Effect of Dietary Protein Quantity on the Activities of Brain Proteases in Rats

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Abstract

In this study, we examined effects of different quantity of dietary protein on the brain proteinases in rats. Male Wistar strain rats (14 wk old) were divided into two groups, and fed the diets containing 0% (protein-free) or 20% casein for 10 d, respectively. The body weight gain in the rats fed with the protein-free diet was significantly low compared with rats fed the 20% casein diet. On the other hand, the protein concentration in various brain regions were unaffected by quantity of dietary protein. However, the activities of intracellular proteolytic enzymes were decreased along with the reduction of dietary protein, especially cathepsins were significantly decreased. These results suggest that the proteolytic enzymes in the brain declines along with a decrease in dietary protein quantity in order to compensate the loss of protein, and finally protein levels is maintained in the brain.

Key Words：dietary protein; proteases; brain; rat

INTRODUCTION

The protein concentration in tissues are easily affected by nutritional alteration such as changes in quantity of dietary proteins. These changes in protein metabolism may be reflected in the rate of protein synthesis and breakdown, especially in the liver and muscle [1-6]. There is an evidence indicated that protein synthesis in the brain was also susceptible of the quality or quantity of dietary protein [7-9] and the internal environment [10,11]. However, in comparison with the studies on the liver or muscle, there are few documentations about the effect of dietary protein on brain proteolysis.

In the present study, we conducted to examine the effects of dietary protein on the brain proteolysis in rats. As indices of the capacity for brain proteolysis, the activities of intracellular proteolytic enzymes such as calpain (m- plus μ-calpain), proteasome (20S plus 26S proteasome), and cathepsins were determined.

MATERIALS AND METHODS

Animal experiment. Male Wistar strain rats (12 wk old, Japan SLC Inc, Shizuoka, Japan) were individually kept in stainless cages in a temperature- and humidity-controlled room (24 ± 1℃ and 55 ± 5% relative humidity) with a 12 h light-dark cycle (light on 07:00-19:00). All rats had free access to 20% casein diet and water for 14 d as adaptation period. Then the rats were divided into two groups, one fed ad

libitum a 20% casein diet and the other a protein-free (0% casein) diet (Table 1) for 10 d. On the last day at the experiment, the rats were decapitated, and then brains were immediately isolated. Finally, brain regions such as cerebral cortex, hippocampus, cerebellum and brain stem were quickly removed and frozen in liquid nitrogen, and stored at -80℃ until assay.

Table 1. Composition of experimental diets

ingredient	Protein-free	20% Casein
	(%)	
Casein[1]	0.0	20.0
Corn starch[1]	56.9	43.5
Sucrose[1]	28.4	21.8
Cellulose[1]	5.0	5.0
Corn oil	5.0	5.0
AIN-93 vitamin mixture[2]	1.0	1.0
AIN-93 mineral mixture[2]	3.5	3.5
Choline-Cl	0.2	0.2

1 Supplied by Oriental Yeast, Tokyo, Japan.
2 Supplied by Nihon Nosan K.K., Yokohama, Japan.

Chemical analysis. Each protease activities, such as calpain, proteasome and cathepsins, were analyzed as described previously [12]. Each brain region was homogenized in 2 volumes of ice-cold 20 mM Tris-HCl buffer (pH 7.5) containing 5 mM EDTA and 10 mM dithiothreitol. Then the homogenate was centrifuged at 30,000 ×g for 30 min at 4℃ . The supernatant was used for measurement of calpain and proteasome activities. The resulting protein pellet was suspended in 50 mM acetate buffer (pH 5.0) containing 0.2 M NaCl and 0.1% Triton X-100. The suspension was centrifuged at 13,000 ×g for 30 min at 4℃ . The supernatant was used for measurement of cathepsin B+L and cathepsin D activities.

Calpain activity was measured by the method of Sasaki et al. [13] by using 0.2 mM succinyl-Leu-Leu-Val-Tyr-MCA, fluorogenic synthetic peptide as a substrate at pH 7.3. Proteasome activity was analyzed by the method of Tanaka et al. [14] determined with succinyl-Leu-Leu-Val-Tyr-MCA as a substrate at pH 8.0. The activity of cathepsin B+L was measured by the method of Barrett and Kirschke [15] by using fluorogenic peptides. It was assayed with 10 mM Z-Phe-Arg-MCA as a substrate at pH 5.5. This synthetic substrate is hydrolyzed by cathepsin B and cathepsin L, so the activity is shown as cathepsin B+L activity. The activity of cathepsin D was measured by the method of Barrett and Kirschke [16] using 2% hemoglobin as a substrate.

Protein concentration was measured by Lowry’ s method by using bovine serum albumin as a standard [17].

Statistical analysis. Data were analyzed by Student's *t*-test. A *p* value of less than 0.05 was considered to be statistically significant. Each result was expressed as the means ± standard errors.

RESULT AND DISCUSSION

The body weight gain in the rats fed the protein-free diet was significantly low compared with the rats fed the 20% casein diet (Table 2). However, the weight and protein concentration in various brain regions did not differ.

Table 2. Effect of quantity of dietary protein on body weight gain, and tissue weight and protein concentration of brain regions in rats

Values represent means±SEM, n=5. Student's *t*-test was performed

*Significantly different from that of the 20% casein diet group ($p<0.05$)

	Protein-free	20% Casein
Initial body weight (g)	272.3 ± 2.7	274.2 ± 2.6
Body weight gain (g)	-30.8 ± 1.8*	27.1 ± 1.5
Tissue weight (g)		
Cerebral cortex	0.165 ± 0.003	0.163 ± 0.005
Hippocampus	0.060 ± 0.002	0.057 ± 0.002
Cerebellum	0.131 ± 0.005	0.138 ± 0.004
Brain stem	0.248 ± 0.005	0.229 ± 0.006
Protein concentration (mg/g tisuue)		
Cerebral cortex	157.6 ± 5.2	150.7 ± 4.7
Hippocampus	156.1 ± 6.8	145.9 ± 4.3
Cerebellum	177.3 ± 4.8	164.8 ± 5.4
Brain stem	144.5 ± 1.8	143.6 ± 2.3

The activities of calpain, proteasome, cathepsin B+L and cathepsin D were determined in cerebral cortex, hippocampus, cerebellum and brain stem (Table 3). The activities of proteolytic enzymes in some brain regions declined with the decrease in quantity of dietary protein, especially cathepsin B+L activity was significantly decreased by the protein-free diet.

Table 3. Effect of quantity of dietary protein on the activity of proteolytic enzymes in brain regions of rats
Values represent means±SEM, n=5. Student's *t*-test was performed
*Significantly different from the 20% casein diet grooup($p<0.05$)

	Protein-free	20% Casein
Calpain (nmol AMC/h/mg protein)		
Cerebral cortex	17.72 ± 1.56	18.58 ± 1.62
Hippocampus	24.31 ± 0.62	26.83 ± 1.32
Cerebellum	18.54 ± 0.48	19.81 ± 0.81
Brain stem	14.29 ± 1.07	14.88 ± 0.76
Proteasome (nmol AMC/h/mg protein)		
Cerebral cortex	3.31 ± 0.31	4.00 ± 0.61
Hippocampus	11.38 ± 1.76	10.06 ± 4.96
Cerebellum	2.89 ± 1.23	6.12 ± 2.48
Brain stem	3.73 ± 0.38	4.29 ± 0.62
Cathepsin B+L (nmol AMC/h/mg protein)		
Cerebral cortex	20.81 ± 1.14*	34.42 ± 4.50
Hippocampus	38.17 ± 3.28	37.21 ± 1.83
Cerebellum	29.91 ± 1.56*	38.31 ± 3.85
Brain stem	32.32 ± 1.70	27.26 ± 3.24
Cathepsin D (μg Tyr/min/mg protein)		
Cerebral cortex	0.195 ± 0.008	0.218 ± 0.038
Hippocampus	0.122 ± 0.006	0.162 ± 0.017
Cerebellum	0.114 ± 0.004	0.119 ± 0.004
Brain stem	0.145 ± 0.022	0.173 ± 0.014

The intracellular proteolytic process is composed of lysosomal and nonlysosomal pathways in which intracellular proteases are directly responsible for the degradation of proteins. Calpain is a cysteine protease ubiquitously and constitutively expressed in the cytosol of mammalian cells, and is thought to be the main agent of nonlysosomal Ca2+-dependent proteolysis [18]. Proteasome is a multicatalytic ATP-dependent proteolytic enzyme that needs ubiquitination of its substrate [19]. The multiple proteolytic pathways are essential to complete proteolysis. Lysosomal cathepsin B, L and D are endopeptidases of neurons that might play major roles in intracellular protein catabolism [20]. Cathepsin B is the most abundant lysosomal protease, being required for the housekeeping function of lysosomes in protein turnover by cells. In contrast, cathepsin L has been implicated in tumor progression and bone resorption [21,22].

In previous study, Yokogoshi *et al*. reported that the fractional rates of protein synthesis in the brain declined with a decrease in quantity of dietary protein, and the aggregation of polyribosomes in the brain decreased with a decrease in dietary protein after only 5 h of feeding, and that there was a correlation between the polysomal profile and RNA activity [8]. Generally, the fractional rates of protein degradation in the liver or skeletal muscle are increased with the reduction of protein intake, accompanied by ascent of the activities and the protein expression levels of proteolytic enzymes, and then the masses of these organs are decreased. In this study, the weight and protein concentration in the brain regions were unaffected by quantity of dietary protein, and there was not ascent of the brain proteolytic enzyme activities. So, the rate of protein degradation might decrease in the brain, although not determined in the present study. It seems

that the regulation of brain protein metabolism differ from the other organs.

In conclusion, these results indicate that the brain protein metabolism is declined with the reduction of dietary protein through the reduction of protein synthesis and not ascent of the proteolytic enzyme activities, which will compensate to protein loss in the brain.

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REFERENCES

1) Millward, D. J., Garlick, P. J., Stewart, R. J. C., Nnanyelugo, D. O., and Waterlow, J. C., Skeletal muscle growth and protein turnover. *Biochem. J*., **150**, 235-243 (1975).

2) Millward, D. J., Garlick, P. J., Nnanyelugo, D. O., and Waterlow, J. C., The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. *Biochem. J.*, **156**, 185-188 (1976).

3) Funabiki, R., Watanabe, Y., Nishizawa, N., and Hareyama, S., Quantitative aspect of the myofibrillar protein turnover in transient state on dietary protein depletion and repletion revealed by urinary excretion of N^t-methylhistidine. *Biochim. Biophys. Acta*, **451**, 143-150 (1976).

4) Yokogoshi, H., Sakuma, Y., and Yoshida, A., Relationships between nutritional quality of dietary proteins and hepatic polyribosome profiles in rats. *J. Nutr*., **110**, 383-387 (1980).

5) Yokogoshi, H., Sakuma, Y., and Yoshida, A., Effect of dietary protein quality and quantity on hepatic polyribosome profiles in rats. *J. Nutr*., **110**, 1347-1353 (1980).

6) Lewis, S. E. M., Kelly, F. J., and Goldspink, D. F., Pre- and post-natal growth and protein turnover in smooth muscle, heart and slow- and fast-twitch skeletal muscles of the rat. *Biochem. J*., **217**, 517-526 (1984).

7) Beverly, J. L. 3rd., Gietzen, D. W., and Rogers, Q. R., Protein synthesis in the prepyriform cortex: effects on intake of an amino acid-imbalanced diet by Sprague-Dawley rats. *J. Nutr*., **121**, 754-761 (1991).

8) Yokogoshi, H., Hayase, K., and Yoshida, A., The quality and quantity of dietary protein affect brain protein synthesis in rats. *J. Nutr*., **122**, 2210-2217 (1992).

9) Hayase, K., Koie, M., and Yokogoshi, H., The quantity of dietary protein affects brain protein synthesis rate in aged rats. *J. Nutr*., **128**, 1533-1536 (1998).

10) Hayase, K., Naganuma, Y., Moriyama, M., Yoshida, A., and Yokogoshi, H., Effect of a thyroid hormone treatment on brain protein synthesis in rats. *Biosci. Biotech. Biochem*., **61**, 1536-1540 (1997).

11) Haysase, K., Tanaka, M., Tujioka, K., Hirano, E., Habuchi, O., and Yokogoshi, H., 17-β-Estradiol

affects brain protein synthesis rate in ovariectomized female rats. *J. Nutr*., **131**, 123-126 (2001).

12) Tada, O., and Yokogoshi, H., Effect of different dietary protein composition on skeletal muscle atrophy by suspension hypokinesia/hypodynamia in rats. *J. Nutr. Sci. Vitaminol*., **48**, 115-119 (2002).

13) Sasaki, T., Kikuchi, T., Yumoto, N., Yoshimura, N., and Murachi, T., Comparative specificity and kinetic studies on porcine calpain I and calpain II with naturally occurring peptides and synthetic fluorogenic substrates. *J. Biol. Chem*., **259**, 12489-12494 (1984).

14) Tanaka, K., Ii, K., Ichihara, A., Waxman, L., and Goldberg, A. L., High molecular weight protease in the cytosol of rat liver. *J. Biol. Chem*., **261**, 15197-15203 (1986).

15) Barret, A. J., and Kirschke, H., Cathepsin B, cathepsin H, and cathepsin L. *Methods Enzymol*., **80**, 535-561 (1977).

16) Barrett, A. J., and Kirschke, H., Cathepsin D and other carboxyl protease. *In*: A Laboratory Hand Book (Dingle, J. T., ed), North-Holland, Amsterdam, 19-147 (1977).

17) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem*., **193**, 265-275 (1951).

18) Sorimachi, H., Ishiura, S., and Suzuki, K., Structure and physiological function of calpains. *Biochem. J*., **328**, 721-732 (1997).

19) Coux, O., Tanaka, K., and Goldberg, A. L., Structure and functions of the 20S and 26S proteasomes. *Annu. Rev. Biochem*., **65**, 801-847 (1996).

20) Kutunuma, N., and Kominami, E., Structure and functions of lysosomal thiol porteinases and their endogenous inhibitor. *Curr. Top Cell Regul*., **22**, 71-101 (1983).

21) Keppler, D., Sameni, M., Moin, K., Mikkelsen, T., Diglio, C. A., and Sloane, B. F., Tumor progression and angiogenesis: cathepsin B & Co. Biochem. *Cell Biol*., **74**, 799-810 (1996).

22) Chapman, H. A., Riese, R. J., and Shi, G. P., Emerging roles for cysteine proteases in human biology. *Annu. Rev. Physiol*., **59**, 63-88 (1997).