Note

Effect of γ-Aminobutyric Acid-enriched Tempeh-like Fermented Soybean (GABA-Tempeh) on the Blood Pressure of Spontaneously Hypertensive Rats

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GABA-enriched tempeh-like fermented soybean (GABA-tempeh) was supplemented to the AIN-76 diet and fed for 2 months to spontaneously hypertensive rats (SHRs), an animal model of spontaneously developed hypertension, to compare the antihypertensive activity with that of authentic GABA. The elevation of systolic blood pressure in SHRs was significantly retarded in the GABA-tempeh group as well as that with authentic GABA when compared with the controls, and the effect lasted for two months of the feeding period. The blood urea nitrogen level tended to be higher in the control group than in the GABA-supplemented groups. On the other hand, no effect was apparent on the plasma levels of cholesterol, triacylglycerol and glucose, or on the urinary excretion of Na and K.

Key words: γ-aminobutyric acid; fermented soybean; GABA; SHR; tempeh

γ-Aminobutyric acid (GABA) is a depressive neurotransmitter in the sympathetic nervous system,1,2) and also has physiological functions to depress the elevation of systolic blood pressure,3) improve discrimination learning,4) and relieve the discomfort symptoms that appear during the menopausal or presenile period such as sleeplessness, depression and autonomic disorder.5) GABA is present in a variety of daily foods such as yogurt, Korean kimchi and a type of Japanese pickle, “shibazuke.”6) Feeding with GABA-enriched foods such as gabaron tea,7,8) red-mold rice9) and Chlorella10) has been reported to depress the elevation of systolic blood pressure in spontaneously hypertensive rats (SHRs). We have previously developed a new procedure for the preparation of GABA-enriched tempeh-like fermented soybean (GABA-tempeh) by aerobic and successive anaerobic fermentation with Rhizopus.11) The resulting GABA-tempeh contained more than 1 g of GABA in 100 g of soybean (dry basis) and was also richer in free amino acids than conventional tempeh. In this study, we report the effect of supplementing GABA-tempeh to the AIN-76 diet on the blood pressure in SHRs.

GABA-tempeh was prepared as previously described.11) Briefly, dehulled soybeans were soaked overnight in 0.2% acetic acid and then steamed at 121°C for 5 min. The steamed beans (200 g) were inoculated with 1 ml of a suspension of spores of R. oligosporus IFO 32002 at a level of about 1 × 10⁷ W ml. The inoculated beans were aerobically incubated at 37°C for 20 h and then incubated under anaerobic conditions at 37°C for 20 h. Dry GABA-tempeh contained 1.3 W of GABA together with protein (45.7%), fat (25.7%), carbohydrate (22.4%), ash (4.3%), and water (1.9%).

Male SHRs 9 weeks old were purchased from Charles River Japan (Atsugi, Kanagawa, Japan). Twenty-four male SHRs (215–277 g) were caged individually in a room under the following conditions: the temperature of the room was set to 22 ± 3°C, the humidity to 55 ± 15%, the ventilation frequency to at least 10 times per h by an all-fresh-air system, and lighting for 12 h from 6 a.m. to 6 p.m. at 150–300 lux. After being fed for 14 d on the basal diet, which had been prepared on the basis of the AIN-76 preparation, the animals were divided into the following four dietary groups of 6 rats: control, authentic GABA, 0.1 W GABA-tempeh, and 0.5 W GABA-tempeh groups. The compositions of the experimental diets are shown in Table 1. The level of GABA for the authentic GABA group matched the GABA level for the 0.1 W GABA-tempeh group. Free access to the feed and drinking water was provided for 8 weeks, and the food and water intake was recorded every day. The body weight was measured once a week, and the blood pressure and pulse were mea-
Table 1. Composition of the Experimental Diets (%)

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Control</th>
<th>Authentic GABA</th>
<th>0.1% GABA-tempeh</th>
<th>0.5% GABA-tempeh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>22.0</td>
<td>22.0</td>
<td>22.0</td>
<td>21.8</td>
</tr>
<tr>
<td>Lard</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>9.9</td>
</tr>
<tr>
<td>Mineral mixture (AIN76)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture (AIN76)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Cellulose</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>59.2</td>
<td>59.2</td>
<td>59.1</td>
<td>59.0</td>
</tr>
<tr>
<td>GABA</td>
<td>—</td>
<td>0.001</td>
<td>—</td>
<td>0.1</td>
</tr>
<tr>
<td>GABA-tempeh</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of GABA-Tempeh on the Systolic Blood Pressure in SHR.

Symbols: ○, control group; ●, authentic GABA; △, 0.1% GABA-tempeh; ▲, 0.5% GABA-tempeh. Each value is the mean ± SE of 6 animals. Significantly different from the control group: *p<0.05, **p<0.01, ***p<0.001.

ured with a non-invasive blood pressure meter (MK-1100, Muromachi Kikai, Tokyo, Japan) once a week. Before the period of experimental feeding and after 4 and 8 weeks of feeding, 0.5 ml of blood was collected from the caudal vein. The levels of plasma glucose, total cholesterol, triacylglycerol, and urea nitrogen were determined with an automatic analyzer (7070, Hitachi, Tokyo), using commercial assay kits (Wako Pure Chemical Ind., Osaka) as follows: glucose, L-type Wako GLU; total cholesterol, L-type Wako CHO; triacylglycerol, TG EII-HA 7070; and urea nitrogen, UN II-HA 7070. After feeding the experimental diets for 7 weeks, the animals were transferred to metabolic cages and fasted for one day before cumulative urine was collected. The urine volume was calculated from the specific gravity and the weight. Then Na and K levels in the urine were determined by the ion-selective electrode method with an automatic analyzer (7070, Hitachi, Tokyo, Japan). Each result is presented as the mean ± SE.

Differences among the dietary groups were analyzed by repeated-measure ANOVA and followed by Dunnett’s multiple comparison. Differences were judged to be significant at p<0.05.

There was no significant difference in body weight during the experimental feeding among the dietary groups, although the 0.5% GABA-tempeh group showed a slightly greater increase in body weight than the other groups. The food and water consumption of all dietary groups was similar and not significantly different.

The systolic blood pressure in the control group rose to 213 mm of Hg after 2 weeks of feeding, but it did not rise any more during the experimental period as shown in Fig. 1. On the other hand, the authentic GABA, and 0.1% and 0.5% GABA-tempeh groups showed a slightly greater increase in body weight than the other groups. The food and water consumption of all dietary groups was similar and not significantly different.

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The effects of dietary GABA and GABA-tempeh on the levels of plasma cholesterol, triacylglycerol, glucose, and urea nitrogen, together with the urinary excretion of Na and K were assessed. Dietary GABA and GABA-tempeh did not have any significant effects on the blood total cholesterol, triacylglycerol, and glucose levels. On the other hand, the blood urea nitrogen level tended to be higher in the control group (18.2 mg/100 ml) than in the GABA-supplemented groups, i.e., in the authentic GABA, and 0.1% and 0.5% GABA-tempeh groups at 8 weeks (about 12 mg/100 ml). Furthermore, the urinary levels of Na and K were not significantly different among the dietary groups, although the Na level was slightly lower in the 0.5% GABA-tempeh group than in other groups, while the K level was lower in the control than in the GABA-fed groups.

We have previously developed a new procedure to prepare the unique fermented soybean, GABA-tempeh, containing a high level of GABA. In this present study, we assessed the antihypertensive effect of dietary GABA-tempeh on SHR. The addition of GABA-tempeh at the 0.1% level together with the addition of authentic GABA at the 0.001% level to the basal AIN-76 diet significantly depressed the elevation of the systolic blood pressure. However, increasing the supplementation of GABA-tempeh to 0.5% did not have a more depressing effect on the blood pressure.
In the present studies, we found that a GABA intake as low as about 0.3 mg per rat per day showed significant antihypertensive activity in SHR. This GABA level is less than 1/10 of that in feeding experiments on other GABA-enriched foods such as gabaron tea\(^7,8\) and Chlorella.\(^9\) Among the GABA-supplemented groups, the GABA level of the 0.001% authentic GABA and the 0.1% GABA-tempeh groups were almost equivalent. Therefore, GABA would have been responsible for the antihypertensive activity of GABA-tempeh in SHR.

As the addition of five-fold more GABA-tempeh had little further lowering effect on the blood pressure, it was found that feeding a larger GABA-tempeh dose did not lower the systolic blood pressure much. A similar result has been reported for the case of red-rice mold.\(^9\)

The plasma urea nitrogen level in the GABA-supplemented groups, i.e., the authentic GABA, and 0.1% and 0.5% GABA-tempeh groups tended to be lower than in the control group. This result may suggest that dietary GABA-tempeh protected the filtration function of the kidneys from the damage induced by high blood pressure. A similar effect has been reported for GABA-enriched Chlorella\(^10\) which improved the kidney function.

The hypotensive mechanism for GABA has not been fully resolved, but it has been reported that the systemic administration of GABA decreased the blood pressure by a peripheral mechanism that is dependent upon an intact sympathetic nervous system and that the ganglionic-blocking activity of GABA most likely contributes to its vasodepressive effect upon systemic administration.\(^3\) The results of the present studies preclude the involvement of Na metabolism on the antihypertensive effect, because no significant difference was observed in the urinary excretion of Na among the dietary groups.

The present studies show that feeding GABA-tempeh at the 0.1% level had significant antihypertensive activity in SHR. GABA-tempeh is rich not only in GABA but also in various free amino acids and peptides.\(^13\) Free amino acids and oligopeptides in foods have been reported not only to improve the umami taste, but also to have such nutritional advantages as rapid absorption,\(^12,13\) muscle protein maintenance,\(^13\) and antioxidogenic activity.\(^14\) These physiological effects can also be expected for GABA-tempeh, but they do need to be assessed by feeding experiments.

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**References**