Antihypertensive Effect of \(\gamma\)-Aminobutyric Acid-Enriched Brown Rice on Spontaneously Hypertensive Rats

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Summary ‘Haiibuki’ is a giant embryo rice cultivar that contains abundant \(\gamma\)-aminobutyric acid (GABA) compared with conventional rice cultivars. Here, we performed a functional evaluation of ‘GABA-enriched brown rice’ (GEBR) prepared by modifying the ‘Haiibuki’ cultivar to contain more GABA. Study 1: Spontaneously hypertensive rats were divided into three groups [control (cornstarch), normal brown rice, and GEBR] and fed an orally administered diet for 4 wk. A significant blood pressure elevation-inhibitory effect was observed in the GEBR group as compared with the other groups. Study 2: Rats were divided into two groups and fed ad libitum for 12 wk. Body weight, blood pressure, food consumption, and water intake were measured during the study period, and blood chemistry was analyzed after the study. Plasma 8-hydroxy-2′-deoxyguanosine (8-OHdG) and urinary isoprostane were measured 12 and 10 wk after the start of the study, respectively. A significant blood pressure elevation-inhibitory effect was observed in the GEBR group. The 8-OHdG and isoprostane levels were significantly lower in the GEBR group than in the control group, demonstrating an oxidative stress-reducing effect. Therefore, GEBR exhibited an antihypertensive action under the conditions of this study. The antihypertensive action may occur secondarily to the antihypertensive action of GABA, suggesting that the long-term ad libitum ingestion of GEBR prevents hypertension. A reduction in oxidative stress could reduce the chances of complications in cardiovascular diseases.

Key Words \(\gamma\)-aminobutyric acid (GABA), spontaneously hypertensive rat (SHR), blood pressure, giant embryo rice, anti-oxidation

When diseases caused by lifestyle factors such as excessive cigarette smoking and alcohol drinking are left untreated due to the absence of pain, serious diseases such as stroke and myocardial infarction may develop. One in two Japanese people already have hypertension, obesity, diabetes, and/or hyperlipidemia. Thus, drastic policy measures are necessary to maintain quality of life in the aging Japanese society and prevent an increase in national medical expenses. According to the World Health Organization, there are nearly one billion patients with hypertension worldwide, and the prevention and improvement of hypertension are key challenges faced by humanity.

Along with this, the function of food has changed from nutrition (primary function) to taste (secondary function), and now to disease prevention (tertiary function). Rice-based ‘traditional Japanese diets’ have been revived through dietary education. Based on the concept that ‘a balanced diet leads to a healthy body,’ the accumulation of several functional components (peptides, amino acids, vitamins, and minerals) in rice and its daily ingestion may prevent lifestyle-related diseases (1).

\(\gamma\)-Aminobutyric acid (GABA) is a non-protein amino acid widely existing in nature. It has been elucidated that GABA is an important neurotransmitter of the central nervous system in mammals (2). GABA is abundantly contained in grains, cereals, and green tea, and its blood pressure (BP)-lowering effects have been reported (3, 4). However, the polished rice that is most commonly eaten as cooked rice is the rice endosperm region, and the embryo buds, which have a relatively high GABA content, are mostly removed during the rice polishing process. Recently, GABA-enriched rice has been experimentally developed by gene manipulation (5). However, transgenic rice cannot be sold in Japan. In

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addition, GABA-abundant sprouted brown rice is sold and a ‘grain GABA-enriching device’ to increase the GABA content of polished rice has been developed (6, 7). In this study, the functionality of ‘GABA-enriched brown rice’ (GEBR) containing a high concentration of GABA was investigated with regard to its influence on BP by ad libitum ingestion in spontaneously hypertensive rats (SHR).

MATERIALS AND METHODS

Animals. A total of 40 male and female SHR/Imz rats aged 6 wk (Disease Model Cooperative Research Association, Kyoto, Japan) were used in this study. The animal room was maintained at a constant temperature (23±2°C), humidity (55±10%), and mean ventilation frequency (10–13/h) with 12-h light/dark cycles (light: 07:00–19:00). The animals were fed a commercial diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and provided with filtered tap water ad libitum. Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at Shimane University (approval number: IZ27-52).

Sample preparation. The giant embryo rice cultivar ‘Haiibuki’ (8), which was produced by cultivar improvement, contains abundant GABA compared with conventional rice cultivars. Using the brown rice of this cultivar, GEBR containing a high concentration of GABA was manufactured employing a technique to synthesize GABA from glutamic acid accumulated in the embryo buds of rice and enhance natural GABA absorption by the endosperm. The resultant ‘Haiibuki’ brown rice (Satake Co., Ltd., Hiroshima, Japan) had a GABA content of 47.0 mg/100 g dry biomass, which is 8 times more than that of normal brown rice (GABA content: 5.8 mg/100 g dry biomass, Satake Co., Ltd.) (9).

Experimental procedure. Study 1: To evaluate the functional effects of GEBR, an oral administration experiment was performed. Previous studies on the oral administration of functional foods to SHR [0.5–1.0 mg GABA/kg body weight (BW)/d] resulted in a BP-lowering effect (10, 11). We prepared three different kinds of samples containing different doses of GABA in a range determined from the BP-lowering effects reported by the former studies. After rice grains were polished into powder, they were suspended in distilled water. For each sample type, a 10-mL suspension of rice grains was administered to the rats because they are easy to handle. BP was measured once a week.

Study 2: Male rats aged 7 wk were divided into two groups (control and GEBR groups, 8 animals in each) and fed the experimental diets ad libitum during the 12-wk experimental period. For the control group, 5% cornstarch (GABA content: 0 mg, Mylan N.V.) was mixed with commercial feed. For the GEBR group, 5% GEBR was mixed with commercial feed. BW and BP were measured once every 3 wk, while food consumption and water intake were measured at 1, 4, 7, and 10 wk of the experimental period. Using a metabolic cage (CT-10S, CLEA Japan Inc., Tokyo, Japan), urine was collected for 24 h after the initiation of feeding and again after 10 wk. The samples were stored at −80°C until analysis. After 12 wk, the rats were fasted for 16 h and blood was collected from the abdominal inferior vena cava under anesthesia with isoflurane, followed by macroscopic observation of the organs (brain, heart, kidney, liver, spleen, and pancreas).

Measurement of BW and BP. BW and BP were measured during the experimental period. BW was measured using an electronic balance (HF-3000, A&D Co., Ltd., Tokyo, Japan). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automatic sphygmomanometer (BP-98A, Softron Co., Ltd., Tokyo, Japan) at the tail vein employing the tail-cuff method (12) without anesthesia after warming the rat at 38°C for 8 min. BP was measured 5 times for each rat and the mean was calculated.

Blood chemistry. Collected blood was transferred to a micro blood-sampling tube (CAPIJECT, Ethylenediami-
netraacetic acid (EDTA)-2Na, Terumo Co., Ltd., Tokyo, Japan), and centrifuged at 860 \( g \) for 15 min at 4˚C, and the supernatant was collected as a plasma sample.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), \( \gamma \)-glutamyltranspeptidase (GGT), total cholesterol (T-Cho), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), total protein (T-Pro), albumin (ALB), urea nitrogen (BUN), uric acid (UA), and creatinine (Cre) were measured using a benchtop chemistry analyzer (SPOTCHEMTM EZ SP-4430, ARKRAY, Inc., Kyoto, Japan).

Assay of plasma 8-hydroxy-2′-deoxyguanosine (8-OHdG) and urinary isoprostane. The plasma samples were stored at −80˚C until measurement. After thawing to room temperature, 8-OHdG in plasma was measured. Macromolecular components were removed from plasma by ultrafiltration as a pretreatment, followed by 8-OHdG measurement using a commercial enzyme-linked immunosorbent assay kit (8-OHdG Check kit, Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd., Tokyo, Japan) (13). After thawing to normal temperature, urine samples were centrifuged at 860 \( g \) for 15 min at 4˚C, and the supernatant, excluding precipitates, was used for measurement. Urinary isoprostane was measured using a commercial enzyme-linked immunosorbent assay kit (urinary isoprostane kit, Japan Institute for the Control of Aging). The results were expressed as the urinary isoprostane concentration multiplied by the urine volume.

Statistical analysis. All values are expressed as the mean±standard error (SE). Statistical significance among three groups was determined using one-way ANOVA and the Scheffe post hoc test. The Wilcoxon rank-sum test was used to compare the changes in food consumption, water intake, BW, SBP, and DBP values with a few exceptions. Statistical significance between two groups was determined by analyses performed using Student’s t-test. A value of \( p<0.05 \) was considered to be significant.

RESULTS

To confirm the antihypertensive effect caused by GABA, we compared the antihypertensive effects of GEBR and normal brown rice upon oral administration to SHR. Figure 1 shows the changes in SBP during the oral administration of GEBR to the rats in Study 1. The SBP in the GEBR group was significantly lower than that in the control group at 3 and 4 wk, resulting in a decrease in SBP of about 15 mmHg. Additionally, the SBP in the GEBR group was significantly lower than that in the normal brown rice group at 3 wk.

In Study 2, we prepared different diets containing cornstarch or GEBR and provided them ad libitum in the feed described during the 12-wk study period. The food consumption of the rats is shown in Fig. 2A. The GEBR
group consumed significantly more food than the control group did in the first week. No significant difference was noted between the GEBR and control groups thereafter. There were no significant differences in the calorie intake between the GEBR groups (62.4±1.1 kcal) and the control group (59.9±0.9 kcal) during 7 and 10 wk.

Water consumption is shown in Fig. 2B and was significantly lower in the GEBR group than in the control group at week 7 and 10. The BW measurements of the rats are shown in Fig. 3. No significant difference in BW was noted among the groups throughout the experimental period. All rats grew linearly, and there were no

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**Table 1.** Comparison of the biochemical parameters of plasma in male SHRs.

<table>
<thead>
<tr>
<th></th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>GGT (IU/L)</th>
<th>T-Cho (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>TG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.3±2.9</td>
<td>8.3±1.5</td>
<td>7.5±0.2</td>
<td>60.9±1.7</td>
<td>17.3±0.6</td>
<td>42.5±3.0</td>
</tr>
<tr>
<td>GABA</td>
<td>68.0±2.6</td>
<td>9.0±1.8</td>
<td>7.5±0.2</td>
<td>66.8±1.3</td>
<td>18.4±0.6</td>
<td>45.0±2.3</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>T-Pro (g/dL)</th>
<th>ALB (g/dL)</th>
<th>BUN (mg/dL)</th>
<th>UA (mg/dL)</th>
<th>Cre (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.0±0.1</td>
<td>3.0±0.0</td>
<td>21.6±0.6</td>
<td>2.4±0.1</td>
<td>0.4±0.0</td>
</tr>
<tr>
<td>GABA</td>
<td>7.0±0.1</td>
<td>3.2±0.0</td>
<td>21.4±1.2</td>
<td>2.2±0.2</td>
<td>0.3±0.0</td>
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Each value represents the mean±SE of 8 rats.
major differences in the growth compared with the control group.

Changes in SBP are shown in Fig. 4A. No significant difference in SBP was noted among the groups until 3 wk after the initiation of the experiment, but BP elevation was significantly inhibited at 6, 9, and 12 wk in the GEBR group. The changes in DBP during the experimental period are shown in Fig. 4B. No significant difference in DBP was noted among the groups throughout the experimental period.

The results of the blood chemistry analysis are shown in Table 1. No abnormal values were detected in any of the liver or kidney function parameters, and no rats showed any differences between the GEBR and control groups. The plasma 8-OHdG levels are shown in Fig. 5A. They were 0.79 ± 0.02 ng/mL in the control group and significantly lower at 0.70 ± 0.02 ng/mL in the GEBR group. The urinary isoprostane levels are shown in Fig. 5B. They were 73.1 ± 9.5 ng/d in the control group and significantly lower at 48.2 ± 5.2 ng/d in the GEBR group.

DISCUSSION

The objective of this experiment was to investigate the BP elevation-inhibitory action of the ad libitum ingestion of GEBR containing a high concentration of GABA using SHR. In similar studies using SHR, GABA was accumulated to a high concentration by the forcible oral administration of food materials such as rice (14), fermented beans (13), mushrooms (15), or tea leaves (16), and a BP elevation-inhibitory action was demonstrated in each case.

We demonstrated that SHR administered a 1 mg/kg BW dose of GEBR grains resulted in an antihypertensive effect of about 15 mmHg SBP in SHR. However, the normal brown rice (0.12 mg of GABA/kg BW) did not show a significant SBP-lowering effect (Study 1). As a result, it is suggested that components other than GABA are not involved in the antihypertensive action. Further, SHRs were given ad libitum access to a diet containing GEBR and its effects were investigated (Study 2), resulting in a significant inhibition of BP elevation at 6, 9 and 12 wk in the GEBR group. A similar effect was observed throughout the present experiment, suggesting that the BP-lowering effect of GEBR persisted during the experimental period.

Regarding the BP elevation-inhibitory effect of GABA, Aoki et al. (17) reported that the administration of GABA at 0.3 mg/rat/d as the lowest effective dose to SHR inhibited BP elevation. Concerning the long-term administration of GABA, it has been reported that BP elevation was inhibited by mixed feeding at approximately 10 mg/kg BW/d, and GABA acted in a dose-dependent manner (18). The daily required intake of GABA in the diet for BP improvement in humans was specified as 10–20 mg (19). The GABA concentration in the GEBR used in this experiment was 47 mg/100 g dry biomass. The male rats continuously ingested approximately 2 mg/kg BW/d of GABA by free feeding, suggesting that the anti-hypertensive action was maintained throughout the study period.

Measurements of BW and food consumption are useful methods for the early detection of the onset of disease or debilitation in animals. The GEBR group consumed significantly more food than the control group did in the first week. It was considered to be a temporary increase due to changes in taste when the food was changed (20). There were no significant differences in food consumption or calorie intake between the control and GEBR groups. In the GEBR group, there was no association between food consumption and the BP-lowering effect. Additionally, to avoid affecting the BP measurements, estimations of the amount of food intake were carried out at week 1, 4, 7 and 10. In the GEBR group, water consumption was significantly lower than in the control group at week 7 and 10. Nevertheless, it should be noted that no increase in BP occurred in the GEBR group. A similar suppressive effect of GABA on water consumption was previously observed in SHR (21, 22). Although water consumption is thought to be closely related to BP, the mechanism of the effect of GABA on water consumption needs to be investigated further.

In SHR, the production of superoxide by NADH/NADPH oxidase, which may be activated by norepinephrine, is enhanced, resulting in the inactivation of nitric oxide (NO) and the impairment of the endothelial modulation of vascular contractions. Vascular oxidative stress may contribute to the altered circulation in hypertension by impairing the endothelial modulation of vascular contractions (23). To investigate whether an antioxidative action was involved in the BP elevation-inhibitory action of GABA, plasma 8-OHdG and urinary isoprostane levels were measured. 8-OHdG that is synthesized in tissue and organs under excessive oxidative stress is normally cleaved by repair enzymes and finally excreted into urine via the circulation (24). The plasma 8-OHdG concentration was significantly lower in the GEBR group than in the control group. Urinary isoprostane is a prostaglandin-like compound formed by the oxidation of lipids. The oxidation of lipids in the body can be evaluated and used as a marker of the oxidative damage of cell membrane phospholipids (25). The urinary isoprostane level was significantly lower in the GEBR group. In addition, if hypoxia occurs in peripheral tissues due to an increase in peripheral vascular resistance, which is one of the causes of hypertension, oxidative stress caused by NO production associated with the expression of inducible nitric oxide synthase occurs (26). However, in the GEBR group, a reduction in DBP was not observed (Fig. 4B), which suggests that there is no possibility of reducing oxidative stress via peripheral blood flow improvement in SHR.

In general, regarding the mechanism of the BP-lowering effects of GABA, a vasodilatory action (10, 27, 28), sympathetic nerve-suppressive action (29), and antidiuretic hormone secretion-inhibitory action (30) were considered. In the mechanism involving the peripheral sympathetic nervous system, blood vessels constrict and elevate BP when the sympathetic nervous system activity increases. GABA inhibits this activity at the nerve ending through binding to the GABAA receptor present.
in the periphery, and relaxes excess arterial constriction by inhibiting noradrenaline secretion (11). Treatment with antioxidants has been suggested to lower oxidative stress and the resulting signal transduction cascades, and therefore, to decrease BP. However, to date, clinical studies investigating antioxidant supplements have failed to show any consistent benefit. It is noteworthy that lowering BP with antihypertensive medications is associated with reduced oxidative stress and signal transduction (31). Therefore, there is a possibility that an antioxidative action may occur secondarily to the antihypertensive action caused by GABA. A reduction in oxidative stress could reduce the chances of complications in cardiovascular diseases.

It was demonstrated in this study that the ad libitum ingestion of GEBR significantly inhibited BP elevation in SHR, suggesting its contribution to inhibiting the development of hypertension. It may be feasible to improve and prevent hypertension by the consumption of foods such as GEBR without using drugs in humans.

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