Changes in Thyroid Hormone Are Not Involved in Regulating Brain Protein Synthesis in Adults Rats Fed Ornithine

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Summary Brain protein synthesis and the plasma concentration of growth hormone (GH) are sensitive to dietary ornithine. However, dietary ornithine does not increase brain protein synthesis in hypophysectomized rats. Because hypophysectomy may decrease the secretion of thyroid stimulated hormone (TSH), we assessed whether the regulation of brain protein synthesis was mediated by changes in the plasma concentrations of thyroid hormone and ghrelin in the 6-propyl-2-thiouracil (PTU, thyroid inhibitor)-treated or control adult rats fed ornithine. The four experimental groups consisted of PTU-treated and control (24-wk-old) male rats given 0% or 0.7% ornithine-HCl added to a 20% casein diet. The plasma concentrations of GH and ghrelin, and the fractional rates of protein synthesis and RNA activity [g protein synthesized/(g RNA·d)] in the brains were significantly increased after treatment with the 20% casein diet compared with the 20% casein diet alone in both the PTU-treated and control groups. Ornithine supplementation to the basal diet did not affect the plasma concentration of T3. The RNA concentration (mg RNA/g protein) was not related to the fractional rate of protein synthesis in the brain regions. The results suggest that dietary ornithine likely increases the rate of brain protein synthesis in control and PTU-treated rats, and that the ornithine-induced increase in the GH concentration may stimulate mainly brain protein synthesis via ghrelin. RNA activity is at least partly related to the fractional rate of brain protein synthesis.

Key Words ornithine, thyroid hormone, brain protein synthesis, growth hormone, ghrelin

The protein content of tissues is affected by alterations in dietary proteins, age and hormonal factors. Changes in protein metabolism may be reflected in the protein synthesis, especially in the liver, muscle and intestine (1–5). In comparison to studies of visceral organs, regulatory mechanisms of brain protein synthesis have not been well understood. However, previous reports showed that protein synthesis in the brain of young rats depends on dietary proteins and amino acids (6, 7).

Ornithine is an amino acid widely distributed in the liver of mammals and various foods such as Corbicula (Asian clam). Ornithine is also a urea cycle intermediate and the substrate for citrulline synthesis. Furthermore, ornithine ingestion increases the plasma concentration of growth hormone (GH) in humans and rats (8, 9). In many investigations, GH deficiency has been shown to affect many functions related to the central nervous system in mammals (10). GH is well known as an anabolic hormone in protein metabolism. Several studies have demonstrated that the protein synthesis in visceral organs, skeletal muscle and brains was increased by GH in rats (11, 12).

Recently, Tujio et al. (13) reported that dietary ornithine increased the plasma concentration of GH and the rates of brain protein synthesis in sham-operated rats, but not hypophysectomized rats, thus suggesting that the regulation of brain protein synthesis was mediated through changes in the GH concentration. Ghrelin in the gastrointestinal tract can regulate GH secretion from the pituitary via the afferent vagal nerve (14, 15). However, the effect of dietary ornithine on the plasma concentration of ghrelin in adult rats remains largely unknown. On the other hand, hypophysectomy in rats may decrease the secretion of thyroid stimulated hormone (TSH) and thus change the plasma concentration of thyroid hormone. The thyroid hormone is essential for normal growth in the brain (16). In the previous
study, we (17) demonstrated that the thyroid hormone increased the rate of protein synthesis in the rat brain. However, the role of dietary ornithine in maintaining thyroid hormone status in adult rats is unknown.

Here, we sought to determine if ornithine affects the rate of brain protein synthesis in 6-propyl-2-thiouracil (PTU, thyroid inhibitor)-treated adult rats, and if the regulation of brain protein synthesis is mediated by changes in the concentration of GH or thyroid hormone (T3) in rats treated with and without ornithine. In our previous report (7), 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 adult rats. However, the reduction with age in protein synthesis in the brain was related to a fall in the RNA concentration (18). Four questions were addressed in the present study: 1) whether the dietary addition of ornithine increases the plasma concentration of T3 in control and PTU-treated adult rats, 2) whether the dietary addition of ornithine affects brain protein synthesis in PTU-treated adult rats, 3) whether a higher concentration of ghrelin in rats given ornithine leads to an increase in the plasma concentration of GH and stimulates brain protein synthesis, and 4) whether higher RNA concentrations or RNA activity in control and PTU-treated rats fed ornithine results in a greater protein synthesis rate in the brain than in rats fed a basal diet. Therefore, we examined the effects of ornithine treatment on the plasma concentrations of T3, GH, and ghrelin and three brain protein synthesis parameters (its rate, RNA concentration and RNA activity) in control and PTU-treated rats. We previously reported that the plasma concentration of T3 was significantly lower in rats administered a 20% casein diet containing 0.01% PTU when compared with control rats (16). Furthermore, the plasma concentration of GH was the highest in rats fed a 20% casein diet supplemented with 0.7% ornithine (13). Thus, in this study, we used four groups of adult rats fed 0%, or 0.7% ornithine-HCl and 0%, or 0.01% PTU added to a 20% casein diet.

MATERIALS AND METHODS

Chemicals. L-Tyrosine decarboxylase, L-leucyl-L-alanine, 6-phenethylamine and 6-propyl-thiouracil (PTU) were purchased from Sigma Chemical (St. Louis, MO). L-[2,4-3H]Phenylalanine (2.2 TBq/mmol) was obtained from Moravek (Brea, CA). L-Ornithine–HCl was obtained from KYOW A HAKKO BIO CO., LTD. (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and diet. 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 given to the experimental diets after being fed a 20% casein diet for 10 d. The experimental diets contained 0% or 0.7% ornithine-HCl added to the 20% casein diet (basal diet) or the 20% casein + 0.01% PTU diet (Table 1). All animals were individually housed and given free access to food and water. The Aichi University of Education Animal Care and Use Committee approved these animal experiments (approval number, 201402001).

Experimental design. Two experiments were conducted on four groups of rats. In Experiment 1, the effect of dietary ornithine on brain protein synthesis rates and the plasma concentration of T3 were investigated in PTU-treated and control rats. Rats in each group were divided into two subgroups that were fed experimental diets for 10 d ad libitum. The experimental diets contained 0% or 0.7% ornithine added to the 20% casein diet (Table 1). The fractional rates of protein synthesis in the brain and liver were measured by the method of Garlick et al. (19). The rats were decapitated between 1000 and 1200 h. Brain regions (20) and the liver were quickly removed and frozen in liquid nitrogen. The concentrations of protein and RNA in the brain and liver were measured according to the methods of Lowry et al. (21) with bovine serum albumin as a standard, and Fleck and Munro (22), respectively. Plasma was collected in glass tubes and stored at −80°C. The concentration of plasma T3 was measured by EIA (Endocrine Technologies, Newark, CA). In Experiment 2, the effect of dietary ornithine on the plasma concentrations of GH and ghrelin was investigated in PTU-treated and control rats. In our previous experiment, the plasma concentration of GH rose very rapidly after ornithine treatment (9). Therefore, in the present study, the plasma concentrations of GH and ghrelin were measured after a single 3-h feeding period of the experimental diets. After being fed a 20% casein diet for 10 d (one 3-h feeding period per day, from 9:00–12:00), the rats were given experimental diets for a single 3-h period and then immediately decapitated. The concentrations of GH and ghrelin (active ghrelin) in the plasma were measured by EIA (Bertin Pharma, Montigny le Bretonneux, France). To measure the concentrations of ornithine, the cerebral cortex, liver, and plasma were treated with ice-cold sulfosalicylic acid to precipitate the protein (23). The ornithine concentration was measured by an amino acid

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>20% Casein</th>
<th>20% Casein +0.7% Ornithine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Ornithine–HCl&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Cornstarch&lt;sup&gt;2&lt;/sup&gt;</td>
<td>43.3</td>
<td>42.9</td>
</tr>
<tr>
<td>Sucrose&lt;sup&gt;3&lt;/sup&gt;</td>
<td>21.7</td>
<td>21.4</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>AIN-93M mineral mix&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>AIN-93VX vitamin mix&lt;sup&gt;5&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cellulose&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>1</sup>Supplied by KYOWA HAKKO BIO CO., LTD., Tokyo, Japan.  
<sup>2</sup>Supplied by Oriental Yeast Co., Ltd., Tokyo, Japan.  
<sup>3</sup>Supplied by CLEA Japan, Inc., Tokyo, Japan (40).
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The specific radioactivity of \( \text{[3H]} \)phenylalanine was determined by the method described in our previous report (19). The means and SE values are shown in Table 2. Values are means and SE, n=6. * Significantly different from corresponding value in rats of control group (p<0.05).

Table 2. Effect of the addition of ornithine to a basal diet on body weight gain, relative weights in liver and brain regions, and plasma concentration of thyroid hormone in PTU-treated adult rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + Ornithine</th>
<th>PTU2</th>
<th>PTU + Ornithine</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g/10 d)(^1)</td>
<td>23.8±3.6</td>
<td>23.2±2.1</td>
<td>22.0±2.0</td>
<td>21.6±3.4</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>19.5±0.4</td>
<td>19.8±0.3</td>
<td>19.1±0.4</td>
<td>19.1±0.4</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Tissue weight (g/100 g body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.00±0.09</td>
<td>2.97±0.07</td>
<td>2.98±0.03</td>
<td>2.92±0.09</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.117±0.003</td>
<td>0.116±0.003</td>
<td>0.111±0.004</td>
<td>0.110±0.003</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.095±0.003</td>
<td>0.099±0.003</td>
<td>0.099±0.002</td>
<td>0.098±0.003</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.040±0.002</td>
<td>0.039±0.002</td>
<td>0.040±0.002</td>
<td>0.038±0.001</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Plasma T(_1^3) ((\mu)g/L)</td>
<td>1.17±0.07</td>
<td>1.22±0.09</td>
<td>0.46±0.01(^*)</td>
<td>0.47±0.01(^*)</td>
<td>NS p&lt;0.01 NS</td>
</tr>
</tbody>
</table>

1 Values are means and SE, n=6. * Significantly different from corresponding value in rats of control group (p<0.05).
2 6-Propyl-2-thiouracil.
3 Initial body weight of rats was 360–390 g.
4 Triiodothyronine.

Fractional rate of protein synthesis in tissues. Radioactive l-[2,4, \(\text{[3H]} \)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 7 mL/100 g of body weight. Rats were quickly decapitated 10 min after the injection. The specific radioactivities of \( \text{[3H]} \)phenylalanine in tissue samples were determined according to the method described in our previous report (24). Tissue samples were homogenized with 10 volumes of 0.2 mol/L perchloric acid and then centrifuged at 2,800 \( \times \) 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 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 \( \text{[3H]} \)phenylalanine involved its enzymatic conversion into phenethylamine using tyrosine decarboxylase, followed by a radioactivity counting (Accflex LSC 7400, Aloka Co., Tokyo, Japan) and fluorometric determination (F-3000, Hitachi Co.). The \( \beta \)-phenethylamine concentration was determined by the method of Suzuki and Yagi (25) using ninhydrin and l-leucyl-l-alanine. In a preliminary experiment, we determined whether the method of Garlick et al. (19) could be used to measure the rate of protein synthesis in the brain under this experimental condition. The specific radioactivities of free phenylalanine in the plasma, cerebral cortex and cerebellum of rats from the two groups were constant in each tissue (the data are not shown). Moreover, the values were also not significantly different among the plasma, cerebral cortex, and cerebellum, indicating that the precursor pool of labeled phenylalanine was similar. In our previous report (7), a decrease in the labeling of free phenylalanine at 3, 5, and 10 min in the brain was not significant after an injection of a large dose of \( \text{[3H]} \)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 was reported by Garlick et al. (19), i.e.

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

where t is the incorporation time in days.

RNA activity was calculated by dividing the fractional rate of protein synthesis by the RNA/protein ratio.

Statistical analysis. The means and SE values are reported. Student’s t-test was used to compare means after a 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

Fractional rates of protein synthesis in tissues and plasma concentration of thyroid hormone (Experiment 1)

Body weight gain and food intake were not significantly different among experimental groups. The weights of the brain regions and liver did not differ...
among experimental groups when expressed relative to body weight (Table 2). Compared with the control rats, the plasma concentration of T₃ was significantly lower in rats given PTU and there was no difference between rats treated with or without ornithine (Table 2). The fractional rates of protein synthesis (Ks) in the liver and brain regions, such as the cerebral cortex and cerebellum, increased significantly in rats fed the 20% casein + 0.7% ornithine diet when compared with the 20% casein diet alone in the control and PTU-treated groups (Table 3). In pooled samples of hippocampus, this rate tended to be higher in ornithine-treated rats. In the control and PTU-treated groups, the RNA activity [g protein synthesized/(g of RNA·d)] in the liver and brain regions was increased significantly in rats fed the 20% casein + 0.7% ornithine diet when compared with the 20% casein diet alone (Table 4). Treatment with PTU alone caused a significant decrease in the fractional rates of protein synthesis, or RNA activity in the liver and brain regions, when compared with control rats.

Table 3. Effect of the addition of ornithine to a basal diet on protein synthesis in liver and brain regions of PTU-treated adult rats.¹

<table>
<thead>
<tr>
<th>Protein synthesis, Ks (%/d)</th>
<th>Control</th>
<th>Control + Ornithine</th>
<th>PTU²</th>
<th>PTU + Ornithine</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>81.6±1.4b</td>
<td>97.7±1.5a</td>
<td>71.4±0.7ab</td>
<td>94.4±1.7a</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>18.8±0.3b</td>
<td>22.9±0.2a</td>
<td>16.9±0.1b</td>
<td>22.0±0.4a</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>20.1±0.4b</td>
<td>25.1±0.3a</td>
<td>18.2±0.1b</td>
<td>24.4±0.5a</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Hippocampus¹</td>
<td>15.7</td>
<td>15.8</td>
<td>15.6</td>
<td>15.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RNA/protein (mg RNA/g protein)</th>
<th>Control</th>
<th>Control + Ornithine</th>
<th>PTU²</th>
<th>PTU + Ornithine</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>37.8±0.5</td>
<td>37.5±0.6</td>
<td>33.2±0.2a</td>
<td>33.4±0.1a</td>
<td>NS</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>15.7±0.1</td>
<td>15.7±0.1</td>
<td>15.7±0.2</td>
<td>15.7±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>15.0±0.1</td>
<td>15.1±0.1</td>
<td>15.2±0.2</td>
<td>15.2±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Hippocampus¹</td>
<td>15.7</td>
<td>15.8</td>
<td>15.6</td>
<td>15.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RNA activity (g protein synthesized/(g RNA·d))</th>
<th>Control</th>
<th>Control + Ornithine</th>
<th>PTU²</th>
<th>PTU + Ornithine</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>21.6±0.2b</td>
<td>26.0±0.3a</td>
<td>21.7±0.2b</td>
<td>28.2±0.4a</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>12.0±0.1b</td>
<td>14.7±0.2a</td>
<td>10.9±0.2b</td>
<td>14.3±0.1a</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>13.4±0.2b</td>
<td>16.6±0.2a</td>
<td>12.1±0.1b</td>
<td>16.2±0.2a</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Hippocampus¹</td>
<td>13.3</td>
<td>16.3</td>
<td>12.1</td>
<td>15.8</td>
<td></td>
</tr>
</tbody>
</table>

¹ 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 ornithine treatment within control or PTU groups.
² 6-Propyl-2-thiouracil.
³ Data were obtained by a single analysis of pooled samples from six rats.

Table 4. Effect of the addition of ornithine to a basal diet on plasma concentrations of growth hormone and ghrelin, concentrations of ornithine in plasma, liver and cerebral cortex of PTU-treated adult rats.¹

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + Ornithine</th>
<th>PTU³</th>
<th>PTU + Ornithine</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>365.4±5.4</td>
<td>365.8±4.5</td>
<td>367.2±3.8</td>
<td>367.8±1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>11.5±0.4</td>
<td>11.2±0.2</td>
<td>11.3±0.3</td>
<td>11.3±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma GH (μg/L)</td>
<td>10.7±1.1b</td>
<td>52.4±2.1a</td>
<td>10.3±1.3b</td>
<td>52.0±2.3a</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Plasma ghrelin (ng/L)</td>
<td>133±4b</td>
<td>280±7a</td>
<td>134±3b</td>
<td>277±5a</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ornithine</th>
<th>Plasm (mmol/L)</th>
<th>Liver (μmol/g)</th>
<th>Cerebral cortex (μmol/g)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.195±0.011b</td>
<td>0.339±0.015b</td>
<td>0.152±0.007b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.240±0.010a</td>
<td>0.387±0.013a</td>
<td>0.180±0.007a</td>
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</tr>
<tr>
<td></td>
<td>0.190±0.013b</td>
<td>0.337±0.014b</td>
<td>0.150±0.006b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.238±0.012a</td>
<td>0.385±0.013a</td>
<td>0.179±0.008a</td>
<td></td>
</tr>
</tbody>
</table>

¹ Values are means and SE, n=6. The superscript letters indicate significant differences of means (p<0.05) due to type of ornithine treatment within control or PTU groups.
² Growth hormone.
³ 6-Propyl-2-thiouracil.
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rats given the 20% casein diet alone (Table 3). Correlations between the fractional rate of protein synthesis and RNA activity were significant in the liver \((r=0.898, p<0.001)\), cerebral cortex \((r=0.941, p<0.001)\) and cerebellum \((r=0.920, p<0.001)\). Compared with control groups, lower RNA concentrations (mg RNA/g protein) in the liver were observed in each group given PTU regardless of ornithine treatment (Table 4). However, the RNA concentration in brain regions was the same for all groups. The fractional rate of protein synthesis correlated with RNA concentration in the liver \((r=0.795, p<0.01)\). A two-way ANOVA revealed that the interactions between ornithine and PTU were significantly different between the fractional protein synthesis in the cerebral cortex and liver, and RNA activity in the brain regions and liver.

**Plasma concentrations of growth hormone and ghrelin, and the concentrations of ornithine in tissues and plasma (Experiment 2)**

In both the control and PTU-treated groups, the plasma concentrations of GH and ghrelin, and the concentrations of ornithine in the liver, cerebral cortex and plasma were significantly increased with the 20% casein + 0.7% ornithine diet when compared with the 20% casein diet alone. PTU treatment did not affect the plasma concentrations of GH or ghrelin in groups treated with or without ornithine (Table 4).

**DISCUSSION**

The amino acid supply is important for protein synthesis in the brain regions. In our previous study, we (13) reported that the plasma concentration of GH and protein synthesis in the brains were increased in control rats, but not hypophysectomized rats. In addition, the regulation of brain protein synthesis was mediated through changes in the plasma GH concentration. However, when rats are hypophysectomized, the secretion of thyroid stimulated hormone from the pituitary gland may decrease. The thyroid hormone stimulates protein synthesis in the liver, muscle and brain regions (17). However, limited information is available on the role of thyroid hormone on the rate of protein synthesis in the brain of rats given ornithine. We hypothesized that the rate of brain protein synthesis would increase in PTU (thyroid inhibitor)-treated rats fed ornithine. Therefore, we assessed if the dietary addition of ornithine affected the T3 concentration in plasma and brain protein synthesis in PTU-treated and control rats.

The plasma concentration of T3 was significantly lower in rats given PTU when compared with control rats; however, ornithine-supplemented diets did not affect the plasma concentration of T3 in groups treated with or without PTU (Table 2). Thus, ornithine did not regulate plasma concentration of T3 in the present investigation. In the brain regions, PTU treatment reduced the fractional rates of protein synthesis in rats, but the dietary addition of ornithine significantly elevated the rate of brain protein synthesis in control and PTU-treated rats (Table 3). On the other hand, a two-way ANOVA of the fractional protein synthesis in the brain revealed an interaction between ornithine and PTU. Thus, the thyroid hormone may not be the main factor to regulate brain protein synthesis when dietary ornithine is manipulated. Future studies are needed to determine the detailed mechanism behind the interaction between ornithine and thyroid deficiency.

As mentioned above, the ornithine-induced increase in the concentration of GH may be primarily responsible for changes in brain protein synthesis. Recently, ghrelin in the gastrointestinal tract was shown to regulate GH secretion from the pituitary via the role of the afferent vagal nerve (14, 15). In the present study, the plasma concentrations of GH and ghrelin were significantly higher in rats fed ornithine with or without PTU (Table 4). PTU treatment did not affect the plasma concentrations of GH or ghrelin. Therefore, dietary ornithine may regulate the GH concentration via the changes of ghrelin in the gastrointestinal tract and stimulate brain protein synthesis. Ohinata reported that the injection of ornithine directly to the duodenum increased the plasma concentration of GH and the mRNA level of ghrelin in the duodenum (27). However, limited information on the effect of dietary ornithine on the mRNA level of ghrelin in the gastrointestinal tract is available for adult rats. On the other hand, several investigators have reported the inhibitory effect of a ghrelin antagonist ([L-Lys3]-GHRP-6) on GH secretion (27). The concentration of GH in the plasma and mRNA level of ghrelin in the gastrointestinal tract should be measured in rats fed ornithine treated with or without ghrelin antagonist to determine the mechanism by which dietary ornithine elevates plasma GH concentration and increases the brain protein synthesis.

In weaned rats, a reduction in protein synthesis in the brain and skeletal muscle with age was related to a fall in RNA concentration (15, 28). However, a positive correlation between the rate of protein synthesis and RNA activity was found in the brain of aged rats when dietary protein and amino acids were manipulated (29, 30). Hormonal treatments such as GH and thyroid hormone also elevated the rate of protein synthesis and RNA activity in the brain (12, 17). Within the control and PTU-treated groups, RNA activity in the brain regions, rather than RNA concentration, was higher in rats fed the 20% casein + ornithine diet than rats fed the 20% casein diet alone (Table 3). Higher RNA activity in control rats fed the 20% casein + ornithine diet may have increased the rate of brain protein synthesis in this group. The dietary addition of ornithine increased not only the rates of protein synthesis but also RNA activity in the brain regions of PTU-treated rats.

RNA activity was defined as the amount of protein synthesized per unit of RNA in each tissue, and calculated by dividing the fractional rate of protein synthesis by the RNA/protein ratio. Several studies have suggested that RNA activity represents changes in the translational phase of protein synthesis (31). There is limited information on the mechanism by which dietary ornithine affects RNA activity in the brain of adult rats. In both liver and muscle, the stimulation of protein synthe-
sis caused by amino acids and hormonal factors is medi- 
ated by the increase in the initiation of mRNA transla-
tion (32–34). We reported that the ingestion of a higher quanti-ty of dietary protein and the dietary addition of GABA enhanced brain protein synthesis through the activation of the mRNA binding to the 40S ribosomal subunit (35, 36). Kato (11) suggested that GH stimu-
lated the translational phase of tissue protein synthesis. Measure-ment of the initiation factors of mRNA translation in the brain should be included in future studies on the effect of dietary ornithine on brain protein synthesis in adult rats.

Recently, ornithine has attracted attention as a func-
tional food that improves hepatic function and growth hormone release (8). In the present study, we showed that the ingestion of ornithine resulted in higher rates of brain protein synthesis in adult rats, and that this change in brain protein synthesis in rats given the orni-
thine depends on growth hormone, not thyroid hor-
mon. Several studies have shown that GH affects many functions related to the central nervous system. Tre-
ment of adult GH-deficient patients with human GH improved psychological well-being and memory func-
tion (10, 37). Le Greves et al. (38) suggested that GH induced the gene expression of hippocampal N-methyl-
D-aspartate receptor in rats, coinciding with improved learning and memory capabilities. We demonstrated that the ingestion of a high-protein diet increased the plasma concentration of GH, and the rate of protein synthesis and mRNA of nerve growth factor (NGF) in the brain, which is important for learning and memory (39). In the present investigation, dietary ornithine pro-
duced higher rates of brain protein synthesis in adult rats, suggesting that brain function can be modulated by ornithine. Determining the effect of ornithine on the mRNA of NGF in the brain should be included in future studies in adult rats.

In the present study, ornithine treatment stimulated the fractional rates of protein synthesis and RNA activ-
ity in the liver of control and PTU-treated groups, but did not affect the RNA concentration of protein synthe-
sis in liver of either group. The rates of protein synthesis in the liver and plasma concentration of GH increased in young rats fed ornithine (9). These observations demon-
strate that ornithine ingestion mainly controls the con-
centration of GH, not thyroid hormone, and increases the in vivo protein synthesis in the liver of adult rats.

In conclusion, these results show that ornithine increases the rate of brain protein synthesis not only in control rats but also in PTU-treated rats, and that the ornithine-induced increase in the GH concentration may stimulate mainly brain protein synthesis via ghre-
lin. RNA activity is at least partly related to the frac-
tional rate of brain protein synthesis.

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