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The role of growth hormone and amino acids on brain protein synthesis in aged rats given proteins of different quantity and quality

M. Ohsumi [1], **X. Shi** [1], **T. Tuchiya** [1], **K. Tujioka** [2], **S. Lyou** [2], **K. Hayase** [1], and **H. Yokogoshi** [2]

[1] Department of Home Economics, Aichi University of Education, Kariya, Aichi, Japan
[2] Laboratory of Nutritional Biochemistry, School of Food and Nutritional Sciences, COE Program in the 21st Century, The University of Shizuoka, Yada, Shizuoka, Japan

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Summary. The purpose of the present study was to determine whether the regulation of brain protein synthesis was mediated through changes in the plasma concentrations of insulin and growth hormone (GH), and whether the concentrations of amino acids in the brain and plasma regulate the brain protein synthesis when the quantity and quality of dietary protein is manipulated. Two experiments were done on three groups of aged rats given diets containing 20% casein, 5% casein or 0% casein (Experiment 1), and 20% casein, 20% gluten, or 20% gelatin (Experiment 2) for 1 d (only one 5-h period) after all rats were fed the 20% casein diet for 10 d (only 5-h feeding per day). The aggregation of brain ribosomes, the concentration in plasma GH, and the branched chain amino acids in the plasma and cerebral cortex declined with a decrease of quantity and quality of dietary protein. The concentration of plasma insulin did not differ among groups. The results suggest that the ingestion of a higher quantity and quality of dietary protein increases the concentrations of GH and several amino acids in aged rats, and that the concentrations of GH and amino acids are at least partly related to the mechanism by which the dietary protein affects brain protein synthesis in aged rats.

Keywords: Dietary protein – Brain protein synthesis – Amino acids – Insulin – Growth hormone – Rats

Abbreviation: GH, growth hormone

Introduction

Concentrations of tissue proteins are affected by alterations in dietary proteins and also by age. These changes in protein metabolism may be reflected in the rate of protein synthesis and in the polyribosomal profile of the endoplasmic reticulum, especially in the liver, muscle and small intestine (Goldspink et al., 1984; Lewis et al., 1984; Millward et al., 1974, 1975, 1976; Symmons et al., 1972; Yokogoshi et al., 1980a, b). Protein synthesis in the brain is also sensitive to the alteration of dietary amino acid in young rats (Beverly et al., 1991; Yokogoshi et al., 1992).

Many investigators have demonstrated that protein synthesis declined in specific tissues (e.g., the liver, muscle or brain) and in the whole body during development in mammals after weaning (Attaix et al., 1988; Goldspink and Kelly, 1984; Waterlow et al., 1978; Hayase and Yokogoshi, 1994). In older rats, we reported that the rate of protein synthesis and the polysome profile in the brain depended on the quantity and quality of dietary protein consumed, and that a correlation between the protein synthesis and the RNA activity was found in the brain when the dietary protein was manipulated (Hayase et al., 1998; Koie et al., 1999; Hirano et al., 2002).

The purpose of our present study was to discover the mechanism by which the quantity and quality of dietary protein affect the brain protein synthesis in aged rats. Insulin and growth hormone (GH) have been shown to stimulate tissue protein synthesis (Jefferson, 1980; Jepson et al., 1988; Kato, 2002). Recently several studies have demonstrated that there were receptors of insulin and GH in brain regions, and that the insulin and GH might directly stimulate the protein synthesis and gene expression in the brain (LeRoith et al., 1988; Hayase and Yokogoshi, 1995; Le Greves et al., 2002). The possible direct effects of insulin and GH on the brain protein synthesis of rats are of interest.

Several early studies revealed that provision of amino acids improved the decline in the protein synthesis in the liver and skeletal muscle associated with food deprivation, suggesting that amino acids not only have a role as the substrates for protein synthesis but also have a unique role in regulating mRNA translation (Buse and Reid, 1975;

Li and Jefferson, 1978; Yoshizawa et al., 1998). More recent studies have extended the earlier investigations to show that branched chain amino acids, especially leucine, are the most potent amino acids in enhancing the initiation phase of mRNA translation (Anthony et al., 2000a, b; Yoshizawa et al., 2004).

Two questions were considered in the present study:

1) whether the quantity and quality of dietary protein might control the plasma concentrations of insulin or GH and regulate the brain protein synthesis in aged rats, and
2) whether the concentrations of amino acids might regulate the protein synthesis in the brain when the quantity and quality of dietary protein were manipulated.

Therefore, we examined the plasma concentrations of insulin and GH, and the free amino acid concentrations in the plasma and cerebral cortex of aged rats. The effects of the quantity and quality of dietary protein on the polysome profiles in the cerebral cortex were also investigated. Gelatin and gluten are known to be lower quality protein than casein because of a deficiency in several essential amino acids (e.g. tryptophan) in gelatin, and a deficiency in lysine in gluten, and also because of lower nitrogen retention (Steele and Harper, 1990). In particular, as the gelatin has less of many essential amino acids, the body weight decreased in rats fed a 20% gelatin diet in young and aged rats (Yokogoshi et al., 1992; Koie et al., 1999). Thus, in this experiment, 20% gelatin, 20% gluten and 20% casein diets were chosen to investigate the mechanism by which the quality of dietary protein altered the brain protein synthesis.

Materials and methods

Chemicals

All reagents were purchased from Wako Pure Chemical (Osaka, Japan).

Animals and diets

Male Wistar rats (24 wk, Japan SLC, Hamamatsu, Japan) were individually housed at 24 °C in a room with a 12-h light-dark cycle. The rats were switched to the experimental diets containing 0, 5 or 20% casein (Experiment 1, Table 1), or 20% gelatin, 20% gluten or 20% casein (Experiment 2, Table 1) after being fed 20% casein diet for 10 d. All rats were given free access to food and water. The approval of Aichi University of Education Animal Care and Use Committee was given for our animal experiments.

Experimental design

Two experiments were done, with 18 rats being divided randomly into three groups. Our previous results demonstrated that the aggregation or disaggregation of brain ribosomes and the plasma concentrations of insulin and GH were affected very rapidly after feeding the test diet in spite of the differences in feeding period. Therefore, in the present study, the changes in the population of ribosomes and the plasma concentrations of hormones were measured after only one 5-h feeding period of the test diet. After all rats were fed the 20% casein diet for 10 d (only 5-h feeding per day, from 9:00–14:00), they were given experimental diets for 1 d (only one 5 h period). After a 5 h feeding period, the rats were decapitated and the plasma was collected in glass tubes and stored at −20 °C. The cerebral cortices were quickly removed. In Experiment 1, the effects of quantity of dietary protein on the polysome profile and amino acids in the cerebral cortex, and the plasma concentrations of insulin, GH and amino acids were investigated. In Experiment 2, the effects of quality of dietary protein on the polysome profile and amino acids in the cerebral cortex, and the plasma concentrations of insulin, GH and amino acids were determined.

Table 1. Composition (g/100 g of diet) of experimental diets

Ingredient	20% Gelatin	20% Gluten	20% Casein	5% Casein	0% Casein
Casein			20.0	5.0	
Gluten		20.0			
Gelatin	20.0				
Sucrose[1]	21.8	21.8	21.8	26.8	28.4
Corn starch[1]	43.5	43.5	43.5	53.5	56.9
Corn oil	5.0	5.0	5.0	5.0	5.0
AIN-93M mineral mix[2]	3.5	3.5	3.5	3.5	3.5
AIN-93VX vitamin mix[2]	1.0	1.0	1.0	1.0	1.0
Cellulose[1]	5.0	5.0	5.0	5.0	5.0
Choline chloride	0.2	0.2	0.2	0.2	0.2

[1] Supplied by Oriental Yeast, Tokyo, Japan
[2] Supplied by Nihon Nosan K. K., Yokohama, Japan (American Institute of Nutrition, 1993)

Analytical procedures

The concentration of plasma GH was measured by the method of EIA (SPI bio, Massy, Cedex, France). The concentration of plasma insulin was determined by the method of EIA (Mercodia, Uppsala, Sweden). For measuring the concentrations of free amino acids, the cerebral cortex and plasma were treated with ice-cold sulfosalicylic acid to precipitate the protein (Millward et al., 1974). The amino acid concentrations were measured by an amino acid analyzer (L-8500, Hitachi, Tokyo, Japan).

Preparation of polysomes from the rat cerebral cortex

The polysome profiles in the cerebral cortex were measured by the method of Yokogoshi and Yoshida (1979). The cerebral cortex was homogenized in two volumes of medium (0.25 mol/l sucrose, 0.05 mol/l Tris–HCl buffer, pH 7.6, 0.025 mol/l KCl and 0.005 mol/l $MgCl_2$ at 4 °C). The postmitochondrial supernatant was prepared by centrifugation at $10{,}000 \times g$ for 10 min, which sedimented mitochondria and cell debris. Sodium deoxycholate was added to the supernatant to give a concentration of 10 g/l. Samples of this preparation were put on the linear 0.3 to 1.2 mol/l sucrose gradients and were centrifuged at $158{,}000 \times g$ for 110 min (SCP-85H ultracentrifuge, Hitachi, Tokyo, Japan). The ultraviolet absorption of the gradient at 254 nm was recorded, and the polysome

profile was calculated by the method previously reported (Yokogoshi and Yoshida, 1999).

Statistical analysis

The means and pooled SEM are reported. Duncan's multiple-range test was used to compare means after a one-way ANOVA (Duncan, 1955; Snedecor and Cochran, 1967). Differences were considered significant at $p < 0.05$.

Results

Effect of the quantity of dietary protein on the polysome profile and the concentrations of amino acids in the cerebral cortex, and the plasma concentrations of hormones and amino acids (Experiment 1)

Compared with rats fed the 20% casein or 5% casein diets, rats fed the 0% casein diet had food intake which was significantly lower. The relative weight of the cerebral cortex was not different among the three groups. The numbers of monomers + dimers per total ribosomes (polysome profile) declined gradually with the decreasing quantity of dietary protein in the cerebral cortex. The plasma concentration of insulin did not differ among groups. The GH concentration in plasma was markedly lower in rats fed the 0% casein or 5% casein diets than in those fed the 20% casein diet (Table 2). In the plasma concentrations of amino acids, several essential amino acids, such as threonine, methionine, branched chain amino acids and lysine, decreased significantly with the 5% casein diet and still more with the 0% casein diet compared with the 20% casein diet (Table 3). In the cerebral cortex, the concentrations of aspartic acid, glutamic acid, glycine, threonine and serine were high in each group. The concentrations of threonine, branched chain amino acids and lysine both in the plasma and in the cerebral cortex showed similar variations resulting from dietary treatment (Tables 3 and 4).

Table 2. Effect of the quantity of dietary protein on the plasma concentrations of insulin and growth hormone, and brain polysome profile in aged rats[1]

	20% Casein	5% Casein	0% Casein	Pooled SEM
Final body weight (g)	365.2	363.6	361.8	6.4
Food intake (g/day)	16.6[a]	15.2[ab]	13.6[b]	0.6
Cerebral cortex weight (g/100 g body weight)	0.105	0.103	0.101	0.004
Plasma insulin (pmol/l)	292	307	305	6
Plasma growth hormone (μg/l)	62.31[a]	9.38[b]	9.83[b]	4.13
Polysome profile[2], % Cerebral cortex	39.7[b]	41.2[b]	45.5[a]	0.5

[1] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)
[2] (Monomers + dimers)/total ribosomes

Table 3. Effect of the quantity of dietary protein on the plasma concentrations of free amino acids in aged rats[1]

	mmol/l of plasma			Pooled SEM
	0% Casein	5% Casein	20% Casein	
Aspartic acid	0.019[b]	0.028[a]	0.023[ab]	0.0026
Threonine	0.092[c]	0.143[b]	0.319[a]	0.008
Serine	0.138[c]	0.180[b]	0.273[a]	0.007
Glutamic acid	0.115	0.145	0.131	0.014
Proline	0.162	0.176	0.176	0.006
Glycine	0.160	0.167	0.155	0.007
Alanine	0.430[c]	0.611[b]	0.744[a]	0.028
Valine	0.086[c]	0.133[b]	0.385[a]	0.009
Methionine	0.026[b]	0.038[b]	0.085[a]	0.0061
Isoleucine	0.039[c]	0.060[b]	0.150[a]	0.0040
Leucine	0.063[c]	0.105[b]	0.268[a]	0.007
Tyrosine	0.047[c]	0.076[b]	0.132[a]	0.0037
Phenylalanine	0.040[c]	0.050[b]	0.083[a]	0.0022
Histidine	0.050[c]	0.064[b]	0.089[a]	0.0028
Lysine	0.189[c]	0.284[b]	0.447[a]	0.010
Arginine	0.050[b]	0.061[b]	0.094[a]	0.0045

[1] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)

Table 4. Effect of the quantity of dietary protein on the concentrations of free amino acids in cerebral cortex of aged rats[1]

	μmol/g of cerebral cortex			Pooled SEM
	0% Casein	5% Casein	20% Casein	
Aspartic acid	3.647	3.548	3.750	0.108
Threonine	0.409[b]	0.411[b]	0.460[a]	0.010
Serine	0.983	0.960	0.983	0.025
Glutamic acid	13.497	13.357	14.019	0.439
Proline	0.606	0.580	0.630	0.017
Glycine	0.623	0.602	0.645	0.018
Alanine	0.616[ab]	0.597[b]	0.662[a]	0.017
Valine	0.073[b]	0.077[b]	0.119[a]	0.0028
Methionine	0.031	0.032	0.036	0.0026
Isoleucine	0.029[b]	0.030[b]	0.045[a]	0.0015
Leucine	0.063[c]	0.071[b]	0.102[a]	0.0025
Tyrosine	0.076[ab]	0.084[a]	0.074[b]	0.0028
Phenylalanine	0.060[a]	0.052[b]	0.044[c]	0.0016
Histidine	0.096[a]	0.085[b]	0.077[b]	0.0029
Lysine	0.204[b]	0.202[b]	0.230[a]	0.007
Arginine	0.109	0.101	0.101	0.003

[1] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)

Effect of the quality of dietary protein on the polysome profile and the concentrations of amino acids in the cerebral cortex, and the plasma concentrations of hormones and amino acids (Experiment 2)

Compared with rats fed the 20% casein or 20% gluten diets, rats fed the 20% gelatin diet had food intake that was significantly lower. The relative weight of the cerebral cortex was not affected by the dietary protein quality.

Table 5. Effect of the quality of dietary protein on the plasma concentrations of insulin and growth hormone, and brain polysome profile in aged rats[1]

	20% Casein	20% Gluten	20% Gelatin	Pooled SEM
Final body weight (g)	365.2	358.0	359.0	6.6
Food intake (g/day)	16.6[a]	14.3[ab]	13.3[b]	0.9
Cerebral cortex weight (g/100 g body weight)	0.105	0.107	0.102	0.003
Plasma insulin (pmol/l)	292	304	305	5
Plasma growth hormone (μg/l)	62.31[a]	13.10[b]	18.16[b]	6.23
Polysome profile[2], % Cerebral cortex	39.6[c]	42.0[b]	47.1[a]	0.7

[1] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)
[2] (Monomers + dimers)/total ribosomes

Table 6. Effect of the quality of dietary protein on the plasma concentrations of free amino acids in aged rats[1]

	mmol/l of plasma			Pooled SEM
	20% Gelatin	20% Gluten	20% Casein	
Aspartic acid	0.035[a]	0.027[ab]	0.023[b]	0.0034
Threonine	0.155[b]	0.178[b]	0.319[a]	0.008
Serine	0.335[a]	0.250[b]	0.273[b]	0.009
Glutamic acid	0.141	0.117	0.131	0.013
Proline	1.020[a]	0.195[b]	0.176[b]	0.047
Glycine	1.054[a]	0.178[b]	0.155[b]	0.045
Alanine	0.704[a]	0.575[b]	0.744[a]	0.034
Valine	0.173[c]	0.207[b]	0.385[a]	0.009
Methionine	0.039[b]	0.038[b]	0.085[a]	0.0060
Isoleucine	0.064[b]	0.045[c]	0.150[a]	0.0035
Leucine	0.114[c]	0.190[b]	0.268[a]	0.007
Tyrosine	0.034[c]	0.096[b]	0.132[a]	0.0039
Phenylalanine	0.061[b]	0.085[a]	0.083[a]	0.0022
Histidine	0.045[b]	0.098[a]	0.089[a]	0.0037
Lysine	0.283[b]	0.080[c]	0.447[a]	0.010
Arginine	0.148[a]	0.083[b]	0.094[b]	0.0064

[1] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)

Table 7. Effect of the quality of dietary protein on the concentrations of free amino acids in cerebral cortex of aged rats[1]

	μmol/g of cerebral cortex			Pooled SEM
	20% Gelatin	20% Gluten	20% Casein	
Aspartic acid	3.800	3.651	3.750	0.061
Threonine	0.384[b]	0.388[b]	0.460[a]	0.016
Serine	1.146[a]	0.987[b]	0.983[b]	0.022
Glutamic acid	14.343	13.779	14.019	0.270
Proline	0.757[a]	0.600[b]	0.630[b]	0.014
Glycine	0.787[a]	0.620[b]	0.645[b]	0.014
Alanine	0.654	0.628	0.662	0.016
Valine	0.098[b]	0.079[c]	0.119[a]	0.0023
Methionine	0.028[b]	0.021[c]	0.036[a]	0.0016
Isoleucine	0.033[c]	0.041[b]	0.045[a]	0.0023
Leucine	0.078[c]	0.090[b]	0.102[a]	0.0020
Tyrosine	0.036[b]	0.069[a]	0.074[a]	0.0019
Phenylalanine	0.059[a]	0.054[b]	0.044[c]	0.0013
Histidine	0.062[c]	0.090[a]	0.077[b]	0.0021
Lysine	0.161[b]	0.111[c]	0.230[a]	0.004
Arginine	0.153[b]	0.172[a]	0.101[c]	0.002

[1] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$)

The numbers of monomers + dimers per total ribosomes decreased with gelatin, gluten and casein diets in that order. The plasma concentration of insulin did not differ among the three groups. The GH concentration in plasma was significantly lower in rats fed the 20% gelatin diet or 20% gluten diet than in those fed the 20% casein diet (Table 5). The plasma concentrations of essential amino acids such as threonine, methionine, branched chain amino acids and lysine decreased significantly with the 20% gelatin diet or 20% gluten diet compared with the 20% casein diet (Table 6). With the specific amino acids such as glutamic acid and aspartic acid, the concentrations of most amino acids in the cerebral cortex were related to their levels in the plasma and in the diet (Tables 6 and 7).

Discussion

In research on changes in brain protein metabolism related to aging, work is necessary to understand the modulating effects of factors (Smicklas-Wright, 1990). Direct evidence that the supply of amino acid influenced the neuronal protein synthesis was provided by the study of Parks et al. (1976). In previous studies, we found that the rate of protein synthesis in the brain declined with age, and decreased with the decrease in dietary protein in aged rats (Hayase and Yokogoshi, 1994; Hayase et al.,

1998). In older rats, we also reported that the rate of protein synthesis in the brain of rats given the gluten or gelatin diets is lower than in rats given the casein diet (Koie et al., 1999). The purpose of the present experiments was to elucidate the mechanism by which the dietary protein affects the brain protein synthesis in aged rats. In the present study, the aggregation of polyribosomes in the cerebral cortex was highest in the group fed the 20% casein diet, followed by the 5% casein and 0% casein diets in Experiment 1, and by the 20% gluten and 20% gelatin diets in Experiment 2, in that order. The changes in the polysome profiles of the cerebral cortex were closely correlated with the quantity and quality of dietary protein as previously demonstrated in the brain of weaned and aged rats (Yokogoshi et al., 1992; Hirano et al., 2002). Therefore, we hypothesized that the plasma concentrations of anabolic hormones such as GH and insulin, and the concentrations of essential amino acids in plasma and the brain increased in aged rats given a higher quantity or quality of dietary protein.

Several investigators have reported that the protein synthesis in visceral organs and skeletal muscle was increased by GH and insulin in rats (Jepson et al., 1988; Kato, 2002). Insulin also stimulates the protein synthesis rates in the brains of diabetic rats (Hayase and Yokogoshi, 1995). In the present study, the plasma concentration of GH, rather than insulin, markedly decreased in rats fed the lower quantity of protein and lower quality of protein. The changes in the plasma concentration of GH depended on the quantity and quality of dietary protein. Recent studies have shown that GH may affect many functions related to the central nervous system. Treatment of adult GH-deficient patients with human GH is reported to improve psychological well being and memory function (Gibney et al., 1999; Deijen et al., 1998). Le Greves et al. (2002) suggested that GH might directly affect gene expression in neurons. Kato (2002) suggested that GH might stimulate the translational phase of protein synthesis. Many investigations suggested that the polysome profile in tissues also represented the changes in the translational phase of protein synthesis, and included initiation, elongation and termination (Siegel et al., 1971; Symmons et al., 1972; Yokogoshi et al., 1980a, 1992). Therefore, in the present study, the quantity and quality of dietary protein may have controlled the concentration of GH and been one of the factors affecting brain protein synthesis in aged rats. The effect of GH treatment on the brain protein synthesis rates in rats is another question to consider in a further examination.

In our previous work (Yokogoshi et al., 1992), we also demonstrated that most of free amino acids, both in blood and in the brain, showed variations in accordance with their concentrations in the dietary protein in weaned rats, and that the alterations in the amino acid concentrations in the blood and brain, as well as in the brain protein synthesis, resulted from changes in the quantity and quality of dietary proteins. In the present study, in the cerebral cortex and plasma, most of the essential amino acids, such as branched chain amino acids, methionine, threonine and lysine, decreased significantly in aged rats given lower quantity or quality protein. Beverly et al. (1991) suggested that the concentrations of dietary-limiting amino acids within the brain influenced the protein synthesis in young rats. Koie et al. (2000) and Lyou et al. (2004) reported that the addition of lysine or methionine to a low-gluten diet or to a low-soy protein diet, respectively, increased the protein synthesis rates in the brains of aged rats. In both the liver (Flaim et al., 1982; Yoshizawa et al. 1998) and skeletal muscle (Preedy and Garlick, 1986; Anthony et al., 2000a), the stimulation of protein synthesis caused by amino acids and protein is reported to be mediated by the increase in the initiation of mRNA translation. Recently, leucine has been shown to be the most potent of the amino acids in enhancing the initiation phase of mRNA translation (Anthony et al., 2000b). Yoshizawa et al. (2001, 2004) demonstrated that leucine stimulated the translation initiation by inhibiting the translational repressor, eukaryotic initiation factor (eIF) 4E-binding protein 1, in the liver and skeletal muscle. Our experiments showed that, when the quantity and quality of dietary protein were high, the concentrations of essential amino acids including leucine in the plasma and consequently in the brain will be improved, which could lead to an improvement in the brain protein synthesis of aged rats. Therefore, the decrease of protein synthesis rates and polysome profile in the brain resulting from the lower quantity or quality of dietary protein may be due to the dietary-limiting amino acids, which were at low levels both in the blood and in the brain. Measurement of the initiation factors of mRNA translation in the brain should be included in further studies of the effect of dietary protein on brain protein synthesis in older rats.

The results suggest that the ingestion of a higher quantity and quality of dietary protein increases the concentrations of GH and several amino acids in aged rats, and that the concentrations of GH and amino acids are at least partly related to the mechanism by which the dietary protein affects brain protein synthesis in aged rats.

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Authors' address: Kazutoshi Hayase, Department of Home Economics, Aichi University of Education, Kariya, Aichi 448-8542, Japan,
Fax: +81-566-26-2476, E-mail: khayase@auecc.aichi-edu.ac.jp