Dietary Genistein Affects Brain Protein Synthesis Rates in Ovariectomized Female Rats

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ABSTRACT The purpose of this study was to determine whether genistein affects the rate of brain protein synthesis in ovariectomized female rats. Experiments were conducted on three groups of 12-wk-old female rats: those in group 1 were ovariectomized to reduce the level of plasma sex hormone; those in group 2 were ovariectomized and fed diets containing 0.01% genistein; and those in group 3 were sham-operated controls. The fractional rates of protein synthesis in the brain of ovariectomized rats fed genistein were significantly greater than those in ovariectomized rats without genistein treatment. In the cerebral cortex and cerebellum, the RNA activity [g protein synthesis/(g RNA*d)] significantly correlated (r> 0.86, P < 0.001) 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. The results suggest that the addition of genistein to the diet of ovariectomized female rats is likely to increase 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. J. Nutr. 132: 2055–2058, 2002.

KEY WORDS: • brain • isoflavone • ovariectomy • protein synthesis • 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 alterations of dietary amino acid composition in young rats (6,7).

Many investigators previously 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). The isoflavones in soybeans have weak estrogenic activity in tissues of mammals (16), although the role of isoflavones in maintaining brain protein synthesis during estrogen deficiency remains unknown under physiological conditions. Therefore, the possible effects of the dietary addition of isoflavones on brain protein synthesis in ovariectomized female rats are of nutritional importance in understanding the role of nutrition in the brain function in mammals.

The purpose of our study was to determine whether soybean isoflavones affect the rate of brain protein synthesis in ovariectomized female rats. In our previous reports (17,18), 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 aged male rats. However, the reduction with age in protein synthesis in the brain was related to a decrease in the RNA concentration (11). Two questions were considered in the present study: 1) whether the dietary addition of isoflavones might affect brain protein synthesis in ovariectomized female rats, and 2) whether greater RNA concentration or RNA activity in ovariectomized female rats fed isoflavones resulted in a greater protein synthesis rate in the brain compared with that in untreated ovariectomized rats. Therefore, we examined three indicators of protein synthesis in rat brains: its rate, RNA concentration and RNA activity.

Ovariectomized female rats were studied as a clinical animal model for postmenopausal osteoporosis, and also used to test the function of isoflavones (19,20). Genistein (4',5,7-trihydroxyisoflavone) and daidzein (4',7-dihydroxyisoflavone) are the major isoflavones in soybeans. From in vitro data of the hormonal effects of soybean isoflavones (relative binding affinity to uterus receptor), the estrogenic potency of genistein was suggested to be higher than that of daidzein (16). Recently, several investigators showed that genistein absorbed quickly through the small intestine, and that oral treatment with genistein at physiological concentrations produce blood levels sufficient to have estrogenic effects in clinical animal studies (20,21). Thus, in this experiment, we used ovariectomized female rats and soybean genistein as the animal and source of isoflavones, respectively.

MATERIALS AND METHODS

Chemicals. l-Tyrosine decarboxylase, β-phenethylamine and leucylalanine were purchased from Sigma Chemical (St. Louis, MO). l-[2,6,3H]Phenylalanine (1.5 TBq/mmol) was obtained from Amer sham (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemicals (Osaka, Japan).

Animals and diet. Female 12-wk-old Wistar rats (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 sham-operated control rats gained less body weight and consumed less food than did either group of ovariectomized rats, which did not differ (Table 1). The relative weights of various brain regions did not differ among groups.

Fractional (Ks) and absolute rates of protein synthesis in some brain regions, such as cerebral cortex and cerebellum, were lower in ovariectomized rats than those in sham-operated controls or ovariectomized rats fed genistein, which did not differ (Table 2). In pooled samples of hippocampus and brain stem, these rates also were lower in the ovariectomized rats.

RNA activity in the brain regions was significantly lower in the untreated ovariectomized groups than that in the control or ovariectomized plus genistein groups (Table 2). Correlations between the fractional rate of protein synthesis and RNA activity were significant in the cerebral cortex (r = 0.882, P < 0.001) and cerebellum (r = 0.901, P < 0.001). The RNA concentrations in all brain regions did not differ among groups (Table 2).

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### DISCUSSION

In older women, the deficiency of sex hormone strongly affects body composition and functions. Ovariectomy decreases brain protein synthesis in female rats (15). The effects of isoflavones on the rat uterus mimic some of the physiological actions of estrogen (e.g., the synthesis of protein and

### TABLE 1

**Effect of dietary genistein on body weight gain and brain region relative weights in ovariectomized rats**

<table>
<thead>
<tr>
<th>Body weight gain, g/14 d</th>
<th>Control</th>
<th>Ovariectomy</th>
<th>Ovariectomy + genistein</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.6b</td>
<td>31.8a</td>
<td>29.6a</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Food intake, g/d

<table>
<thead>
<tr>
<th>Tissue weight, g/100 g body weight</th>
<th>Cerebral cortex</th>
<th>Cerebellum</th>
<th>Hippocampus</th>
<th>Brain stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.20</td>
<td>0.13</td>
<td>0.046</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.12</td>
<td>0.042</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.12</td>
<td>0.040</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>0.006</td>
<td>0.004</td>
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</tbody>
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<th>Food intake, g/d</th>
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<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.6b</td>
<td>14.2a</td>
<td>13.9a</td>
<td>0.3</td>
</tr>
</tbody>
</table>

1 Values are means and pooled SEM, n = 6. Means with different superscript letters are significantly different (P < 0.05).

2 Initial body weight of rats was 170–180 g.

1 Values are means and pooled SEM, 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 2

**Effect of dietary genistein on fractional and absolute protein synthesis rates, RNA concentrations, and RNA activities in brain regions of ovariectomized rats**

<table>
<thead>
<tr>
<th>Protein synthesis (Ks), 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
<tr>
<td>Hippocampus3</td>
</tr>
<tr>
<td>Brain stem3</td>
</tr>
<tr>
<td>Absolute protein synthesis, mg protein synthesized/ (tissue d)</td>
</tr>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
<tr>
<td>Hippocampus3</td>
</tr>
<tr>
<td>Brain stem3</td>
</tr>
<tr>
<td>RNA/protein, mg/g</td>
</tr>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
<tr>
<td>Hippocampus3</td>
</tr>
<tr>
<td>Brain stem3</td>
</tr>
<tr>
<td>RNA activity, mg synthesized/ (g RNA d)</td>
</tr>
<tr>
<td>Cerebral cortex</td>
</tr>
<tr>
<td>Cerebellum</td>
</tr>
<tr>
<td>Hippocampus3</td>
</tr>
<tr>
<td>Brain stem3</td>
</tr>
</tbody>
</table>

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2 Fractional rate of protein synthesis.

3 Data were obtained by a single analysis of pooled samples from six rats.
RNA) (16). However, little information is available on the effects of dietary soybean isoflavones on the rate of brain protein synthesis during sex hormone deficiency. We hypothesized that the rate of brain protein synthesis would increase in ovariectomized rats fed genistein.

Ovariectomized female rats had reduced fractional rates of protein synthesis in brain regions, and dietary genistein reversed the effect of ovariectomy (Table 2). Therefore, the changes in brain protein synthesis likely were attributable to ovariectomy and dietary isoflavones, as previously demonstrated in the uterus of female rats (16,28). Steroid hormones, especially estrogen, increased the transcription rate (13,29). However, ovariectomy with or without estrogen treatment changed the rate of protein synthesis and RNA activity in the brain (15). In the brain regions of rats in the present study, RNA activities, rather than RNA concentrations, in the ovariectomy plus genistein and sham-operated control groups were greater than those in the ovariectomized group (Table 2). Therefore, ovariectomy and treatment with isoflavones may have controlled RNA activity and been one of the factors affecting brain protein synthesis in female rats.

Little information is available on the mechanism by which isoflavones affect RNA activity in the brain of ovariectomized female rats. We previously reported that the aggregation of polyribosomes in the brain of weaned and aged rats after only a 5-h feeding decreased with a decrease in dietary protein quality, and that there was a correlation between the polysomal profile and RNA activity (7,30). In future studies, to determine the effect of soybean isoflavones on brain protein synthesis in ovariectomized female rats, the ribosomal aggregation in the brain will be measured. 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 isoflavone treatment alter brain protein metabolism.

The growth rate and food intake of the sham-control group were significantly lower than those of the two ovariectomized groups (Table 1). However, the rate of brain protein synthesis in ovariectomized rats was less than that of the sham-control and ovariectomy + isoflavone groups. Roy et al. (31) suggested that sex hormones might directly affect gene expression in neurons. These results strongly suggested that sex hormone deficiency and isoflavones, rather than food intake, directly affect brain protein synthesis.

Soybean isoflavones are being recognized as having potential roles in the prevention and treatment of chronic diseases, most notably cancer (32) and heart disease (33,34). A diet rich in soy isoflavones has been shown to produce significant effects on bone density and quality, and that there was a correlation between the polysomal profile and RNA activity (7,30). In future studies, to determine the effect of soybean isoflavones on brain protein synthesis in ovariectomized female rats, the ribosomal aggregation in the brain will be measured. 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 isoflavone treatment alter brain protein metabolism.

The present results indicate that brain protein synthesis was affected by genistein in ovariectomized female rats as evaluated by protein synthesis rates, and suggest that the effects of soybean isoflavones on brain protein synthesis in female rats are also of importance in understanding the relationships among aging, nutrition, sex hormone deficiency and brain function in mammals.

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LITERATURE CITED


