**Note**

**Influence of GABA on Brain Protein Synthesis Mediated by the Mammalian Target on the Rapamycin Pathway**

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This study determined the influence of \(\gamma\)-aminobutyric acid (GABA) on brain protein synthesis via the mammalian target of rapamycin (mTOR) pathway. Experiments were carried out on three groups of 6-wk-old male rats with 0%, 0.5%, and 1% GABA. The percentage-phosphorylated S6K1 and growth hormone (GH) concentration was significantly increased by the GABA administration. The insulin level was not significantly changed, while the insulin-like growth factor 1 (IGF-1) level was significantly decreased by the GABA administration.

**Key words:** \(\gamma\)-aminobutyric acid (GABA); mammalian target of rapamycin (mTOR); S6K1; growth hormone; protein synthesis

Previous studies have demonstrated that amino acids and hormonal factors stimulate protein synthesis and RNA activity in the brain. It is known that mTOR regulates cell growth and metabolism. mTOR signalling is activated by hormones, growth factors and amino acids. mTOR controls a number of components involved in the initiation and elongation of mRNA translation. Holz et al. and Pain have suggested initiation of the translational phase to be the limiting step in protein synthesis. mTOR modulates the activity of ribosomal S6 kinases 1 (S6K1) by the phosphorylation of S6 kinase. The eIF4F initiation factors include eIF4E, eIF4A, eIF4G, and eIF3. Phosphorylation of S6K1 dissociates it from the eIF3 complex, resulting in the phosphorylation of 40S ribosomal protein S6 and eIF4B. Phosphorylated eIF4B then associates with the translational preinitiation complex.

A previous study has shown that the rates of protein synthesis in the liver and skeletal muscle following an oral administration of leucine were associated with an increase in the phosphorylation of S6K1. Furthermore, Tujjoka et al. have demonstrated that dietary GABA increased the fractional rate of protein synthesis in the brain which was correlated with RNA activity and RNA concentration in the cerebral cortex and cerebellum.

GABA has been used as a functional food. A number of studies have shown that GABA stimulates the rate of protein synthesis in the liver, muscle and brain. GABA has been found to be transported through the blood-brain barrier (BBB). However, a previous study has demonstrated that the concentration of GABA in the serum from rats after an oral administration of GABA gradually increased and remained constant for a while before gradually decreasing. It is therefore very likely that GABA affects the alteration of hormones, which in turn influences protein synthesis in the brain. Previous studies have shown that GABA played a role as a paracrine signal molecule in pancreatic islets. Insulin is one of the hormonal factors involved in the stimulation of protein synthesis. IGF-1 is a peptide hormone which has a similar feature to insulin. It has been demonstrated that insulin and IGF-1 could cross the BBB. Additionally, IGF-1 has also affected neuronal growth. We therefore examined in our present study the levels of insulin and IGF-1. Investigators have also demonstrated that GH may be involved in stimulating the translational phase of protein synthesis. There may therefore be some relevance between GH and mTOR, as both play a role in protein synthesis. Moreover, previous studies have shown that the concentration of plasma GH was increased by a GABA intake which in turn immediately increased the protein synthesis rate in the brain.

Male 3-wk-old Wistar rats (Japan SLC, Hamamatsu, Japan) weighing approximately 60 g were housed at 24 ± 1°C and 55 ± 5% humidity in a room with a 12-h light/dark cycle. The rats were randomly assigned to three experimental groups, i.e., control, 0.5% GABA, and 1% GABA. The control group (n = 7) received food and tap water, while the other two groups (n = 7) received food and water containing 0.5% GABA or 1% GABA. The rats were given a standard CE-2 diet (Clea

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Abbreviations: GABA, \(\gamma\)-aminobutyric acid; mTOR, mammalian target of rapamycin; GH, growth hormone; IGF-1, insulin-like growth factor 1; BBB, blood-brain barrier; CNS, central nervous system
Japan, Tokyo, Japan) and water ad libitum for 30 d, before the animals were decapitated. The body weight of the rats was measured daily. The body weight of all the rats increased gradually to approximately 263 g, there being no significant difference among the three groups. In addition, the average amount of food consumed by all three groups was not significantly different. This experiment was conducted under the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka that follows the American Association for Laboratory Animals Science. In addition, the average amount of food consumed by all rats increased gradually to approximately 263 g, there being no significant difference among the three groups.

Sample preparation followed the method of Ohsumi et al. Briefly, brain tissues (the cerebellum and cerebral cortex) were treated by a Dounce homogenizer in a homogenization buffer. The resulting homogenate was centrifuged, and then an equal volume of 2

cortex) were treated by a Dounce homogenizer in a homogenization buffer. The resulting homogenate was centrifuged, and then an equal volume of 2× the SDS sample buffer was added to the supernatant. The diluted sample was subjected to electrophoresis on 7.5% polyacrylamide gel, and then the protein samples were immune-blotted using S6K1 polyclonal antibodies (Santa Cruz Biotechnology, CA, USA). The concentrations of insulin, IGF-1, and GH in the serum were respectively identified with a rat insulin enzyme immunoassay (ELISA) kit (Morinaga, Japan), EK 0377 rat IGF-1 ELISA kit (Boster Biological Technology), and A05104 rat growth hormone ELISA kit (SPI-Bio, France). A statistical analysis was performed with SPSS, and data are presented as the mean ± SE. Comparisons were made by a one-way analysis of variance (ANOVA), this being followed by a post-hoc Tukey test and Dunnett test. Any p values of less than 0.05 are considered to be significantly different.

The phosphorylation states of S6K1 in the cerebral cortex and cerebellum are respectively shown in Fig. 1A and B. S6K1 was resolved into its α, β, and γ forms on SDS-polyacrylamide gels. The most phosphorylated was the γ form which exhibited the lowest electrophoretic mobility. The percentages of the γ and β + γ forms were compared in the present study to identify the phosphorylation state of S6K1 of all groups. Phosphorylated S6K1 (%γ) in both the cerebral cortex and cerebellum was significantly higher than the control after the administration of 1% GABA (Table 1, p < 0.05). However, there was no significant difference in the percentage of phosphorylated S6K1 (%γ) in the cerebral cortex and cerebellum of the 0.5% GABA group when compared to the control (Table 1, p < 0.05). In addition, the percentage of β + γ in the cerebral cortex was significantly higher in 1% GABA group than the control, while there was no significant difference in the percentage of β + γ in the cerebellum of all groups (Table 1, p < 0.05). These results indicate that GABA appeared to enhance the phosphorylation of S6K1 in the cerebral cortex and cerebellum.

The respective concentrations of insulin in the control, 0.5% GABA, and 1% GABA groups were approximately 2,000, 1,700, and 1,400 ng/mL (Table 1). Our results show that the insulin concentration was slightly, although not significantly, decreased by the GABA administration (Table 1, p < 0.05). Gilon et al. have similarly demonstrated that the exogenous GABA and GABA agonists, muscimol and baclofen, did not affect the release of insulin by rat islets. Excessive amino acid consumption has caused insulin resistance, resulting in high plasma levels of glucose and insulin due to a reduced glucose uptake. However, in the present study, insulin resistance did not result after chronic GABA administration.

Table 1 shows the concentrations of IGF-1 in the control, 0.5% GABA, and 1% GABA groups. The concentration of IGF-1 in the 0.5% GABA group (4,294.86 pg/mL) was significantly lower than that in the control group (5,208.57 pg/mL; Table 1, p < 0.05). The IGF-1 concentration in the 1% GABA group (3,254 pg/mL) was also significantly lower than that in the control and 0.5% GABA groups (Table 1, p < 0.05). Our study shows that the administration of GABA significantly enhanced the level of GH when compared to the control value (0.65 ng/mL; Table 1, p < 0.05). However, the GH concentration in rats of the 0.5% GABA (1.42 ng/mL) and 1% GABA (1.76 ng/mL) groups was not significantly different (Table 1, p < 0.05). A number of studies have demonstrated that GABA increased the concentration of plasma GH.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>0.5% GABA</th>
<th>1% GABA</th>
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<tbody>
<tr>
<td>S6K1 in the cerebral cortex</td>
<td></td>
<td></td>
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<tr>
<td>γ (%)</td>
<td>4.12 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.84 ± 1.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.57 ± 1.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>β + γ (%)</td>
<td>46.43 ± 1.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.08 ± 3.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>56.63 ± 2.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S6K1 in the cerebellum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>γ (%)</td>
<td>8.98 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.66 ± 1.79&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15.58 ± 2.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>β + γ (%)</td>
<td>63.46 ± 2.03</td>
<td>67.10 ± 2.65</td>
<td>67.85 ± 1.15</td>
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<tr>
<td>Hormones</td>
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<tr>
<td>Insulin (ng/mL)</td>
<td>2116.75 ± 216.73</td>
<td>1792.43 ± 172.30</td>
<td>1432.36 ± 219.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IGF-1 (pg/mL)</td>
<td>5208.57 ± 195.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4294.86 ± 151.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3254.00 ± 229.82&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>GH (ng/mL)</td>
<td>0.65 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.42 ± 1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.76 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Data are mean value ± SE, n = 7.

Means with different letters in the same row indicate significant differences (p < 0.05).

**Fig. 1.** Phosphorylation States of S6K1 in the Cerebral Cortex (A) and Cerebellum (B).

S6K1 was resolved into α, β, and γ phosphorylated forms on SDS-polyacrylamide gels. The γ form is the most highly phosphorylated which represents the lowest electrophoretic mobility. The phosphorylation of S6K1 was consequently quantified as the percentage of the γ form in S6K1.
A change in GH concentration after GABA intake may affect the secretion of IGF-1. In our study, GABA may have been involved in the increase in GH synthesis and/or secretion by the inhibition of somatostatin, which in turn decreased the IGF-1 concentration.\textsuperscript{22,23} However, there need to be further studies on the effects of GH on IGF-1 and somatostatin secretion to clarify the mechanism of GABA and its influence on the release GH and IGF-1.

In conclusion, GABA administration to young rats led to an increase in the concentration of plasma GH which stimulated the translational phase of protein synthesis.\textsuperscript{8,11} A previous study has shown that GH could cross the BBB and stimulate brain protein synthesis.\textsuperscript{13,24} Investigators have also demonstrated that GABA increased the GH level, leading to an immediate increase in the protein synthesis rate in the brain.\textsuperscript{11} However, we could not find any increased level of GABA in the brain after the GABA treatment (our unpublished laboratory data). It is therefore very likely that GABA enhanced the level of GH in our study, and that GH then crossed the BBB to activate mTOR signalling. This resulted in an increase in the phosphorylation of S6K1 which indicates enhanced protein synthesis.

References