Role of N-Acetylglutamate Turnover in Urea Synthesis of Rats Given Proteins of Different Quality

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Summary The purpose of this study was to find whether the synthesis and degradation of N-acetylglutamate would affect urea synthesis when the dietary protein quality was manipulated. Experiments were done on three groups of rats given diets containing 10 g gluten, 10 g casein or 10 g whole egg protein/100 g for 10 d. The urinary excretion of urea, the liver concentrations of N-acetylglutamate and free glutamate, the liver activity of N-acetylglutamate synthetase increased with the decline in quality of dietary protein. A reverse correlation was observed between the liver N-acetylglutamate degradation and liver N-acetylglutamate concentration. N-Acetylglutamate concentration in the liver was closely correlated with the concentration of glutamate and the N-acetylglutamate synthetase activity in the liver, and excretion of urea. These results suggest that the greater synthesis and the lower degradation rate of N-acetylglutamate in the liver of rats given the lower quality of protein increase the liver concentration of N-acetylglutamate and stimulate urea synthesis.

Key Words dietary protein quality, urea synthesis, N-acetylglutamate synthesis, N-acetylglutamate degradation, rats

Schimke (1, 2) has suggested that the concentrations of urea-cycle intermediates were unchanged under conditions affecting the rate of urea excretion (e.g., ingestion of a high-protein diet) and concluded that the activities of various urea-cycle enzymes were regulatory factors of urea synthesis. However, many investigators have previously reported that there was an increase in urinary urea excretion without a comparable increase in the enzyme activities when a diet containing high-quality protein was replaced by an isonitrogenous diet with low-quality protein (3-6).

Urea formation has been shown to be stimulated by adding N-acetylglutamate in vivo (7), in perfused liver (8-10) and in isolated hepatocytes (11) when substrates for urea production were present in excess. N-Acetylglutamate, an essential activator of carbamylphosphate synthetase (EC 6.3.4.16), plays a critical role in the regulation of urea synthesis in mammalian liver (12, 13). Shigesada and Tatibana (14) have demonstrated that N-acetylglutamate synthetase (EC 2.3.1.1) catalyzed the acetylation of glutamate in liver. This enzyme is activated specifically by arginine. On the other hand, Meijer et al. (15, 16) have suggested that there could be an alteration in the enzyme system catabolizing N-acetylglutamate in hepatic cytosol or in transporting this compound out of the mitochondria under physiological conditions. Thus, the supply of N-acetylglutamate may limit the rate of urea synthesis.

The purpose of the present study was to discover the mechanism by which the dietary protein quality affects urea synthesis. In our previous report (6), positive correlations between the liver concentration of N-acetylglutamate and urea excretion, and between the liver concentration and synthesis in vivo of N-acetylglutamate were found when the dietary protein quality was manipulated. However, the plasma concentration of arginine was not correlated with urea excretion. Two questions were considered in the present study: 1) whether N-acetylglutamate synthetase, glutamate, acetylCoA in the liver might control the N-acetylglutamate synthesis, when the dietary protein quality was manipulated and 2) whether a decreased degradation of N-acetylglutamate by rats fed the low-quality protein might result in elevated N-acetylglutamate concentrations and higher urea synthesis than in those animals given the high-quality protein. We therefore examined the hepatic concentrations of N-acetylglutamate, glutamate and acetylCoA, the activity of N-acetylglutamate synthetase, and the hepatic degradation of N-acetylglutamate in rats given proteins of different quality.

MATERIALS AND METHODS

Chemicals. N-Acetylglutamic acid was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). NaH14CO3 (1.85 GBq/mmol) and U-14C-glutamic acid (9.25 GBq/mmol) were obtained from Amersham (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).
Table 1. Composition of experimental diets.

| Ingredient                     | 10% Gluten (g/kg diet) | 10% Casein (g/kg diet) | 10% Whole egg protein (g/kg diet) |
|-------------------------------|------------------------|------------------------|----------------------------------|
| Whole egg protein¹            |                        |                        | 100                              |
| Casein                        |                        |                        | 100                              |
| Gluten                        | 100                    | 100                    | 100                              |
| Cornstarch²                   | 502                    | 502                    | 502                              |
| Sucrose³                      | 251                    | 251                    | 251                              |
| Corn oil                      | 50                     | 50                     | 50                               |
| AIN-93G mineral mix³          | 35                     | 35                     | 35                               |
| AIN-93VX vitamin mix³         | 10                     | 10                     | 10                               |
| Cellulose²                    | 50                     | 50                     | 50                               |
| Choline chloride              | 2                      | 2                      | 2                                |

¹Supplied by Taiyo Kagaku, Yokkaichi, Japan.  
²Supplied by Oriental Yeast, Tokyo, Japan.  
³Supplied by Nihon Nosan K.K., Yokohama, Japan (38).

Table 2. Time-dependent changes of specific radioactivities of free glutamate in the liver of rats fed diets with different quality of protein.

| Time after injection (min) | 10% Gluten (Bq/μmol) | 10% Casein (Bq/μmol) | 10% Whole egg protein (Bq/μmol) | Pooled SE |
|----------------------------|-----------------------|----------------------|---------------------------------|-----------|
| 15                         | 2,400                 | 2,350                | 2,490                           | 140       |
| 30                         | 430                   | 420                  | 450                             | 30        |
| 60                         | 200                   | 180                  | 210                             | 20        |
| 120                        | 190                   | 180                  | 200                             | 20        |

¹Values are means and pooled SE, n=6. Values within each time period were not significantly different (p>0.05).

Animals and Diets. Young male Wistar rats (110–120 g, Japan SLC, Hamamatsu, Japan) were maintained at 24°C with a 12 h light:dark cycle. The rats were transferred to the experimental diets containing 20% gluten, 20% casein or 20% whole egg protein diet (Table 1) after being fed with a commercial nonpurified diet (MF, Oriental Yeast, Tokyo, Japan) for 5 d. All rats were individually housed and provided free access to food and water. The approval of the Aichi University of Education Animal Care and Use Committee was given for our animal experiments.

Experimental design. Three experiments were done, with 18 rats (Experiments 1 and 2) or 54 rats (Experiment 3) being divided randomly into three groups. In each experiment, animals were fed the experimental diet for 10 d. On days 6–9, urine was collected for 3 d, filtered and used for urea analysis. After the experimental period, the rats were decapitated and the plasma was collected in glass tubes and stored at −20°C. The livers were quickly removed, weighed, and used.

In Experiment 1, the effects of dietary protein quality on the urinary excretion of urea, the hepatic activity of N-acetylglutamate synthetase, and the concentrations of N-acetylglutamate and glutamate in the liver were investigated.

In Experiment 2, the effects of dietary protein quality on the liver concentration of acetylCoA and plasma concentration of urea were determined.

In Experiment 3, the effects of dietary protein quality on the N-acetylglutamate degradation in vivo in the liver were determined.

Analytical procedures. The plasma concentration and urinary excretion of urea were measured by the method of Archibald (17). The N-acetylglutamate concentration was measured by the method of McGivan et al. (18) described in our previous report (19). For measuring the concentration of free glutamate, liver was treated with ice-cold 120 mmol/L sulfosalicylic acid to precipitate the protein (20). The concentration of glutamate was measured using an amino acid analyzer (L-8500; Hitachi, Tokyo, Japan). The concentration of acetylCoA in the liver was determined by the method of Wieland and Weiss (21). The activity of N-acetylglutamate synthetase was assayed according to the method of Shigeshada and Tatibana (14) described in our previous report (22).

N-Acetylglutamate degradation in the liver. The hepatic degradation of N-acetylglutamate was determined 25 by the method of Morita et al. (23). Radioactive 14C-glutamate was combined with unlabelled glutamate to yield a dose of 1.11 MBq and a concentration of 5 μmol/mL of saline. The rats were each given an intraperitoneal injection at a dose of 1 mL/100 g body wt. After 30, 60 or 120 min, the rats were decapitated. The
measurement of N-acetylglutamate degradation involved N-acetylglutamate isolation and then radioactivity counting (23).

In a preliminary experiment, we determined the hepatic specific radioactivity of glutamate 15, 30, 60 and 120 min after the injection of radioactive glutamate under these experimental conditions by the method of Morita et al. (23). The specific radioactivity of glutamate in the liver was the highest 15 min after the injection, and decreased to very low values by 30, 60 and 120 min (Table 2). These values in the three groups were the same, indicating that the prolonged retention of radioactivity in N-acetylglutamate was not due to continued entry of the label into this compound but instead to an increase in the half-life of the compound in Experiment 3.

Statistical analysis. The means and pooled SE are reported. Duncan’s multiple-range test was used to compare means after one-way ANOVA (24, 25). A linear regression analysis was used to assess the liver degradation of N-acetylglutamate. Differences were considered significant at \( p < 0.05 \).

**RESULTS**

Liver concentrations of N-acetylglutamate and glutamate, hepatic N-acetylglutamate synthetase activity, and urinary excretion of urea (Experiment 1)

The rats fed the 10% gluten diet gained less body weight and had less food intake than the other two groups, which did not differ. Compared with the rats fed the 10% whole egg protein or 10% casein diets, rats fed the 10% gluten diet had relative liver weights which were significantly lower. Urinary excretion of urea and the hepatic concentrations of N-acetylglutamate increased significantly with the 10% casein diet and still more with the 10% gluten diet as compared with the 10% whole egg protein diet (Table 3). The concentration of glutamate and activity of N-acetylglutamate synthetase in liver of rats given the 10% gluten diet was significantly greater than that in rats given the 10% casein or 10% whole egg protein diets (Table 3). The liver concentration of N-acetylglutamate was correlated with the volumes of urinary excretion of urea (\( r=0.895, p<0.001 \)). The correlation between the concentrations of N-acetylglutamate and glutamate was significant (\( r=0.798, p<0.01 \)). A similar correlation was found between the liver concentration of N-acetylglutamate and the activity of N-acetylglutamate synthetase (\( r=0.792, p<0.01 \)).

**Hepatic concentration of acetylCoA and plasma concentration of urea (Experiment 2)**

As in Experiment 1, the group fed the 10% gluten diet grew less than the group fed the 10% casein diet or 10% whole egg protein diet. The plasma concentration of urea increased significantly with the 10% casein diet and still more with the 10% gluten diet as compared with the 10% whole egg protein diet. The acetylCoA concentration in the liver did not differ among groups (Table 4).

**Hepatic degradation rate constant and half-life of N-acetylglutamate (Experiment 3)**

The dietary protein quality affected the hepatic deg-
The degradation rate constant of N-acetylglutamate in the liver was higher in rats fed the 10% gluten diet compared with those fed the 10% casein diet. The first limiting amino acid of wheat gluten for the requirement of amino acids is lysine. The concentrations of threonine, sulfur amino acids and branched amino acids are also lower in wheat gluten. Recently, amino acid deficiency was suggested to stimulate the transcription rate in several genes (32). The possible effects of dietary gluten diet on the gene of N-acetylglutamate synthetase in rats are important to elucidate the mechanism by which the dietary protein quality affects the N-acetylglutamate concentration in liver. Measurement of mRNA in the hepatic N-acetylglutamate synthetase should be included in further studies.

The acetylCoA and glutamate were the substrates of N-acetylglutamate synthetase in the liver. Therefore, we examined the effect of dietary protein quality on the concentrations of hepatic glutamate and acetylCoA. The changes in acetylCoA concentration are not considered to be the factor leading to the greater synthesis of liver N-acetylglutamate in rats fed the low-quality protein (Table 4). On the other hand, the greater concentration of hepatic glutamate in the groups given the 10% gluten or 10% casein diets increased the N-acetylglutamate synthesis in these groups (Table 3). Thus corroborating the finding of our previous study (6), these results suggest that the changes in glutamate concentration might control the N-acetylglutamate synthesis in rat liver. Gluten is known to have higher concentrations of glutamine and glutamate than those in whole egg protein or casein. The protein synthesis in tissues is known to depend on the quality of dietary protein (33–35). The lower protein synthesis in tissues of rats given the low-quality of protein may be one of the factors that stimulate the release of these amino acids from tissues and regulate the liver concentration of glutamate. Further studies on the role of the dietary amino acid composition in protein and protein synthesis on the hepatic concentration of glutamate should be included in the examination of the mechanism by which the quality of dietary protein affects the N-acetylglutamate concentration and urea synthesis.

N-Acetylglutamate is transported from mitochondria to cytosol in the liver, and then degraded. The activity of the catalyzing enzyme was detected only in the cytosol (18, 36). The hepatic concentration and half-life of N-acetylglutamate increased markedly after protein ingestion (23), suggesting that the in vivo degradation rate contributed to the concentration of N-acetyl-
glutamate when the ingestion of dietary protein was changed. Therefore, we assume that the quality of dietary protein also affect N-acetylglutamate degradation. In this study, not only greater synthesis but also lower degradation of N-acetylglutamate was observed in the group fed the low-quality protein (Table 5). The regulation of urea synthesis may be partly mediated by an alteration of N-acetylglutamate when the quality of dietary protein was manipulated. The mechanism by which the quality of dietary protein affects the hepatic degradation process of N-acetylglutamate remains to be determined. Since the hepatic cytosol fraction has a high level of N-acetylglutamate catabolizing activity (18, 36), an indication of limiting N-acetylglutamate degradation by its transport out of the mitochondria has been reported in many investigations (15, 16, 37). The rate of N-acetylglutamate efflux through the mitochondrial membrane and its degradation have been found to be lower in rats fed the high protein diet than in those fed the protein-free diet (23). This is another possibility to consider in further examinations of the mechanism by which the quality of dietary protein alters urea synthesis.

Mitochondrial carbamylphosphate synthetase in liver has an absolute requirement for N-acetylglutamate as an activator. Shigesada et al. (27) reported that the increase of N-acetylglutamate concentration under the physiological conditions stimulated urea synthesis in isolated hepatocytes. The Ka value (0.11 mM) of the carbamylphosphate synthetase for the N-acetylglutamate was reported to be close to its physiological concentration in mitochondria (12). From observed hepatic concentration of N-acetylglutamate (Table 3), its average concentration in hepatic mitochondria of rats fed the gluten diet is calculated to be about 0.1 mM based on the estimate of the mitochondrial space in the cellular volume (12). This magnitude concentration appear to be adequate for the function of N-acetylglutamate. These results strongly indicate the control mechanism for the urea synthesis mediated by the hepatic concentration of N-acetylglutamate when the quality of dietary protein was changed.

In this study, we show that the greater synthesis and lower degradation of N-acetylglutamate in the liver of rats given the lower quality of protein increase the liver concentration of N-acetylglutamate and stimulate urea synthesis. The activity of N-acetylglutamate synthetase and the concentration of glutamate in the liver are at least partly related to the hepatic N-acetylglutamate synthesis.

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