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Dietary γ-aminobutyric acid affects the brain protein synthesis rate in young rats

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Summary. The purpose of this study was to determine whether the γ-aminobutyric acid (GABA) affects the rate of brain protein synthesis in male rats. Two experiments were done on five or three groups of young rats (5 wk) given the diets containing 20% casein administrated 0 mg, 25 mg, 50 mg, 100 mg or 200 mg/100 g body weight GABA dissolved in saline by oral gavage for 1 day (d) (Experiment 1), and given the diets contained 0%, 0.25% or 0.5% GABA added to the 20% casein diet (Experiment 2) for 10 d. The plasma concentration of growth hormone (GH) was the highest in rats administrated 50 mg and 100 mg/100 g body weight GABA. The concentration of serum GABA increased significantly with the supplementation groups. The fractional (Ks) rates of protein synthesis in brain regions, liver and gastrocnemius muscle increased significantly with the 20% casein + 0.25% GABA diet and still more 20% casein + 0.5% GABA compared with the 20% casein diet. In brain regions, liver and gastrocnemius muscle, 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 in any organ. Our results suggest that the treatment of GABA to young male rats are 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.

Keywords: γ-Aminobutyric acid – Growth hormone – Protein synthesis – Brain – Rats

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

Introduction

The metabolic response to dietary proteins, age and hormonal factors includes marked changes in protein synthesis, especially in liver, muscle and intestine (Goldspink et al., 1984; Lewis et al., 1984; Millward et al., 1976; Symmons et al., 1972; Yokogoshi et al., 1980). Protein synthesis in the brain is also sensitive to the alteration of dietary amino acid composition in young rats (Beverly et al., 1991; Yokogoshi et al., 1992).

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 (Attaix et al., 1988; Goldspink and Kelly, 1984; Waterlow et al., 1978). Hayase and Yokogoshi (1994) reported that the rate of protein synthesis in the brain decreased with age in rats after weaning. Several investigators have demonstrated that the protein synthesis in visceral organs and skeletal muscle was increased by growth hormone (GH) in rats (Kato, 2002).

γ-Aminobutyric acid (GABA) is a kind of the amino acid widely distributed over the nature. The origin of the GABA difference appears to lie in an asymmetric distribution of the enzyme that synthesizes GABA from glutamate, glutamate decarboxylase, the pathway for subsequent degradation being about equally active in the two types of axons (Kravitz et al., 1965). GABA is the inhibitory transmitter compound at the vertebrate (Roberts and Frankel, 1950; Otsuka et al., 1966). Recently, GABA attracts attention as functional foods such as improvement in memory and study capability, blood-pressure descent action and relax action (Mitsushima et al., 2002; Lyou and Yokogoshi, 2004). The investigators reported that the concentration of plasma GH was increased by GABA treatment (Mitsushima et al., 2002; Lyou and Yokogoshi, 2004), and a positive correlation between the rate of protein synthesis and the plasma concentration of GH. Therefore,

the possible effects of dietary addition of GABA on brain protein synthesis and plasma GH in rats are one of the nutritional 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 brain protein synthesis in young rats. In our previous report (Yokogoshi et al., 1992; Hayase et al., 1998; Koie et al., 1999), 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 young and aged male rats. However, the reduction with age in protein synthesis in the brain was related to a fall in the RNA concentration (Hayase and Yokogoshi, 1994). Three questions were considered in the present study: 1) which dose of GABA increases the concentration of GH, 2) whether the dietary addition of GABA to the basal diet might affect brain protein synthesis in young male rats, and 3) whether greater RNA concentration or RNA activity in young 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 protein synthesis in liver and gastrocnemius muscle, and the GH concentration in plasma were also investigated.

Materials and methods

Chemicals

L-Tyrosine decarboxylase, L-leucyl-L-alanine and β-phenethylamine were purchased from Sigma Chemical (St. Louis, MO, U.S.A.).

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).

Animals and diets

Young male Wistar rats (5 wk, Japan SLC, Hamamatsu, Japan) were individually housed at 24 °C in a room with a 12-h light-dark cycle. The rats were transferred to the basal diet or the experimental diet after they had been fed a commercial non-purified diet (MF, Oriental Yeast, Tokyo, Japan) for 1 d. All rats 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 five groups of rats. In our preliminary experiment, the plasma concentration of GH elevated very rapidly after GABA treatment. Therefore, in this study, after feeding a 20% casein diet (Table 1) for 7 d, these rats were assigned randomly to one of the following five treatments: Control (0 mg), or administrated 25 mg, 50 mg, 100 mg or 200 mg/100 g body weight GABA dissolved in saline by oral gavage. The dose for all treatment of GABA was 1 ml/100 g body weight. One hour after GABA administration, rats were sacrificed. The concentration of plasma GH was measured by the method of EIA (SPI bio, Massy, Cedex, France).

Experiment 2 was conducted on three groups of rats. All rats were fed the experimental diet for 10 d. The experimental diet contained 0%, 0.25% or 0.5% GABA added to the 20% casein diet (Table 1). All rats were provided free access to food and water after 10 d. For measuring the concentration of GABA, the serum was treated with ethyl alcohol to precipitate the protein (Bianchi et al., 1999). The GABA concentration was measured by high pressure liquid chromatography (C-R7A/LC-10A, Shimadzu, Kyoto, Japan). The fractional rates of protein synthesis in the brain were measured by the method of Garlick et al. (1980). The rats were decapitated between 1000 and 1200 h. The liver, brain regions (Reinstein et al., 1979) and gastrocnemious muscle were quickly removed and frozen in liquid nitrogen. The concentrations of protein and RNA in liver, brain and muscle were measured according to the methods of Lowry et al. (1951) with bovine serum albumin as a standard, and Fleck and Munro (1962), respectively.

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

Ingredient	20% casein	20% casein +0.25% GABA	20% casein +0.5% GABA
Casein	20.0	20.0	20.0
GABA	0.00	0.25	0.50
Cystine	0.3	0.3	0.3
Cornstarch[1]	43.3	43.1	43.0
Sucrose[1]	21.7	21.6	21.5
Corn oil	5.0	5.0	5.0
AIN-93G mineral mix[2]	3.5	3.5	3.5
AIN-93VX Vitamin mix[2]	1.0	1.0	1.0
Cellulose[1]	5.0	5.0	5.0
Choline chloride	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)

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 μmol/ml 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 [^{3}H]phenylalanine in tissue samples were determined according to the method described in our previous report (Hayase et al., 1998). In a preliminary experiment, we determined whether the method of Garlick et al. (1980) 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 (data not shown). 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. In our previous report (Yokogoshi et al., 1992), 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. 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.

Statistical analysis

The means and pooled SEM are reported. Duncan's multiple range test was used to compare means after one-way ANOVA (Duncan, 1955; Snedecor and Cochran, 1967). Linear regression analysis was used to assess the relationship between the rate of protein synthesis and RNA activity (Snedecor and Cochran, 1967). 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 plasma concentration of growth hormone was the highest in rats administered 50 mg and 100 mg/100 g body weight GABA by oral gavage compared with control rats (Table 2) and depended on the dose of GABA ingestion.

Serum concentration of GABA and protein synthesis in tissues (Experiment 2)

The body weight gain did not differ among groups. The relative weights of the various brain regions and organs did not differ among the experimental groups. The concentrations of serum GABA (μmol/l) were 0, 1.25 and 3.55 with the 20% casein diet alone, the 20% casein + 0.25% GABA diet and the 20% casein + 0.5% GABA diet, respectively. The concentrations of serum GABA increased significantly with the 20% casein + 0.25% GABA diet and still more with 20% casein + 0.5% GABA compared with the 20% casein diet alone. The fractional (Ks) rate of protein synthesis in liver, gastrocnemius muscle and some brain regions, such as cerebral cortex and cerebellum increased significantly with the 20% casein + 0.25% GABA diet and still more 20% casein + 0.5% GABA compared with the 20% casein diet (Table 3). The RNA concentrations (mg RNA/g protein) of all organs and brain regions did not differ among groups (Table 4). The RNA activity [g protein synthesized/(g of RNA·d)] in the liver, muscle and brain regions increased significantly with the 20% casein + 0.25% GABA diet and still more 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 liver ($r = 0.907$, $p < 0.001$), gastrocnemius muscle ($r = 0.949$, $p < 0.001$), cerebral cortex ($r = 0.927$, $p < 0.001$), cerebel-

Table 2. Effect of the administration of GABA to a basal diet on the plasma concentration of growth hormone in rats [1]

GABA treatment (mg/100 g body weight)	Final body weight (g)	Liver weight (g)	Plasma GH (μg/l)
Control (0)	137.2	6.57	10.3[c]
25	136.2	6.66	34.7[b]
50	135.8	6.84	56.9[a]
100	135.2	6.31	55.6[a]
200	133.4	6.24	43.0[b]
Pooled SEM	2.6	0.24	4.3

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

Table 3. Effect of the addition of GABA to a basal diet on fractional synthesis rate in liver, gastrocnemius muscle and brain regions in rats [1]

	20% casein	20% casein + 0.25% GABA	20% casein + 0.5% GABA	Pooled SEM
Protein synthesis (Ks)[2] (%/day)				
Liver	97.8[c]	107.2[b]	116.4[a]	2.1
Gastrocnemius muscle	11.5[c]	13.4[b]	15.5[a]	0.4
Cerebral cortex	20.6[c]	22.7[b]	25.4[a]	0.5
Cerebellum	24.9[c]	27.4[b]	30.2[a]	0.3
Hippocampus[3]	20.5	22.0	24.6	–

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

Table 4. Effect of the addition of GABA to a basal diet on RNA concentrations and RNA activities in liver, gastrocnemius muscle and brain regions in rats [1]

	20% casein	20% casein + 0.25% GABA	20% casein + 0.5% GABA	Pooled SEM
RNA/protein (mg RNA/g protein)				
Liver	39.5	38.9	39.8	0.6
Gastrocnemius muscle	8.8	8.4	8.6	0.2
Cerebral cortex	16.9	16.5	16.3	0.4
Cerebellum	15.1	15.8	15.3	0.4
Hippocampus[2]	15.8	15.7	15.3	–
RNA activity (g protein synthesized/g RNA·d)				
Liver	25.1[c]	27.6[b]	29.2[a]	0.5
Gastrocnemius muscle	13.2[c]	16.0[b]	18.1[a]	0.6
Cerebral cortex	12.3[c]	13.7[b]	15.6[a]	0.4
Cerebellum	16.2[c]	17.5[b]	19.8[a]	0.5
Hippocampus[2]	13.0	14.0	16.1	–

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

lum ($r=0.894$, $p<0.001$). In pooled samples of hippocampus, this rate tended to be higher in the GABA supplementation groups than control group (Table 3).

Discussion

The γ-aminobutyric acid (GABA) is a kind of the amino acid widely distributed over the nature. The origin of the GABA difference appears to lie in an asymmetric distribution of the enzyme that synthesizes GABA from glutamate, glutamate decarboxylase, the pathway for subsequent degradation being about equally active in the two types of axons (Kravitz et al., 1965). GABA is the inhibitory transmitter compound at the vertebrate (Roberts and Frankel, 1950). Several investigators have reported that the protein synthesis in visceral organs and skeletal muscle was increased by growth hormone (GH) in rats (Kato, 2002), and that the concentration of plasma GH was increased by GABA treatment (Mitsushima et al., 2002). However, little information is available on the effects of dietary GABA on the rate of brain protein synthesis is in young rats. We hopothesized that the rate of brain protein synthesis would increase in rats fed GABA. Therefore, we determined whether administration of GABA by oral gavage also increased the GH concentration in plasma. The plasma concentration of GH was the highest in rats administrated 50 mg and 100 mg/100 g body weight GABA by oral gavage compared with control rats (Table 2), and a positive correlation between the plasma concentration of GABA and the plasma concentration of GH. The treatment of GABA may have regulated plasma concentration of GH in the present investigation.

In the brain regions, GABA treatment to the basal diet elevated the fractional rates of protein synthesis (Table 3). The changes in brain protein synthesis were likely attributable to the dietary GABA. In weaned rats, a reduction with age in protein synthesis in the brain and muscle was related to a fall in RNA concentration (Waterlow et al., 1978; Hayase and Yokogoshi, 1994).

However, the positive correlation between the rate of protein synthesis and RNA activity was found in the brain of weaned rats when the dietary quality and quantity of protein were manipulated (Yokogoshi et al., 1992). Hormonal treatment such as insulin, thyroid hormone and estrogen also appeared to elevate the rate of protein synthesis and RNA activity in the brain (Hayase and Yokogoshi, 1995; Hayase et al., 1997, 2001). 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 factor affecting brain protein synthesis in young rats.

Little information on the mechanism by which the dietary GABA affects RNA activity in the brain of young rats is available. Many investigations suggested that the polysome profile in tissues represented the changes in the translational phase of protein synthesis (Yokogoshi et al., 1992; Flaim et al., 1982). In both liver and muscle, the stimulation of protein synthesis caused by amino acids is reported to be mediated by the increase in the initiation of mRNA translation (Flaim et al., 1982; Anthony et al., 2000b). Of the many steps in the initiation process, eukariotic initiation factor (eIF) 2 and 4E appear to be particularly important in the physiological regulation (Yoshizawa et al., 1998; Anthony et al., 2000a). Measurement of the initiation factors of mRNA translation and the ribosomal aggregation in the brain should be included in the further studies for the effect of the addition of GABA to the basal diet on protein synthesis in rats.

Recently, GABA attracts attention as functional foods such as improvement in memory and study capability, blood-pressure descent action and relax action (Mitsushima et al., 2002; Lyou and Yokogoshi, 2004). The ingestion of GABA resulted in higher rates of brain protein synthesis in 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 (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. 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. Therefore, the effect of GH treatment on the brain protein synthesis rates in rats is the another question to consider in a further examination.

In the present study, GABA treatment stimulated the fractional rates of protein synthesis and RNA activities in liver and gastrocnemius muscle (Tables 3 and 4). GH has been known to increase the protein synthesis of visceral organs and skeletal muscle (Kato, 2002). These observations suggest that in vivo protein synthesis in liver

and muscle also depend on the GABA ingestion, and that the induction in RNA activity associated with GABA treatment was correlated with the increase in protein synthesis rate in these organs.

The mass of the brain regions were unaffected by GABA treatment, yet the fractional rates of protein synthesis increased with the addition of GABA to the 20% casein diet in the present experiment (Table 3). These results may suggest that the protein degradation in brain also increased in rats given GABA, though the role of the protein degradation in maintaining the brain mass remains to be unknown under the physiological conditions (Hayase et al., 1998). This is another possibility to consider in the further examination of the mechanism by which the addition of GABA changes brain protein metabolism.

The present results indicate that visceral organs and brain protein synthesis was affected by GABA in young rats as evaluated by the protein synthesis rates, and suggest the effects of GABA on brain protein synthesis in rats are also of importance in understanding the relationship among nutrition, GH, GABA and brain function in mammals.

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