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Effect of Dietary Protein Quantity and Quality on the Brain Protein Synthesis Rate in Ovariectomized Female Rats

MIYUKI TANAKA,† SUNOK LYOU,† KAZUTOSHI HAYASE,*,†
SATOSHI OKUYAMA,‡ AND HIDEHIKO YOKOGOSHI‡

Department of Home Economics, Aichi University of Education, Kariya, Aichi 448-8542, Japan, and Laboratory of Nutritional Biochemistry, School of Food and Nutritional Sciences, The University of Shizuoka, Yada, Shizuoka 422-8526, Japan

The purpose of this study was to determine whether the quantity and quality of dietary protein affected the rate of brain protein synthesis in ovariectomized female rats. Two experiments were conducted on the ovariectomized female rats (12 weeks old) given diets containing 20%, 5%, or 0% casein (experiment 1) and 20% casein, 20% soy protein, 20% gluten, or 20% gelatin (experiment 2) for 10 d, respectively. The fractional rates of protein synthesis in the brain declined with a decrease of the quantity and quality of dietary protein. In the brain, the RNA activity [g of protein synthesized/((g of RNA) d)] was significantly correlated with the fractional rate of protein synthesis. The RNA concentration (mg of RNA/g of protein) was not related to the fractional rate of protein synthesis in any organ. The results suggest that the rate of protein synthesis in the brain declines with the decrease of the quantity and quality of dietary protein in ovariectomized female rats, and that RNA activity is at least partly related to the fractional rate of brain protein synthesis.

KEYWORDS: Dietary protein; ovariectomy; protein synthesis; brain; rats

INTRODUCTION

The metabolic response to dietary proteins, age, and hormonal factors includes marked changes in protein synthesis, especially in the liver, muscle, and intestine (*1*−*7*). Protein synthesis in the brain is also sensitive to the alteration of dietary amino acid composition (*8*, *9*) in young rats. Many investigators have reported that protein synthesis declined in specific tissues (e.g., liver or muscle) and in the whole body throughout development in mammals after weaning (*10*−*13*). We have demonstrated that the rate of protein synthesis in the brain decreased with age in rats after weaning (*14*). In many investigations, not only age but also sex hormone deficiency affects body composition and function (*15*). Though we reported that estrogen increased the protein synthesis in the brain in ovariectomized female rats (*16*), few studys are available on the effect of dietary protein on brain protein synthesis during estrogen deficiency. Therefore, the possible effects of dietary protein on brain protein synthesis in ovariectomized female rats are of nutritional importance in understanding the role of protein nutrition and sex hormone on the brain function in mammals. The purpose of our study was to determine whether the quantity and quality of dietary protein affects brain protein synthesis in ovariectomized female rats. In our previous paper (*17*, *18*), a positive correlation between the rate of protein synthesis and the RNA activity was found in the brain when the quality or quantity of dietary protein was manipulated in aged male rats. However, the reduction with age in protein synthesis in the brain was related to a fall in the RNA concentration (*14*). Two questions were considered in the present study: (1) whether the quantity and quality of dietary protein might affect brain protein synthesis in ovariectomized female rats and (2) whether greater RNA concentration or RNA activity in rats fed the higher quantity or quality proteins resulted in a greater protein synthesis rate in the brain than that in rats fed the lower quantity or quality proteins. Therefore, we examined three indicators of protein synthesis in rat brains: its rate, RNA concentration, and RNA activity. In the previous paper (*18*), 20% gelatin, 20% gluten, and 20% casein diets were chosen to investigate the effect of dietary protein quality on the brain protein synthesis rate in the aged male rats. In the present experiment, 20% soy protein diet was also used in addition to these dietary proteins. The soy protein has been known to be high-quality protein in adult mammals (*19*); however, little documentation is available for the effect of soy protein on the brain protein synthesis. We used the same experimental conditions as described in the previous paper (*17*) to investigate the effect of dietary protein quantity.

MATERIALS AND METHODS

Chemicals. L-Tyrosine decarboxylase, β-phenethylamine, and leucylalanine were purchased from Sigma Chemical (St. Louis, MO). L-[2,6-^{3}H]Phenylalanine (1.5 TBq/mmol) was obtained from Amersham (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

* To whom correspondence should be addressed. Phone: 81-566-26-2476. Fax: 81-566-26-2476. E-mail: khayase@auecc.aichi-edu.ac.jp.
† Aichi University of Education.
‡ The University of Shizuoka.

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Table 1. Composition (g/100 g diet) of Experimental Diets

ingredient	20% gelatin[a]	20% gluten[a]	20% soy protein[a]	20% casein[a,b]	5% casein[b]	protein-free[b]
casein[c]				20.0	5.0	0.0
soy protein isolate[d]			20.0			
gluten		20.0				
gelatin	20.0					
cornstarch[c]	21.8	21.8	21.8	21.8	26.8	28.4
sucrose[c]	43.5	43.5	43.5	43.5	53.5	56.9
corn oil	5.0	5.0	5.0	5.0	5.0	5.0
AIN-93G mineral mix[e]	3.5	3.5	3.5	3.5	3.5	3.5
AIN-93VX vitamin mix[e]	1.0	1.0	1.0	1.0	1.0	1.0
cellulose[c]	5.0	5.0	5.0	5.0	5.0	5.0
choline chloride	0.2	0.2	0.2	0.2	0.2	0.2

[a] These diets were used in experiment 2. [b] These diets were used in experiment 1. [c] Supplied by Oriental Yeast, Tokyo, Japan. [d] Supplied by Fuji Oil, Osaka, Japan. [e] Supplied by Nihon Nosan K.K., Yokohama, Japan (*34*).

Table 2. Specific Radioactivities (Bq/nmol) of Free Phenylalanine in the Plasma, Cerebral Cortex, and Cerebellum in Ovariectomized Female Rats Fed Diets with Different Quantities of Protein[a]

	protein-free	5% casein	20% casein	pooled SEM
plasma	9.1	8.7	8.6	0.4
cerebral cortex	8.9	8.5	8.4	0.3
cerebellum	8.2	7.8	7.7	0.4

[a] Values are means and pooled SEM, $n = 6$. Values within each tissue were not significantly different ($p > 0.05$).

Animals and Diet. Female Wistar rats (12 weeks old, Japan SLC, Hamamatsu, Japan) were housed at 24 ± 1 °C in a room with a 12 h light/dark cycle. All rats were ovariectomized and transferred to the experimental diets containing 0%, 5%, or 20% casein (experiment 1,**Table 1**) or 20% casein, 20% soy protein, 20% gluten, or 20% gelatin (experiment 2, **Table 1**) after consuming a commercial nonpurified diet (MF, Oriental Yeast, Tokyo, Japan) for 2 d. All animals were individually housed and given 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. The experiment was conducted on three (experiment 1) or four (experiment 2) groups of rats. All rats were fed the experimental diets for 10 d. After 10 d, the fractional rates of protein synthesis in the brain were measured by the method of Garlick et al. (*20*). The rats were decapitated between 10 a.m. and noon. The brain regions (*21*) were quickly removed and frozen in liquid nitrogen. The concentrations of protein and RNA in the brain were measured according to the methods of Lowry et al. (*22*) with bovine serum albumin as a standard and Fleck and Munro (*23*), respectively.

Fractional Rate of Protein Synthesis in Tissues. Radioactive L-[2,6-^{3}H]phenylalanine was combined with unlabeled phenylalanine to yield a dose of 1.85 MBq and a concentration of 150 mmol/L saline. Rats were injected with the radioisotope via the tail vein at a dose of 1 mL/100 g of body weight. Specific radioactivities of [^{3}H]phenylalanine in tissue samples were determined according to the method described in our previous report (*17*).

In a preliminary experiment, we determined whether the method of Garlick et al. (*20*) could be used to measure the rate of protein synthesis in the brain under this experimental condition. Specific radioactivities of free phenylalanine in the plasma, cerebral cortex, and cerebellum in rats of the three groups were constant in each tissue (**Table 2**). The values were also not significantly different among the plasma, cerebral cortex, and cerebellum, indicating that the precursor pool of labeled phenylalanine was not altered. The decrease in labeling of free phenylalanine at 3, 5, and 10 min in the brain was not significant after an injection of a large dose of [^{3}H]phenylalanine (**Table 3**). Therefore, the protein synthesis rates for the brain regions were calculated for animals killed at a single time point of 10 min after intravenous administration of the radioisotope.

Table 3. Time-Dependent Changes of Specific Radioactivities (Bq/nmol) of Free Phenylalanine in the Brains of Ovariectomized Female Rats Fed the 20% Casein Diet[a]

	3 min[b]	5 min[b]	10 min[b]	pooled SEM
cerebral cortex	8.9	8.8	8.5	0.3
cerebellum	8.3	8.2	7.8	0.3

[a] Values are means and pooled SEM, $n = 6$. Values within each tissue were not significantly different ($p > 0.05$). [b] Time after injection of radioactive phenylalanine.

Table 4. Effect of the Quantity of Dietary Protein on Body Weight Gain and Relative Weights and Fractional Protein Synthesis Rates in the Brain Regions of Ovariectomized Female Rats[a]

	protein-free	5% casein	20% casein	pooled SEM
initial body weight (g)	174.4	173.6	173.8	2.6
body weight gain (g/l0 d)	–12.8[c]	10.4[b]	30.4[a]	1.8
food intake (g/d)	10.0[b]	14.6[a]	15.9[a]	0.7
tissue weight (g/100 g of body weight)				
cerebral cortex	0.20	0.20	0.18	0.006
cerebellum	0.13	0.13	0.12	0.004
hippocampus	0.057	0.052	0.054	0.002
brain stem	0.079	0.075	0.077	0.002
protein synthesis rate (K_s),[b] %/d				
cerebral cortex	14.8[c]	18.5[b]	21.1[a]	0.7
cerebellum	19.8[c]	24.2[b]	27.6[a]	0.4
hippocampus[c]	20.2	24.7	29.8	
brain stem[c]	28.7	33.7	36.3	

[a] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$). [b] Fractional rate of protein synthesis. [c] Data were obtained by a single analysis of pooled samples from six rats.

Statistical Analysis. The means and pooled SEMs are reported. Duncan's multiple range test was used to compare the means after one-way ANOVA (*24*, *25*). Linear regression analysis was used to assess the relationship between the rate of protein synthesis and RNA activity (*25*). Differences were considered significant at $p < 0.05$. In the hippocampus and brain stem, the rates of protein synthesis were determined from a pool of each region.

RESULTS

Experiment 1. The rats fed the protein-free diet lost body weight and consumed less food than the other two groups, which did not differ except the one fed the 5% casein diet, the body weight gain of which was significantly lower. The relative weights of the brain regions did not differ. The fractional rates of protein synthesis (K_s) in some brain regions, such as the cerebral cortex and cerebellum, declined gradually with a decrease of the quantity of dietary protein (**Table 4**). In the hippocampus and brain stem, these rates tended to be lower with each decrease of dietary protein quantity. RNA activity [g of protein synthesized/((g of RNA) d)] in the brain regions was significantly lower in rats fed the protein-free diet or 5% casein diet than in rats fed the 20% casein diet (**Table 5**). Correlations between the fractional rate of protein synthesis and RNA activity were significant in the cerebral cortex ($r = 0.899$, $p < 0.001$) and cerebellum ($r = 0.881$, $p < 0.001$). The RNA concentrations (mg of RNA/g of protein) in all brain regions did not differ among the groups (**Table 5**).

Experiment 2. The rats fed the 20% gelatin diet did not gain body weight and had less food intake as compared to the other

Table 5. Effect of the Quantity of Dietary Protein on RNA Concentrations and RNA Activities in the Brain Regions of Ovariectomized Female Rats[a]

	protein-free	5% casein	20% casein	pooled SEM
RNA/protein, mg/g				
cerebral cortex	11.2	10.9	11.1	0.4
cerebellum	12.4	12.3	11.6	0.3
hippocampus[b]	11.2	11.9	11.6	
brain stem[b]	8.3	8.9	8.7	
RNA activity, g of protein synthesized/((g of RNA) d)				
cerebral cortex	13.3[c]	17.0[b]	19.0[a]	0.6
cerebellum	16.0[c]	19.8[b]	23.8[a]	0.5
hippocampus[b]	18.0	20.8	25.7	
brain stem[b]	34.6	37.8	41.7	

[a] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$). [b] Data were obtained by a single analysis of pooled samples from six rats.

Table 6. Effect of the Quality of Dietary Protein on Body Weight Gain and Relative Weights and Fractional Protein Synthesis Rate in the Brain Regions of Ovariectomized Female Rats[a]

	20% gelatin	20% gluten	20% soy protein	20% casein	pooled SEM
initial body weight (g)	185.8	186.4	185.0	185.8	2.5
body weight gain (g/l0 d)	–16.8[c]	24.0[b]	30.6[a]	31.4[a]	1.7
food intake (g/d)	11.1[b]	16.7[a]	17.5[a]	18.2[a]	0.6
tissue weight (g/100 g of body weight)					
cerebral cortex	0.17	0.18	0.18	0.17	0.004
cerebellum	0.11	0.12	0.12	0.12	0.002
hippocampus	0.061	0.058	0.058	0.056	0.003
brain stem	0.078	0.078	0.077	0.073	0.003
protein synthesis rate (K_S),[b] %/d					
cerbral cortex	14.0[c]	17.8[b]	21.3[a]	22.8[a]	0.7
cerebellum	14.5[c]	19.5[b]	25.4[a]	26.7[a]	0.6
hippocampus[c]	22.2	27.5	30.6	30.6	
brain stem[c]	23.4	27.5	32.9	33.4	

[a] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$). [b] Fractional rate of protein synthesis. [c] Data were obtained by a single analysis of pooled samples from six rats.

three groups, which did not differ except the one fed the 20% gluten diet, the body weight gain of which was significantly lower (**Table 6**). The relative weights of the brain regions were not different among the four groups.

The fractional rates of protein synthesis (K_s) in the cerebral cortex and cerebellum did not change with the 20% soy protein diet as compared with the 20% casein diet, but decreased significantly with the gluten diet and still more with the gelatin diet. The same observation could be made with respect to the hippocampus and brain stem as K_s was unchanged with both casein and soy protein diets but tended to decrease with the gluten diet, and to a higher extent with the gelatin diet (**Table 6**).

RNA activity [g of protein synthesized/((g of RNA) d)] in the brain regions was significantly lower in rats fed the 20% gelatin diet or 20% gluten diet than in rats fed the 20% casein diet or 20% soy protein diet (**Table 7**). Correlations between the fractional rate of protein synthesis and RNA activity were significant in the cerebral cortex ($r = 0.888$, $p < 0.001$) and cerebellum ($r = 0.879$, $p < 0.001$). The RNA concentrations (mg of RNA/g of protein) in all regions did not differ among the groups (**Table 7**).

Table 7. Effect of the Quality of Dietary Protein on RNA Concentrations and RNA Activities in the Brain Regions of Ovariectomized Female Rats[a]

	20% gelatin	20% gluten	20% soy protein	20% casein	pooled SEM
RNA/protein, mg/g					
cerebral cortex	12.8	13.3	11.9	12.4	0.5
cerebellum	17.2	17.5	16.4	16.9	0.3
hippocampus[b]	13.3	13.8	13.7	13.9	
brain stem[b]	11.0	11.3	10.5	10.5	
RNA activity, g of protein synthesized/((g of RNA) d)					
cerebral cortex	10.9[c]	13.5[b]	17.9[a]	18.5[a]	0.6
cerebellum	8.5[c]	11.2[b]	15.5[a]	15.9[a]	0.5
hippocampus[b]	16.7	19.9	22.3	22.0	
brain stem[b]	21.3	24.3	31.3	31.8	

[a] Values are means and pooled SEM, $n = 6$. Means with different superscript letters are significantly different ($p < 0.05$). [b] Data were obtained by a single analysis of pooled samples from six rats.

DISCUSSION

In ovariectomized female animals, the deficiency of sex hormone affects the body composition and function. Estrogen has been known to stimulate tissue protein synthesis (*16*, *26*, *27*). The ovariectomy decreases the brain protein synthesis in female rats (*16*). However, little information is available with regard to the effects of the quantity and quality of dietary protein on brain protein synthesis during sex hormone deficiency. Therefore, the elucidation of the possible roles of dietary protein on brain protein synthesis in ovariectomized female rats was of nutritional interest. We hypothesized that the brain protein synthesis also decreased with a decrease in the quantity or quality of dietary protein in ovariectomized female rats. In the brain regions, the fractional rates of protein synthesis declined with a decrease of dietary protein quantity or quality (**Tables 4** and **6**). In the present study, the changes in the rate of brain protein synthesis were considered to depend on the quantity and quality of dietary protein as previously demonstrated in the brain regions of male rats (*9*, *17*, *18*). In weaned rats, a reduction with age in protein synthesis in the brain and muscle was related to a fall in RNA concentration (*13*, *14*). However, a positive correlation between the rate of protein synthesis and RNA activity was found in the brain of weaned and aged male rats when the dietary proteins were manipulated (*9*, *18*). In the brain regions of rats in the present study, RNA activity, rather than RNA concentration, decreased with a decrease of dietary protein quantity or quality (**Tables 5** and **7**).

The lower RNA activity in rats fed the protein-free and 5% casein diets as compared to those fed the 20% casein diet, on one hand, and the rats fed the 20% gelatin and 20% gluten diets as compared to those fed the 20% soy protein and 20% casein diets, on the other hand, may result in a decreased rate of brain protein synthesis in these groups. Therefore, the changes in dietary protein quantity and quality may have controlled RNA activity and been one of the factors affecting brain protein synthesis in female ovariectomized rats. Cherel et al. (*28*) reported that 24 h of starvation induced a decrease of fractional rates of protein synthesis in the brain, and that this decline resulted from a decrease of RNA activity. Our results with protein feeding were consistent with those for the brief fasting. Little information is available on the mechanism by which the dietary protein affects RNA activity in the brain of ovariectomized rats. Insulin treatment of diabetic rats also appeared to elevate the rate of protein synthesis and RNA activity in the brain (*29*). The ingestion of a high-protein diet elevates the

concentration of plasma insulin when the dietary level of carbohydrates is not manipulated (*30*). Therefore, to determine the effect of dietary protein on brain protein synthesis in ovariectomized female rats, measurement of the concentration of plasma insulin under physiological conditions and varying the dietary protein should be included in future studies. The treatment of a flooding dose of phenylalanine has been known to increase the insulin secretion, whereas this treatment did not affect the rate of tissue protein synthesis directly (*20*). The higher concentration of plasma insulin by the large dose method of phenylalanine should be considered if the relationship among the insulin secretion, dietary protein, and brain protein synthesis is measured.

We also reported that the aggregation of polyribosomes in the brain of weaned rats after only a 5 h feeding decreased with a decrease of dietary protein quantity, and that a correlation between the polysomal profile and RNA activity was observed (*9*). Saito et al. (*31*) measured the polypeptide chain assembly time to understand the mechanism of decreased hepatic protein synthesis in rats fed a low-protein diet, and demonstrated that protein depletion caused a depression in the polypeptide elongation rate. To determine the mechanism by which dietary protein affects brain protein synthesis in ovariectomized female rats, the ribosomal aggregation and polypeptide elongation rates in the brain will be separately reported. In the present study, we did not determine the concentration of mRNA in the brain regions. In further examination, quantitative features of mRNA should also be measured in the brain regions (*32*).

In the present experiment, soy protein was also used as dietary protein in addition to casein, gluten, and gelatin when the dietary protein quality was manipulated. Research on the potential health benefits of soy foods has been particularly intriguing with respect to cancer prevention and hypocholesterolemic effects (*19*). Many investigators reported that the soybean is an important source of high-quality protein (*19*); however, little documentation is available on the effect of soy protein on brain function. In the present study, the ingestion of soy protein resulted in a higher fractional rate of brain protein synthesis in ovariectomized female rats than the groups given gluten or gelatin diets, and this result may suggest that the brain protein synthesis was affected by the soy protein. The isoflavones in soybeans work as weak estrogen in tissues of mammals, though the role of isoflavones in maintaining brain protein synthesis remains unknown under physiological conditions (*33*). In our previous work (*9*), we also demonstrated that most of the free amino acids, both in the serum and 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 brain protein synthesis resulted from changes in the quantity and quality of dietary proteins. Beverly et al. (*8*) reported that the concentrations of dietary limiting amino acids within the brain influenced protein synthesis. In ovariectomized female rats, the decrease of protein synthesis rates resulting from the low quantity or quality of dietary protein may also be an influence of the dietary amino acids, which are at low levels in the brain. Therefore, this is another possibility to consider in further examination of the mechanism by which the dietary protein alters brain protein metabolism in aged rats. The present results indicate that brain protein synthesis was affected by the dietary protein quantity or quality in ovariectomized female rats as evaluated by the protein synthesis rates, and suggest that the effects of dietary protein on brain protein synthesis in ovariectomized female rats is also of importance in understanding the relationship among protein nutrition, sex hormone deficiency, and brain function in mammals.

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