

Dietary γ -Aminobutyric Acid Affects the Brain Protein Synthesis Rate in Ovariectomized Female Rats

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Summary The purpose of this study was to determine whether γ -aminobutyric acid (GABA) affects the rate of brain protein synthesis in ovariectomized female rats. Experiments were done on two groups of 24-wk-old ovariectomized female rats given 0 or 0.5% GABA added to the 20% casein diet. The concentrations of plasma growth hormone (GH) increased significantly with the 20% casein+0.5% GABA compared with the 20% casein diet alone. In the brain regions, GABA treatment to the basal diet elevated significantly the fractional and absolute rates of protein synthesis. In brain regions, the RNA activity [g protein synthesized/(g RNA·d)] significantly correlated with the fractional rate of protein synthesis. The RNA concentration (mg RNA/g protein) was not related to the fractional rate of protein synthesis. The results suggest that the administration of GABA to ovariectomized female rats is likely to increase the concentrations of plasma GH and the rate of protein synthesis in the brain, and that RNA activity is at least partly related to the fractional rate of brain protein synthesis.

Key Words γ -aminobutyric acid, growth hormone, protein synthesis, brain, rats

The metabolic response to dietary proteins, age and hormonal factors includes marked changes in protein synthesis, especially in liver, muscle and intestine (1–5). Protein synthesis in the brain is also sensitive to the alteration of dietary amino acid composition in young rats (6, 7).

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 (8–10). We demonstrated that the rate of protein synthesis in the brain decreased with age in rats after weaning (11).

In many investigations, not only age but also sex hormone deficiency has been shown to affect body composition and function in postmenopausal women (12). Estrogen increases tissue protein synthesis by stimulating transcriptional activity (13, 14). We also reported that estrogen increased protein synthesis in the brain of ovariectomized female rats (15). On the other hand, several investigators demonstrated that estrogen stimulated the release of γ -aminobutyric acid (GABA) in the brain (16). GABA is a kind of the amino acid widely distributed in nature, and is an inhibitory transmitter compound in vertebrates (17, 18). Recently, GABA has

been attracting attention as a food with functions such as improvement of memory and study capability, blood-pressure lowering action and relaxation (16, 19). In a previous study, we reported that the administration of GABA to young male rats increased the concentration of plasma growth hormone (GH) and the rate of protein synthesis in the brain, and resulted in a positive correlation between the rate of protein synthesis in the brain and the plasma concentration of GH (20). GH is well known as an anabolic hormone in protein metabolism. Ohsumi et al. (21) demonstrated that hypophysectomy has been shown to decrease the rate of protein synthesis in the brain regions of rats, whereas treatment with GH reversed the effect of hypophysectomy. However, the role of GABA treatment in maintaining the rate of protein synthesis remains unknown in ovariectomized female rats. Therefore, the possible effects of the dietary addition of GABA on the brain protein synthesis and plasma GH in ovariectomized female aged rats are of importance in understanding the role of nutrition on the brain function in mammals.

The purpose of our study was to determine whether the GABA affects the rate of protein synthesis in ovariectomized female rats. In our previous report (7, 22), a positive correlation between the rate of protein synthesis and RNA activity was found in the brain when the quality or quantity of dietary protein was manipulated in young and aged rats. However, the reduction with age in protein synthesis in the brain was related to

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Abbreviations: GABA, γ -aminobutyric acid; GH, growth hormone.

a fall in the RNA concentration (11). Three questions were considered in the present study: 1) whether the dietary addition of GABA to the basal diet might increase the plasma concentration of GH in ovariectomized female rats, 2) whether the dietary addition of GABA might affect brain protein synthesis in ovariectomized female rats, and 3) whether greater RNA concentration or RNA activity in ovariectomized female rats given GABA resulted in a greater protein synthesis rate in the brain than those in rats fed the basal diet. Therefore, we examined three indicators of protein synthesis in rat brains: its rate, RNA concentration and RNA activity. The effects of GABA treatment on the GH concentration in plasma were also investigated.

MATERIALS AND METHODS

Chemicals. L-Tyrosine decarboxylase, β -phenethylamine and L-leucyl-L-alanine were purchased from Sigma Chemical (St. Louis, MO, USA). L-[2,6-³H]phenylalanine (1.5 TBq/mmol) was obtained from GE Healthcare Bio-Sciences (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and diet. Ovariectomized female 24-wk-old Wistar rats (Japan SLC, Inc., Hamamatsu, Japan) were housed at 24°C in a room with a 12-h light/dark cycle. The rats were transferred to the experimental diets after being fed a 20% casein diet for 10 d. The experimental diets contained 0 or 0.5% GABA added to the 20% casein diet (Table 1). All animals were individually housed and 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. Experiment 1 was conducted on two groups of rats. In our previous experiment, the plasma concentration of GH rose very rapidly after GABA treatment (20). Therefore, in the present study, the change in the plasma concentration of GH was measured after only one 3-h feeding period of the test diet. After all rats were fed the 20% casein diet for 10 d (one 3-h feeding period per day, from 9:00–12:00), they were given the experimental diets for 1 d (only one 3 h period). After the 3 h feeding period, the rats were decapitated and the plasma was collected in glass tubes and stored at –80°C. The concentration of plasma GH was measured by the EIA method (SPI bio, Massy, Cedex, France). Experiment 2 was conducted on two groups of rats. All rats were fed the experimental diet for 10 d ad libitum. The experimental diets contained 0 or 0.5% GABA added to the 20% casein diet (Table 1). All rats were provided free access to food and water. The fractional rates of protein synthesis in the brain were measured by the method of Garlick et al. (23). The rats were decapitated between 1000 and 1200 h. Brain regions (24) 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. (25) with bovine serum albumin as a standard, and Fleck and Munro (26), respectively.

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

Ingredient	20% Casein	20% Casein +0.5% GABA ¹
Casein	20.0	20.0
GABA	0.00	0.50
Cystine	0.3	0.3
Cornstarch ²	43.3	43.0
Sucrose ²	21.7	21.5
Corn oil	5.0	5.0
AIN-93G mineral mix ³	3.5	3.5
AIN-93VX vitamin mix ³	1.0	1.0
Cellulose ²	5.0	5.0
Choline chloride	0.2	0.2

¹ γ -Aminobutyric acid.

² Supplied by Oriental Yeast Co., Ltd., Tokyo, Japan.

³ Supplied by Nihon Nosan K.K., Yokohama, Japan (47).

Fractional rate of protein synthesis in tissues. Radioactive L-[2,6-³H]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 body weight. At 10 min after injection, rats were quickly decapitated. Specific radioactivities of [³H]phenylalanine in tissue samples were determined according to the method described in our previous report (22).

In a preliminary experiment, we determined whether the method of Garlick et al. (23) could be used to measure the rate of protein synthesis in the brain under these experimental conditions. Specific radioactivities of free phenylalanine in the plasma, cerebral cortex and cerebellum in rats of the two groups were constant in each tissue (The data are not shown). Moreover, the values were not significantly different among the plasma, cerebral cortex and cerebellum, indicating that the precursor pool of labeled phenylalanine was not altered. In our previous report (7), 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 [³H]phenylalanine. Therefore, the protein synthesis rates for brain regions were calculated for animals killed at a single time point of 10 min after intravenous administration of the radioisotope.

The fractional rate of protein synthesis (Ks) for brain regions was calculated from the specific radioactivity of phenylalanine in protein (Sb) at 10 min and the specific radioactivity of free phenylalanine in the tissue (Sa) at 10 min. The formula for calculating Ks has been given by Garlick et al. (23), i.e.

$$Ks (\%/d) = Sb \times 100 / Sa \times t$$

where t is the incorporation time in days.

The RNA activity was calculated by dividing the fractional rate of protein synthesis by the RNA/protein ratio. The absolute protein synthesis was calculated by multiplying the fractional rate of protein synthesis by the protein contents of tissues.

Table 2. Effect of the addition of GABA¹ to a basal diet on plasma concentration of growth hormone in ovariectomized female rats.²

	Control	GABA ¹
Final body weight (g)	217±4	215±5
Plasma GH ³ (μ g/L)	18.6±2.6 ^b	51.6±7.8 ^a
¹ γ -Aminobutyric acid		
² Values are means and pooled SE, <i>n</i> =5. Means with different superscript letters are significantly different (<i>p</i> <0.05).		
Table 3. Effect of the addition of GABA ¹ to a basal diet on body weight gain and brain relative weights in ovariectomized female rats. ²		
	Control	GABA ¹
Body weight gain (g/10 d) ³	28.2±2.2	27.6±1.7
Food intake (g/d)	16.7±0.5	16.9±1.1
Tissue weight (g/100 g body weight)		
Cerebral cortex	0.17±0.01	0.17±0.01
Cerebellum	0.11±0.004	0.12±0.004
Hippocampus	0.046±0.003	0.047±0.003
Brain stem	0.063±0.001	0.070±0.004
Tissue protein (mg/g tissue)		
Cerebral cortex	155±2	159±2
Cerebellum	158±3	158±2
Hippocampus ⁴	157	158
Brain stem ⁴	166	164

¹ γ -Aminobutyric acid.² Values are means and pooled SE, *n*=5.³ Initial body weight of rats was 190–220 g.⁴ Data were obtained by a single analysis of pooled samples from five rats.

Statistical analysis. The means and SE are reported. Student's *t*-test was used to compare means after one-way ANOVA (27). Linear regression analysis was used to assess the relationship between the rate of protein synthesis and RNA activity (27). 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

Plasma concentration of growth hormone (Experiment 1)

The food intake did not differ among experimental groups. The plasma concentration of GH increased significantly with the 20% casein+0.5% GABA compared with the 20% casein diet alone (Table 2).

Protein synthesis in brain regions (Experiment 2)

The body weight gain and food intake did not differ between the two groups (Table 3). The relative weights of various brain regions did not differ among the experimental groups. The fractional (Ks) and absolute rates of protein synthesis in some brain regions, such as cerebral cortex and cerebellum increased significantly with the 20% casein+0.5% GABA diet compared with the

Table 4. Effect of the addition of GABA¹ to a basal diet on protein synthesis in brain regions of ovariectomized female rats.²

	Control	GABA ¹
Protein synthesis, Ks (%/d)		
Cerebral cortex	14.7±0.8 ^b	21.0±1.1 ^a
Cerebellum	15.9±0.7 ^b	21.2±0.9 ^a
Hippocampus ³	21.9	26.8
Brain stem ³	20.5	29.5
Absolute protein synthesis (mg protein synthesized/(tissue·d))		
Cerebral cortex	8.7±0.5 ^b	12.8±0.9 ^a
Cerebellum	6.3±0.3 ^b	8.8±0.4 ^a
Hippocampus ³	3.6	4.6
Brain stem ³	5.0	7.7
RNA/protein (mg RNA/g protein)		
Cerebral cortex	13.3±0.2	12.9±0.4
Cerebellum	13.0±0.3	13.4±0.2
Hippocampus ³	14.2	13.4
Brain stem ³	9.6	10.4
RNA activity (g protein synthesized/g RNA·d)		
Cerebral cortex	11.1±0.5 ^b	16.3±0.8 ^a
Cerebellum	12.2±0.4 ^b	16.0±0.8 ^a
Hippocampus ³	15.0	20.0
Brain stem ³	21.4	28.4

¹ γ -Aminobutyric acid.² Values are means and pooled SE, *n*=5. Means with different superscript letters are significantly different (*p*<0.05).³ Data were obtained by a single analysis of pooled samples from five rats.

20% casein diet alone (Table 4). In pooled samples of hippocampus and brain stem, these rates also tended to be higher in the GABA-treated rats. The RNA activity [g protein synthesized/(g of RNA·d)] in the brain regions increased significantly with the 20% casein+0.5% GABA compared with the 20% casein diet alone (Table 4). Correlations between the fractional rate of protein synthesis and RNA activity were significant in the cerebral cortex (*r*=0.960, *p*<0.001) and the cerebellum (*r*=0.968, *p*<0.001). The RNA concentrations (mg RNA/g protein) did not differ among groups in any brain region (Table 4).

DISCUSSION

More research concerning age-related changes in brain composition and function (e.g., nutrient metabolism), is necessary to understand the modulating effects of nutritional factors (28). In older women, the deficiency of sex hormones strongly affects body composition and functions. Ovariectomy decreases brain protein synthesis in female rats (15). Recent studies have shown that estrogen stimulated the release of GABA from the hypothalamus (16). In the previous study, we demonstrated that the protein synthesis in the brain regions of hypophysectomized aged rats was increased by GH, and that the concentration of plasma GH and protein synthesis in the brains of young male rats were also increased by GABA treatment (20, 21). However,

little information is available on the effects of GABA treatment on the rate of brain protein synthesis during sex hormone deficiency. We hypothesized that the rate of brain protein synthesis would increase in ovariectomized aged female rats fed GABA. Therefore, we determined whether the dietary addition of GABA also increased the GH concentration in plasma of ovariectomized female rats. The plasma concentration of GH was significantly higher in rats given GABA (only one 3 h period) than that in control rats (Table 2). The treatment of GABA may have regulated plasma concentration of GH in the present investigation.

In the brain regions, GABA supplementation to the basal diet elevated the fractional and absolute rates of protein synthesis (Table 4). The changes in brain protein synthesis were likely attributable to the dietary GABA in ovariectomized female rats. In weaned rats, a reduction with age in protein synthesis in the brain and skeletal muscle was related to a fall in RNA concentration (10, 11). However, a positive correlation between the rate of protein synthesis and RNA activity was found in the brain of aged rats when the quantity and quality of dietary protein were manipulated (22, 29). Hormonal treatment such as GH also appeared to elevate the rate of protein synthesis and RNA activity in the brain (21). In the brain regions of rats in the present study, RNA activity, rather than RNA concentration in the group fed the 20% casein+GABA diet group was higher than in the group fed the 20% casein diet alone (Table 4). The higher RNA activity in rats fed the 20% casein+GABA diet may have increased the rate of brain protein synthesis in this group. Therefore, the addition of GABA may have controlled RNA activity and been one of the factors affecting brain protein synthesis in ovariectomized female rats.

The RNA activity is calculated by dividing the fractional rate of protein synthesis by the RNA/protein ratio, and means the protein content synthesized per unit RNA in each tissue. Many studies have suggested that the RNA activity represents the changes in the translational phase of protein synthesis (30, 31). Little information on the mechanism by which dietary GABA affects RNA activity in the brain of ovariectomized female rats is available. In the previous studies, we reported that the aggregation of polyribosomes in the brain of weaned and aged rats decreased with a decrease in dietary protein, and that there was a correlation between the polysome profile and RNA activity (7, 32). In both liver and muscle, the stimulations of protein synthesis caused by amino acids and hormonal factors are reported to be mediated by an increase in the initiation of mRNA translation (33–35). Of the many steps in the initiation process, eukaryotic initiation factor (eIF) 2 and 4E appear to be particularly important in the physiological regulation (36, 37). Kimball et al. (35, 38) demonstrated that insulin stimulated protein synthesis in the skeletal muscle by enhancing the association of eIF 4E and eIF 4G. Kato (39) suggested that GH might stimulate the translational phase of tissue protein synthesis. Measurement of the initiation factors of

mRNA translation and the ribosomal aggregation in the brain should be included in further studies on the effect of the addition of GABA to the basal diet on protein synthesis in ovariectomized female rats.

Recently, GABA has been attracting attention as a food with functions such as improvement of memory and study, and relaxation (16, 19). The ingestion of GABA resulted in higher rates of brain protein synthesis in ovariectomized female rats, suggesting that brain function is affected. Recently several 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 the psychological well being and memory function (40, 41). Le Greves et al. (42) suggested that GH induced the gene expression of hippocampal *N*-methyl-D-aspartate receptor in rats, coinciding with improved learning and memory capabilities. As mentioned above, we demonstrated that hypophysectomy has reduced the rate of protein synthesis in the brain regions of rats, whereas treatment with GH reversed the effect of hypophysectomy, and that the changes in the brain protein synthesis likely depended on the body GH concentration (21). The GH-binding receptor has been identified in the brains of humans and rats (43). The possibility that GH itself may pass the blood-brain barrier is supported by several studies (44). Several investigators have reported that the protein synthesis in visceral organs and skeletal muscle was increased by GH in rats (39). The rates of protein synthesis of liver and skeletal muscle have been also shown to increase in young male rats fed GABA (20). In the present study, the plasma concentration of GH rapidly increased after GABA treatment. The increase of brain protein synthesis rates resulting from the GABA ingestion may be due to the changes in concentration of GH in ovariectomized female rats. On the other hands, in order to determine whether the regulation of brain protein synthesis was mediated through changes in the plasma GH when GABA was added to the diet, it is also important to investigate the role of GABA treatment on the brain protein synthesis in hypophysectomized rats. The effect of GABA treatment on the brain protein synthesis rates in hypophysectomized rats is another question to consider in a further study.

A deficiency of sex hormones also affects brain function. Sherwin (45) reported that there was a beneficial effect of estrogen on memory tasks in postmenopausal women, and that estrogen deficiency may be partly responsible for the neurodegeneration of Alzheimer's disease. Data in ovariectomized rats demonstrated that treatment with estrogen increased the mRNA of cholineacetyltransferase in the brain, which is important for learning and memory processes (46). In the present study, we did not determine the concentration of mRNA in the brain regions. This is another possibility to consider in further examination of the mechanism by which the ovariectomy and GABA treatment alter brain protein metabolism.

The mass of the brain regions were unaffected by

GABA treatment, yet the fractional and absolute rates of protein synthesis increased with the addition of GABA to the 20% casein diet in the present experiment (Tables 3 and 4). These results may suggest that the protein degradation in the brain also increased in ovariectomized female rats given GABA, though the role of protein degradation in maintaining the brain mass remains to be discovered under the physiological conditions. The effect of GABA treatment on the brain protein degradation in rats is the another question to consider in a further examination.

The present results indicate that brain protein synthesis was affected by GABA in ovariectomized aged female rats as evaluated by the protein synthesis rates, and suggest that the changes in concentration of GH are at least partly involved in regulating the brain protein synthesis in ovariectomized aged female rats given GABA.

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