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 synthesized/(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 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-phenylethylamine 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

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the experimental diet after they had been fed a commercial nonpurified diet (MF; Oriental Yeast, Tokyo, Japan) for 2 d. The experimental diet contained 0.01% genistein added to the basal diet. All rats were 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.** The experiment was conducted for three groups of rats. All rats were fed the basal diet or the experimental diet for 14 d. On d 1, two groups were ovariectomized and fed the basal diet or the experimental diet. The sham-operated control was fed the basal diet. After 14 d, the fractional rates of protein synthesis in the brain were measured by the method of Garlick et al. (22). The rats were decapitated between 1000 and 1200 h. Brain regions (23) were quickly removed and frozen in liquid nitrogen. The concentrations of protein and RNA in 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-[2,6-3H]phenylalanine was combined with unlabeled phenylalanine to yield a dose of 1.85 MBq and a concentration of 150 mmol saline/L. Rats were injected with the radioisotope through 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 [3H]phenylalanine in tissue samples were determined according to the method described in our previous report (17).

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 the experimental conditions of their study. 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). Moreover, the values in the plasma, cerebral cortex and cerebellum were not significantly different, indicating that the precursor pool of labeled phenylalanine was not altered. 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 rats killed at 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 (20,27). Linear regression was used to assess the relationship between the rate of protein synthesis and RNA activity (27). Differences were considered significant if \( P < 0.05 \). In the hippocampus and brain stem, the rates of protein synthesis were determined from a pool of six rats.

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**RESULTS**

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).

**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...
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). 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 deficiency of sex hormones also affects brain function. Sherwin (35) reported that there was a beneficial effect of estrogen on memory tasks in postmenopausal women, and that estrogen deficiency may be partly responsible for the neurodegeneration of Alzheimer's disease. Data in ovariectomized rats demonstrated that treatment with estrogen and soy isoflavones increased the mRNAs of neuron growth factor and choline acetyltransferase in the brain, which are important for learning and memory processes (36). In the present study, the ingestion of genistein resulted in higher fractional rates of brain protein synthesis in ovariectomized female rats, suggesting that not only cancer prevention and hypercholesterolemic effects but also brain function are affected by dietary isoflavones.

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