives (CH₃-H₄PteGluₙ) were synthesized from the PteGluₙ by the procedure of Suzuki and Wagner (10). Each solution of the derivatives prepared above was used as specimen for HPLC analysis without desalting.

*Animals and diets.* Male weanling Wistar rats (obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals) (46–55 g) were housed individually in wire mesh cages at 22 ± 1°C in a room with a 12-h light-dark cycle. They were supplied with water and a 9.7% casein basal diet containing 0.6% methionine, and the basal diet supplemented with 3.5% histidine as shown in Table 1. Histidine supplements were added at the expense of equal amount of cornstarch. These diets were fed ad libitum. The folic acid content of all diets was 2 mg/kg.

*Experimental design.* After 10-day feeding on a basal diet and 16-h fast on day 11, rats (body weight: 83.0 ± 1.2 g) were further maintained for 16 days on their respective diets. Daily weight gains of rats and daily food intakes were measured through 16 days. After 16-day feeding on experimental diets, they were anesthetized with 0.1 ml of sodium pentobarbital solution (55 mg/kg of body weight) by intraperitoneal injection. The livers were removed from the animals (body weight: J. Nutr. Sci. Vitaminol.
Table 1. Composition of experimental diet.

| Ingredient        | Basal diet (%) | 3.5% histidine diet (%) |
|-------------------|---------------|------------------------|
| Casein            | 9.7           | 9.7                    |
| DL-Methionine     | 0.6           | 0.6                    |
| L-Histidine       | 0             | 3.5                    |
| Cornstarch        | 59.7          | 56.2                   |
| Sucrose           | 15.0          | 15.0                   |
| Corn oil          | 8.0           | 8.0                    |
| Vitamin mixture¹  | 1.0           | 4.0                    |
| Mineral mixture²  | 4.0           | 4.0                    |
| Cellulose powder  | 2.0           | 2.0                    |

¹ Obtained from Oriental Yeast. Composition (mg/kg of diet): calcium phosphate, dibasic, 582.4; potassium phosphate, monobasic, 1,028.8; sodium phosphate, monobasic, 186.4; calcium lactate, 1,403.6; ferric citrate, 127.2; magnesium sulfate 286.8; zinc carbonate, 4.4; manganese sulfate, 4.8; cupric sulfate, 12; and potassium iodide, 0.4.

² Obtained from Oriental Yeast. Composition (mg/kg of diet): retinyl acetate, 1.72; cholecalciferol, 0.025; α-tocopheryl acetate, 50; menadione, 52; thiamin·HCl, 12; riboflavin, 40; pyridoxine·HCl, 8; cyanocobalamin, 0.005; ascorbic acid, 300; biotin, 0.2; folic acid, 2; calcium pantothenate, 50; p-aminobenzoic acid, 50; niacin, 60; inositol, 60; choline chloride, 2,000; and cellulose powder, 7,312.

137.9 ± 1.4 g for controls and 117.7 ± 4.0 g for histidine-supplemented rats), weighed, frozen in liquid nitrogen, and stored at −20°C before use.

**Extraction of folylpolyglutamate derivatives from livers.** The livers were excised, sliced, and homogenized with a Biomixer (Nihonseiki, Tokyo) at 16,000 rpm for 20 s in 5 volumes of a cold 0.2 N HCl solution containing 70% (v/v) methanol, 0.1% sodium ascorbate and 5 mM 2-mercaptoethanol. The homogenate was dispersed for 5 min in a Branson ultrasonic cleaner B-2200 J-1 (Branson Cleaning Equipment, Japan Branch) and centrifuged at 10,000 × g for 30 min at 2°C. The supernatant was passed through a Sep-pak C₁₈ cartridge #51910 (Waters Japan, Tokyo), which was previously activated with 5 ml methanol and 20 ml water in this order. The effluents were lyophilized. The residue was suspended by sonication in 100 μl of 0.1 M 2-mercaptoethanol. After centrifugation at 13,000 × g for 30 s, the supernatants were filtered through a Millipore HV 0.45-μm filter (Nihon Millipore Kogyo) and 2 μl of the sample was used for the high-performance liquid chromatographic (HPLC) analysis.

**HPLC.** The tetrahydro and its 5-methyl derivatives of folylpolyglutamates in rat liver were analyzed by HPLC with amperometric detection, which method has previously been shown to be the most sensitive and convenient method for analyzing both types of tetrahydrofolylmonoglutamates (11) and has been used for analyzing tetrahydrofolylpolyglutamate standards (12). Reversed-phase HPLC was
performed using a JASCO TRI ROTAR-V system (Japan Spectroscopic) consisting of a 50×4.6 mm stainless-steel column, filled with a Cosmosil 5 phenyl packing (Nakarai Chemicals, Kyoto). Peak detection was with an Irica E-502 amperometric detector (Irica Instruments, Kyoto) equipped with 3 electrode potentiostats, and adjusted for a +350 mV operating potential versus Ag/AgCl reference electrode. The mobile phase, containing 0.05 M disodium EDTA and 1% methanol in 50 mM potassium dihydrogen phosphate, pH 2.2, was used. Quantitation was based upon measuring peak height with a JASCO DP L-220 data processor in comparison to that produced by chromatographing standard solutions of the H$_4$PteGlu$_n$ and the CH$_3$-H$_4$PteGlu$_n$ derivatives or cochromatography with standards. Peak height is directly proportional to the concentration of each derivative, providing that the elution time remains relatively constant from analysis to analysis. Other chemicals were purchased from Nakarai Chemicals, Kyoto, Japan.

Statistics. Student's t-test (13) was used to determine the statistical significance between two means.

RESULTS

Growth and food efficiency

As shown in Table 2, the average daily weight gain and the food intake in histidine-supplemented rats did not significantly differ from those for controls during 2 to 16 days. Body weights of rats fed a 3.5% histidine-supplemented diet, however, were consistently lower than those for the unsupplemented controls during 16-day feeding (Fig. 1).

The food efficiencies, i.e., the average daily weight gain per average daily food intake, are given in Table 2.

Table 2. Effect of histidine supplementation on average daily food intake and average daily weight gain.1

|                     | Control diet | Histidine diet |
|---------------------|--------------|---------------|
|                     | day 1        | days 2–16     | day 1        | days 2–16     |
| Weight gain (g/day per rat) | 7.8 ± 1.4 | 3.80 ± 0.8 | 5.0 ± 0.7 | 2.62 ± 0.98* |
| Food intake (g/day per rat)   | 10.5 ± 0.2 | 13.2 ± 3.4 | 7.9 ± 1.1 | 8.6 ± 1.2*   |
| Food efficiency² (%)         | 74.3 ± 13.4 | 28.8 ± 9.6 | 63.3 ± 12.5 | 30.4 ± 12.1 |

1 Values expressed as means ± SE; n = 4 for controls, n = 5 for histidine diets. Rats were fed 9.7% casein diets, containing a 0.6% methionine and/or a 3.5% histidine ad libitum for the number of days indicated. ²(Average daily weight gain)/(average daily food intake) × 100. * Not significantly different from mean of control group during days 2–16 (p > 0.05).

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intake, in histidine-supplemented rats also equaled the controls, although efficiencies in both groups were 40–50% lower than those on 1-day feeding after starvation.

**HPLC Analysis of hepatic folylpolyglutamates**

The recovery of the H₄PteGlu₅ and CH₃-H₄PteGluₖ (n = 1–6) added to the liver homogenate and extracted under the procedure described in MATERIALS and METHODS was over 99%. The concentration of hepatic folylpolyglutamates on day 16 is shown in Table 3. When folylpolyglutamates (tetrahydropteroylpolyglutamates) were considered, di- and pentaglutamates predominated under the control diet, but not so much difference was noted between mono- to hexaglutamate concentrations under the 3.5% histidine diet. CH₃-H₄PteGlu₅ and CH₃-H₂PteGlu₆ were, however, predominant under both the control and the histidine diets. The H₄PteGlu₅ concentration was 76% lower in the histidine-supplemented rats, although its 5-methyl derivative concentration was 5.7-fold higher than controls.

**Increase of liver weight**

Liver weights (g/kg of body weight; means ± SE) on day 16 were 55.0 ± 1.5 g (n = 4) and 81.8 ± 5.2 g (n = 5) for rats fed the control and histidine diets, respectively.
Table 3. Effect of histidine supplementation on the amount of folylpolyglutamates in rat liver.

| Tetrahydropteroyl polyglutamate compound | Amount of folylpolyglutamate on day 16 |
|-----------------------------------------|---------------------------------------|
|                                         | Control diet (nmol/g liver) | 3.5% histidine diet (nmol/g liver) |
| H2PteGlu_n                               |                          |                                     |
| Total (n = 1–6)                          | 5.62 ± 0.69               | 4.57 ± 0.37                         |
| n = 1                                    | 0.40 ± 0.02               | 0.89 ± 0.27                         |
| n = 2                                    | 1.80 ± 0.63               | 1.25 ± 0.20                         |
| n = 3                                    | 0.60 ± 0.18               | 1.00 ± 0.18                         |
| n = 4                                    | 0.23 ± 0.07               | 0.32 ± 0.04                         |
| n = 5                                    | 2.12 ± 0.21               | 0.55 ± 0.07*                        |
| n = 6                                    | 0.47 ± 0.08               | 0.56 ± 0.06                         |
| 5-Methyl H2PteGlu_n                      |                          |                                     |
| Total (n = 1–6)                          | 2.99 ± 0.53               | 9.20 ± 0.42                         |
| n = 1                                    | 0.01 ± 0.00               | 0.01 ± 0.00                         |
| n = 2                                    | 0.07 ± 0.01               | 0.06 ± 0.01                         |
| n = 3                                    | 0.01 ± 0.00               | 0.01 ± 0.00                         |
| n = 4                                    | 0                         | 0                                    |
| n = 5                                    | 1.30 ± 0.22               | 7.40 ± 0.38*                        |
| n = 6                                    | 1.60 ± 0.48               | 1.72 ± 0.19                         |

1 Values (nmol/g of liver) expressed as means ± SE (n = 4 for controls and n = 5 for histidine diets). Rats were fed 9.7% casein diets containing 0.6% methionine and/or a 3.5% histidine load ad libitum for 16 days. * Significantly different from mean of control group, p < 0.001.

DISCUSSION

In spite of a similarity in food efficiency between the controls and the 3.5% histidine-supplemented rats on day 16 (Table 2), a 1.5-fold enlargement of the liver was found to be the case for rats fed a histidine-excess diet. Aoyama et al. (1) have reported the similar hepatic enlargement induced by feeding 25% casein diets with 5% histidine. They indicate that the liver enlargement might be due to the accumulation of hepatic glycogen and stimulation of hepatic cholesterogenesis. This might account for our finding of the hepatic enlargement in the 3.5% histidine-supplemented rats.

The large excretion of formiminoglutamate (FIGLU) is known as an index for folate deficiency (14). In rats fed 10% casein diets containing 0.6% methionine and 1% histidine, the excretion of urinary FIGLU has been elevated 4.6-fold more than control rats fed casein diets containing 1% methionine without histidine (15). We used the casein diets containing 0.6% methionine as a basal diet, since a 200% growth stimulation has been attained on this methionine level without histidine, and
a 123% growth has been observed by feeding a 1% methionine diet comparing the growth with a 10% casein diet without further addition of methionine.

The total amount of $H_4PteGlu_n$ and $CH_3-H_4PteGlu_n$ ($n=1-6$) of control rats was similar to that obtained by the chemical degradation method (16). It is not known why the tetrahydropteroyldiglutamate concentration in this experiment (Table 3) was so much higher than that in normal rats reported by Krumdieck and Eto (17). Evaluation of the method demonstrated that this extraction procedure is not susceptible to chemical conversion of reduced folates and depends on HCl for protection from conjugase which catalyzes deconjugation of folylpolyglutamates. Unlike other tissue extraction methods using heating, we used methanol for removal of protein. The technique should also be applicable to other tissues with appropriate modification and should help clarify the various metabolic status on cellular folylpolyglutamate metabolism.

In vitamin A-deficient rats (15), and nitrous oxide-treated rats (8), histidine catabolism is impaired, and urinary FIGLU excretion is elevated. Total hepatic folate levels was not changed, but there occurred a shift of $H_4PteGlu$ to its 5-methyl derivative in these rats. This suggests that a phenomenon known as the methylfolate trap (18–20) might occur, and a folate homeostasis has also been maintained in these rats as well as in normal animals (21). This evidence suggests that a 5-methylfolylpolyglutamate might be accumulated in the liver of rats which have impaired histidine catabolism and fed a restricted methionine level.

In the present study, a characteristic pattern of pteroylpentaglutamates and monoglutamates was observed in hepatic folate pools in rat fed a histidine-excess diet. $H_4PteGlu_5$ was exhausted likely to its basal level, and $H_4PteGlu$ concentration was 2-fold higher than control, although $CH_3-H_4PteGlu_5$ was markedly accumulated in the 3.5% histidine-supplemented rats. A bifunctional enzyme for histidine oxidation system has preferred $H_4PteGlu_5$ as substrate (9). This evidence suggests that the activity for catabolizing the large influx of histidine might be elevated and its activity might overcome the regenerating activity for folate cofactors such as methionine synthase. An apparent methylfolate trap could be attained rather on a pteroylpentaglutamate level than the monoglutamate level in the histidine-supplemented rats.

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