Effect of Dietary γ-Aminobutyric Acid on the Brain Protein Synthesis Rate in Hypophysectomized Aged Rats

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Summary The purpose of this study was to determine whether the regulation of brain protein synthesis was mediated through changes in the plasma concentration of growth hormone (GH) when dietary γ-aminobutyric acid (GABA) treatment was manipulated in hypophysectomized or sham-operated aged rats. Experiments were done on four groups of hypophysectomized and sham-operated (24-wk-old) male rats given 0% or 0.5% GABA added to a 20% casein diet. The concentrations of plasma GH and fractional rates of protein synthesis in the brains increased significantly with the 20% casein+0.5% GABA compared with the 20% casein diet alone in the sham-operated rats. However GABA treatment to the basal diet did not affect the rates of protein synthesis in the hypophysectomized rats. In the cerebral cortex and cerebellum, 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 also related to the fractional rate of protein synthesis in these organs. The results suggest that treatment with GABA is likely to increase the concentrations of GH and the rate of brain protein synthesis in sham-operated rats only, not in hypophysectomized rats, and that the GABA-induced increase in the concentration of GH may be primarily responsible for changes in the brain protein synthesis. The 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 the liver, muscles and intestines (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 declines 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 decreases with age in rats after weaning (11). In many investigations, not only age but also growth hormone (GH) deficiency has been shown to affect many functions related to the central nervous system in mammals (12). GH is well known as the anabolic hormone in protein metabolism. Several investigators have demonstrated that the protein synthesis in visceral organs, skeletal muscle and brains was increased by GH in rats (13, 14).

γ-Aminobutyric acid (GABA) is a kind of amino acid widely distributed over the nature, and is an inhibitory transmitter compound in vertebrates (15, 16). Recently, GABA was attracted attention as a functional food for improvement in memory and study capability, blood-pressure reduction and relaxation inducement (17, 18). In a previous study, we reported that administration of GABA to young and aged rats increased the concentration of plasma GH and the rate of protein synthesis in the brain, and that a positive correlation existed between the rate of protein synthesis in the brain and the plasma concentration with GH (19, 20). Ohsumi et al. (14) demonstrated that hypophysectomy decreased 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 hypophysectomized rats.

The purpose of our study was to determine whether GABA affects the rate of brain protein synthesis in hypophysectomized aged rats, and whether the regulation of brain protein synthesis was mediated through changes in the concentration of GH in rats treated with or without GABA. In our previous report (7, 21), a positive correlation between the rate of protein synthesis and RNA activity was found in the brain when the
Table 1. Composition (g/100 g of diet) of experimental diets.

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

1: y-Aminobutyric acid.
2: Supplied by Oriental Yeast, Co., Ltd., Tokyo, Japan.
3: Supplied by Nihon Nosan K.K., Yokohama, Japan (40).

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 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 affect the plasma concentration of GH in hypophysectomized aged rats, 2) whether the dietary addition of GABA might affect brain protein synthesis in hypophysectomized aged rats, and 3) whether greater RNA concentration or RNA activity in hypophysectomized and sham-operated rats given GABA resulted in a greater protein synthesis rate in the brain than in those rats fed the basal diet. Therefore, we examined the effects of GABA treatment on the GH concentration in plasma and three indicators of protein synthesis in rat brains: its rate, RNA concentration and RNA activity in hypophysectomized and sham-operated rats.

MATERIALS AND METHODS

Chemicals. L-Tyrosine decarboxylase, β-phenethylamine and L-leucyl-L-alanine were purchased from Sigma Chemical (St. Louis, MO, USA). L-[2,4-1H]Phenylalanine (2.2 TBq/mmol) was obtained from Moravek (Brea, CA, USA). All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and diet. Hypophysectomized and sham-operated male 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 the 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. Two experiments were conducted on four groups of rats. On day 1 of the experimental period, two groups were hypophysectomized and the other two groups were the sham-operated controls; all were fed the 20% casein diet for 10 d. In Experiment 1, the effect of dietary GABA on the plasma concentration of GH was investigated in hypophysectomized and sham-operated control rats. In our previous experiment, the plasma concentration of GH rose very rapidly after GABA treatment (20). Therefore, in the present study, the plasma concentration of GH was measured after only one 3-h feeding period of the test diet. After feeding on 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 3h 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 method of EIA (SPI bio, Massy, Cedex, France). In Experiment 2, the effect of dietary GABA on the brain protein synthesis rates was investigated in hypophysectomized and sham-operated control rats. The rats of each group were divided into two groups and fed the experimental diets for 10 d ad libitum. The experimental diets contained 0% or 0.5% GABA added to the 20% casein diet (Table 1). The fractional rates of protein synthesis in the brain were measured by the method of Garlick et al. (22). The rats were decapitated between 10:00 and 12:00 h. Brain regions (23) 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. (24) with bovine serum albumin as a standard, and Fleck and Munro (25), respectively.

Fractional rate of protein synthesis in tissues. Radioactive L-[2,4-1H]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 [1H]phenylalanine in tissue samples were determined according to the method described in our previous report (21). Tissue samples were homogenized with 10 volumes of cold 0.2 mol/L perchloric acid and then centrifuged at 2,800 ×g for 15 min at 4°C. The supernatant was used for the measurements of specific radioactivity after adjusting the pH to 6.0–7.0 with saturated potassium citrate. The precipitate containing protein was washed three times with 5 mL of 0.2 mol/L perchloric acid, suspended in 10 mL of 0.3 mol/L NaOH and incubated at 37°C for 1 h. Protein-bound phenylalanine was obtained by reprecipitating the protein with 2 mL of 2 mol/L perchloric acid, washing the pellet with 5 mL of 0.2 mol/L perchloric acid twice and hydrolyzing the protein in 10 mL of 6 mol/L HCl for 24 h at 110°C. The HCl was evaporated to dryness, and the amino acids were dissolved in citrate buffer (pH 6.3). The determination of the specific radioactivity of [1H]phenylalanine involved its enzymatic conversion into phenethylamine, followed by a radioactivity counting (Accflex LSC 7400, Aloka Co., Tokyo, Japan) and fluoro-
metric determination (F-3000, Hitachi Co., Tokyo, Japan).

In a preliminary experiment, we determined whether the method of Garlick et al. (22) 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 different. 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. (22), i.e.:

$$K_s = \frac{100}{S_b} \times \frac{S_a}{t}$$

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.

Statistical analysis. The means and SE are reported. Student’s t-test was used to compare means after two-way ANOVA (26). Linear regression analysis was used to assess the relationship between the rate of protein synthesis and RNA activity (26). Differences were considered significant at p<0.05. In the hippocampus, the rates of protein synthesis were determined from a pool of each region.

RESULTS

Plasma concentration of growth hormone (Experiment 1)

In sham-operated control groups, the plasma concentration of GH increased significantly with the 20% casein+0.5% GABA compared with the 20% casein

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

|                | Control          | Control +GABA     | Hypophysectomy | Hypophysectomy +GABA |
|----------------|------------------|-------------------|----------------|----------------------|
| Final body weight (g) | 359.6±10.5 10.8±1.2³ | 357.8±11.1 45.7±7.6⁴ | 279.6±29.5 4.3±0.8³ | 277.1±30.9 4.0±0.5³ |
| Plasma GH¹ (μg/L) | 30.9±4.0 4.0±0.5³ | 30.9±4.0 4.0±0.5³ | 30.9±4.0 4.0±0.5³ | 30.9±4.0 4.0±0.5³ |

¹ γ-Aminobutyric acid.
² Values are means and SE, n=6. * Significantly different from corresponding value in rats of control group (p<0.05). The superscript letters indicate significant differences of means (p<0.05) due to type of GABA treatment within control or hypophysectomy groups.
³ Growth hormone.

Table 3. Effect of the addition of GABA¹ to a basal diet on protein synthesis in liver and brain regions of hypophysectomized aged rats.²

|                | Control | Control +GABA | Hypophysectomy | Hypophysectomy +GABA |
|----------------|---------|--------------|----------------|----------------------|
| Body weight gain (g/10 d)³ | 26.2±4.4 | 24.6±1.0     | 0.4±3.2*       | 5.4±3.0*             |
| Food intake (g/d)       | 19.3±1.0 | 18.9±0.9     | 12.9±1.0*      | 13.2±1.3*            |
| Tissue weight (g/100 g body weight) | 3.25±0.10 | 3.24±0.11     | 2.82±0.23      | 2.83±0.19            |
| Liver               | 0.115±0.003 | 0.119±0.002   | 0.121±0.003    | 0.120±0.004          |
| Cerebral cortex      | 0.093±0.002 | 0.090±0.001   | 0.094±0.002    | 0.092±0.002          |
| Cerebellum           | 0.038±0.001 | 0.039±0.002   | 0.041±0.002    | 0.040±0.002          |
| Hippocampus          | 199±2     | 200±3        | 200±2          | 201±2                |
| Tissue protein (mg/g tissue) | 139±1     | 138±2        | 137±2          | 138±1                |
| Liver               | 146±3     | 148±4        | 147±4          | 148±3                |
| Cerebral cortex      | 148±1     | 150          | 150            | 149                  |
| Cerebellum           | 148±1     | 150          | 150            | 149                  |

1 γ-Aminobutyric acid.
2 Values are means and SE, n=6. * Significantly different from corresponding value in rats of control group (p<0.05).
3 Initial body weight of rats was 220–320 g.
4 Data were obtained by a single analysis of pooled samples from six rats.
diet alone. The hypophysectomy caused a decrease in the plasma concentration of GH in each group treated with or without GABA, which did not differ (Table 2). **Protein synthesis in liver and brain regions (Experiment 2)**

We reported that the rate of protein synthesis in brain regions and liver increased with the 20% casein diet in the control groups (Table 4). In older people, the deficiency of GH affects body composition and functions. Treatment of adult GH-deficient patients with human GH is reported to improve the psychological well being and memory function (12, 27). In the previous study, we demonstrated that the fractional (Ks) and absolute rates of protein synthesis in liver and brain regions did not differ among the experimental groups.

In the control groups, the RNA activity [g protein synthesized/(g of RNA·d)] in the liver and brain regions increased significantly with the 20% casein diet alone (Table 4). However, when rats were hypophysectomized, the dietary GABA did not affect the fractional or absolute rates of protein synthesis, or RNA activity in the liver or brain regions (Table 4). Correlations between the fractional rates of protein synthesis, or RNA activity in the liver or brain regions and liver increased with the 20% casein diet in the control groups (Table 4).

**Table 4. Effect of the addition of GABA**

| Protein synthesis, Ks (%/d) | Control | Control + GABA | Hypophysectomy | Hypophysectomy + GABA | ANOVA |
|---------------------------|---------|---------------|----------------|-----------------------|-------|
| Liver                     | 82.6±1.2| 97.9±1.3      | 64.5±2.0       | 66.6±1.7              | p<0.05 |
| Cerebral cortex           | 18.4±0.7| 22.6±0.4      | 14.3±0.4       | 14.6±0.5              | p<0.05 |
| Cerebellum                | 20.4±0.3| 25.4±0.3      | 15.7±0.2       | 15.9±0.4              | p<0.05 |
| Hippocampus               | 21.1    | 25.5          | 17.5           | 17.9                  |       |

**Absolute protein synthesis (mg protein synthesized/(tissue·d))**

| Liver                     | 1.803±56| 2.140±65      | 889±70         | 966±68                | p<0.05 |
| Cerebral cortex           | 10.0±0.4| 12.6±0.2      | 5.8±0.2        | 6.2±0.2               | p<0.05 |
| Cerebellum                | 9.4±0.3 | 11.4±0.2      | 5.3±0.3        | 5.5±0.2               | p<0.05 |
| Hippocampus               | 4.0     | 5.0           | 2.6            | 2.7                   |       |

**RNA/protein (mg RNA/g protein)**

| Liver                     | 36.4±0.4| 36.5±0.3      | 32.8±0.2       | 32.6±0.1              | NS    |
| Cerebral cortex           | 15.7±0.2| 15.5±0.2      | 14.2±0.3       | 14.2±0.2              | NS    |
| Cerebellum                | 15.3±0.3| 15.3±0.3      | 13.1±0.2       | 13.2±0.1              | NS    |
| Hippocampus               | 15.9    | 16            | 15.8           | 16                    |       |

**RNA activity (g protein synthesized/(g RNA·d))**

| Liver                     | 22.7±0.2| 26.9±0.3      | 19.7±0.5       | 20.5±0.6              | p<0.05 |
| Cerebral cortex           | 11.9±0.4| 14.6±0.3      | 10.1±0.1       | 10.2±0.2              | p<0.05 |
| Cerebellum                | 13.4±0.4| 16.7±0.5      | 12.0±0.3       | 12.0±0.2              | p<0.05 |
| Hippocampus               | 13.3    | 15.9          | 11.1           | 11.2                  |       |

1-α-Aminobutyric acid.
2-Values are means and SE. n=6. *Significantly different from corresponding value in rats of control group (p<0.05). The superscript letters indicate significant differences of means (p<0.05) due to type of GABA treatment within control or hypophysectomy groups.
3-Data were obtained by a single analysis of pooled samples from six rats.
protein synthesis in the brain regions of hypophysectomized aged rats was increased by GH treatment, and that the concentration of plasma GH and protein synthesis in the brains of young and adult rats were also increased by GABA treatment (19, 20). However, little information is available on the effects of GABA treatment on the rate of brain protein synthesis during growth hormone deficiency. We hypothesized that the rate of brain protein synthesis would not increase in hypophysectomized aged rats fed GABA. Therefore, we determined whether the dietary addition of GABA also increased the GH concentration in the plasma of hypophysectomized and sham-operated control rats. The plasma concentration of GH was significantly higher in hypophysectomized and control aged rats. Hypophysectomy 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 plasma concentration of GH when GABA was added to the diet, it is also important to investigate the role of GABA treatment on the gene expression of brain protein. 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 expression of brain protein synthesis in control or hypophysectomized rats. The effect of GABA treatment on the mRNA concentrations in aged rats is another question to consider in a further study.

In the brain regions, GABA supplementation to the basal diet elevated the fractional and absolute rates of protein synthesis in the control rats. Hypophysectomized rats had reduced rates of protein synthesis in the brain regions and the plasma concentration of GH; however, the dietary addition of GABA did not reverse the effect of hypophysectomy (Table 4). The regulation in brain protein synthesis is mediated through changes in the body GH concentration when dietary GABA is manipulated. 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 (21, 28). Hormonal treatment such as GH also appeared to elevate the rate of protein synthesis and RNA activity in the brain (14). 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 control group fed the 20% casein diet alone (Table 4). The higher RNA activity in control rats fed the 20% casein + GABA diet may have increased the rate of brain protein synthesis in this group. The dietary addition of GABA did not affect either the rates of protein synthesis or the RNA activity in the brain regions of hypophysectomized groups.

RNA activity is calculated by dividing the fractional rate of protein synthesis by the RNA/protein ratio, and reflects the protein contents synthesized per unit RNA in each tissue. Many studies suggested that the RNA activity represented the changes in the translational phase of protein synthesis (29). Little information on the mechanism by which dietary GABA affects RNA activity in the brain of aged rats is available. In the previous studies, we reported that the aggregation of polyribosomes in the brain of aged rats decreased with a decrease in dietary protein, and that there was a correlation between the polysome profile and RNA activity (7, 30). In both liver and muscle, the stimulations of protein synthesis caused by amino acids and hormonal factors are reported to be mediated by the increase in the initiation of mRNA translation (31–33). Of the many steps in the initiation process, eukaryotic initiation factor (eIF) 4E and phosphorylation of ribosomal protein S6 appear to be particularly important in the physiological regulation (34, 35). Kimball et al. (33, 36) demonstrated that insulin stimulated protein synthesis in the skeletal muscle by enhancing the association of eIF 4E and eIF 4G. Anthony et al. (32, 35) and Yoshizawa et al. (34) demonstrated that the oral administration of leucine stimulated the rates of protein synthesis in liver and skeletal muscle concomitant with increased phophorylation of 4E-BP1 and S6K1. Kato (13) suggested that GH might stimulate the translational phase of tissue protein synthesis. Measurement of the initiation factors of mRNA translation in the brain should be included in further studies for the effect of the addition of GABA to the basal diet on protein synthesis in hypophysectomized and control aged rats.

Recently, GABA has been attracting attention as a food with functions such as improvement in memory and study, and relaxation (17, 18). The ingestion of GABA resulted in higher rates of brain protein synthesis in sham-operated control rats, suggesting that brain function is affected. Recently several studies have shown that GH may affect many functions related to the central nervous system. Le Greves et al. (37) 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 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 (14). The GH-binding receptor has been identified in the brains of humans and rats (38). The possibility that GH itself may pass the blood-brain barrier is supported by several studies (39). Several investigators have reported that the protein synthesis in visceral organs and skeletal muscle was increased by GH in rats (13). However, in the present study, we did not determine the effects of GABA treatment on the gene expression of brain protein. 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 transcriptional phase of brain protein synthesis in control or hypophysectomized rats. The effect of GABA treatment on the mRNA concentrations in aged rats is another question to consider in a further study.
tion also controls the body concentration of GH and increase in in vivo protein synthesis in the liver of aged rats.

The results indicate that the treatment with GABA increases the concentrations of GH and the rate of brain protein synthesis in the sham-operated rats only, not in the hypophysectomized rats, and that the GABA-induced increase in the concentration of GH is primarily responsible for changes in the brain protein synthesis. The RNA activity is at least partly related to the fractional rate of brain protein synthesis.

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