Antihypertensive and Natriuretic Effects of Less-Sodium Soy Sauce Containing γ-Aminobutyric Acid in Spontaneously Hypertensive Rats

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We investigated the mechanism of the antihypertensive effect of less-sodium soy sauce containing γ-aminobutyric acid (GABA) in spontaneously hypertensive rats (SHRs). When SHRs were given a diet with less-sodium soy sauce containing GABA (GABA-rich soy sauce group) for 6 weeks, the systolic blood pressure decreased as compared with that in rats fed diets with less-sodium soy sauce or a solution of salt. Renal sympathetic nerve activity (RSNA) and positive Na balance were reduced, and the urinary Na excretion tended to increase in the GABA-rich soy sauce group. Vascular hypertrophy of the thoracic aorta and the coronary and renal interlobular arteries tended to reduce in the GABA-rich soy sauce group. These results suggest that inhibition of Na retention by natriuresis, as a result of inhibition of RSNA by the GABA in the soy sauce contributed to the antihypertensive effect of GABA in the SHRs. Intake of less-sodium soy sauce containing GABA might help to reduce overall cardiovascular risk.

Key words: γ-aminobutyric acid (GABA); soy sauce; spontaneously hypertensive rat (SHR); antihypertensive effect; natriuresis

The development of hypertension is related to several factors, including diet and lifestyle, and nutritional factors such as excessive salt and alcohol intake can lead to an increase in blood pressure. The World Health Organization-International Society of Hypertension (WHO-ISH),3 the “Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure” (JNC VII) guidelines,3 and the Japanese Society of Hypertension for the Management of Hypertension in 2004 (JSH-2004) strongly encourage lifestyle modification to prevent high blood pressure, and recommend reduction of the daily dietary sodium intake to no more than 6 g of sodium chloride.5 However, the daily dietary intake of salt in Japan was estimated to be 11.2 g/d in 2003, remaining largely unchanged since 1980.5 Reduction of the daily dietary intake of salt may be difficult for the Japanese people, due to an increase in the consumption of salt-containing processed foods, and also to regular consumption of salt-containing seasonings such as soy sauce and miso (fermented soybean paste) in Japan. Therefore, the strategy as a first-line therapy appears to be moderate salt restriction in combination with a diet rich in fruits and vegetables and thus rich in potassium (K).

GABA, one of the major inhibitory neurotransmitters in the central nervous system, has been claimed to be of value in cerebral disorders and to have an antihypertensive effect. Recently, GABA has been investigated with keen interest as a potential dietary factor with an antihypertensive effect in Japan. Numerous studies have found reductions in blood pressure following central as well as systemic administration of GABA in both experimental animals6–9) and humans.10,11) Hayakawa et al. examined the mechanism of the antihypertensive effect of GABA by observing the effects of GABA administered per oral (p.o.) on the tone of the resistance vessels in the mesenteric arterial bed.12) They reported that GABA inhibited not only the perivascular nerve stimulation-induced increase in perfusion pressure, but also the accompanying noradrenaline release, and that the antihypertensive effect of GABA might be related to its inhibition of noradrenaline release from sympathetic nerve fibers.12) The findings of this study suggest that the short- or medium-term hypotensive effects of GABA might be related to arterial vasodilatation caused by its

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Abbreviations: GABA, γ-aminobutyric acid; SHR, spontaneously hypertensive rat; SBF, systolic blood pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; p.o., per oral; DOCA, deoxycorticosterone acetate; BUN, blood urea nitrogen; HPLC, high-performance liquid chromatograph
inhibition of noradrenaline release and a resulting reduction in peripheral sympathetic nerve activity.

Activation of the sympathetic nervous system plays an important role in the pathogenesis of hypertension. In particular, the renal sympathetic nervous system influences Na excretion both via arteriolar vasoconstriction and alterations in its tubular reabsorption. It has been found that increased renal sympathetic nerve activity (RSNA) is involved in the development of hypertension through Na retention in SHRs and deoxycorticosterone acetate (DOCA)-salt rats, since renal denervation promoted Na excretion and attenuation of hypertension. Chronic oral administration of GABA caused a significant decrease in blood pressure in sham-operated SHRs, but not in renal-sympathetic-denervated SHRs. Therefore, GABA might attenuate the development of hypertension, mainly as a result of arterial vasodilatation and inhibition of Na retention caused by its inhibition of peripheral sympathetic nerve activity. However, the mechanism of the hypotensive effect of systemically administered GABA remains to be fully elucidated.

In this context, we developed a procedure for the preparation of less-sodium soy sauce containing GABA (GABA-rich soy sauce) by fermentation with lactic acid bacteria. The aim of the present study was to determine whether oral administration of GABA-rich soy sauce can attenuate the rise in blood pressure in SHRs, a rodent model of human essential hypertension. Furthermore, to clarify the mechanisms underlying the antihypertensive effects of GABA-rich soy sauce, we investigated changes in urinary sodium excretion, sodium balance and RSNA in SHRs fed GABA-rich soy sauce as compared with those in SHRs fed soy sauce enriched in K, which also possesses antihypertensive and natriuretic actions, (K-rich soy sauce). We also investigated whether regular intake of GABA-rich soy sauce might prevent the development of vascular hypertrophy in SHRs.

Materials and Methods

Soy sauces. Less-sodium soy sauce (soy sauce), GABA-rich soy sauce and K-rich soy sauce were prepared by Kikkoman Corporation (Chiba, Japan). All contained 8.4 g/100 ml of NaCl. GABA-rich soy sauce was produced from moromi fermented with Lactobacillus rennini derived from the moromi of soy sauce. For our experiments, GABA-rich soy sauce containing 1.0 g/100 ml of GABA was used. The less-sodium soy sauce and K-rich soy sauce did not contain GABA. K-rich soy sauce was produced by the addition of KCl to soy sauce. It contained 3.0 g/100 ml of KCl. The soy sauce and GABA-rich soy sauce did not contain KCl. The compositions of soy sauce and GABA-rich soy sauce were almost the same, except for the concentration of GABA. An 8.4 g/100 ml NaCl solution was used as the control.

Chemicals. GABA of analytical grade was purchased from Sigma Chemical (St Louis, MO).

Animals.

Single oral administration. Forty-two male SHRs/Izm (15 weeks old) were purchased from Japan SLC (Shizuoka, Japan) for the single oral administration experiment, and were maintained under the following conditions: temperature, 23 ± 1 °C; relative humidity, 55 ± 5%; 12-h light/dark cycle. They were fed a normal diet (MF, Oriental Yeast, Kanagawa, Japan). The animals were used for the experiments after a 2-week quarantine period.

Chronic oral administration. Seventy-seven male SHRs/Hos and six male WKYs/Hos (4 weeks old) were purchased from Hoshino Laboratory Animals (Saitama, Japan) for the chronic administration experiments, and were maintained under the same conditions as above. The animals were used for the experiments after a 2-week quarantine period. All the animal experiments were conducted in compliance with the guidelines of the Japanese Association for Laboratory Animal Science (1987) and the “Guideline for Animal Experiments” of the Research and Development Division of Kikkoman Corporation.

Experimental design.

Single oral administration. Soy sauce, GABA-rich soy sauce, K-rich soy sauce (0.033 or 0.33 ml/kg body wt.) was given orally to SHRs/Izm (six/group) via a stomach tube after a 14-h fast. GABA (0.33 or 3.3 mg/kg body wt.) dissolved in distilled water (0.33 ml/kg body wt.) was also given orally to SHRs/Izm (six/group). Systolic blood pressure (SBP) and heart rate (HR) were measured by the tail-cuff method with a BP monitor for rats and mice, Model MK-2000 (Muromachi Kikai Ltd., Tokyo), before administration of the samples, and 1, 3, 6, 8, and 24 h after administration. The diet was administered again to the SHRs after the measurement at 8 h.

Chronic oral administrations. SHRs/Hos were randomly divided into five groups of seven animals each for investigation of the hypotensive effect of GABA-rich soy sauce (expt. 1), five groups of six animals each for investigation of the inhibitory effect of the soy sauce on Na retention (expt. 2), and five groups of six animals each for investigation of the influence of the soy sauce on hypothalamic GABA content (expt. 3). These three experiments were conducted separately. In expts. 1 and 2, SHRs/Hos were fed a standard powdered diet containing 0.19% Na and 0.86% K (MF, Oriental Yeast, Tokyo) (control group), a diet containing 3.9% (w/w) NaCl solution (salt group), a diet containing 3.9% (w/w) soy sauce (soy sauce group), a diet containing 3.9% (w/w) GABA-rich soy sauce (GABA-rich soy sauce group), or a diet containing 3.9% (w/w) K-rich soy sauce (K-rich soy sauce group) for 6 weeks. In expt. 3, SHRs/Hos were fed the standard diet (control group of...
SHR) or a diet containing 3.9% (w/w) GABA-rich soy sauce (GABA-rich soy sauce group); the WKYs/Hos were fed the standard diet (control group of WKY). The basic nutrient composition of the diets was almost the same (crude protein: 23.7–24.5%, crude fat: 5.1–5.5%, crude fiber: 2.7–3.2%, crude ash: 6.2–6.3%, nitrogen-free extract: 45.3–53.8%), but the moisture and Na contents of the standard diet were lower than those of the other diets (moisture: 7.1% in the standard diet vs. 9.3–10.1% in the other diets; Na content: 0.19% in the standard diet vs. 0.28–0.30% in the other diets), and the K content of the diet containing 3.9% K-rich soy sauce was higher than that of the other diets (0.91% in the diet containing K-rich soy sauce vs. 0.84–0.86% in the other diets). Food and water were allowed ad libitum to the animals. In all of the chronic administration experiments, body weight, food intake, and water intake were measured, and the SBP and HR were measured once a week.

In expt. 1, for investigation of the hypotensive effect of GABA-rich soy sauce, the rats were anesthetized with urethane at the end of the administration, and the RSNA was measured; then blood was drawn from the abdominal aorta into a tube and a heparinized tube. Serum and urinary samples were collected as described above. Serum was separated by centrifugation at 1,000 g for 80 min at 4 °C, and the samples were stored at −80 °C. The plasma renin activity and aldosterone concentration were measured by direct radioimmunoassay.9) The plasma norepinephrine concentrations were measured by high-performance liquid chromatography (HPLC).24) Blood urea nitrogen (BUN) was determined using commercial reagents (BUN kainos obtained from Kainos Laboratories, Tokyo). The heart, thoracic aorta, and kidney of the animals were fixed in 10% buffered formalin solution. Cross-sections of the coronary arteries from the lower regions of the left ventricle and thoracic aorta were prepared, and the kidneys were cut vertically, followed by staining of 2-μm-thick sections with Miller’s elastic and van Gieson’s staining.

In expt. 2, for investigation of the inhibitory effect on Na retention of GABA-rich soy sauce, the rats were housed individually in metabolic cages throughout the 6-week period of administration of each diet. The body weight, food, and water intakes, and urinary volume were measured for 42 consecutive d. Twenty-four-h urinary samples were collected and stored at −20 °C. The rats were anesthetized with sodium pentobarbital at the end of the administration period, and blood samples were collected as described above. Serum was separated and the samples were stored at −80 °C. Na and K in the serum and urinary samples were measured with an electrolyte analyzer (AHS/Japan Corporation, Tokyo) using a hydrogen electrode.

In expt. 3, for investigation of the influence of GABA-rich soy sauce on hypothalamic GABA content, the rats were sacrificed by decapitation at the end of the 6-week period of administration of the diet, followed by rapid removal of the brains and isolation of the hypothalamus, according to the method of Balcom et al.25) The specimens of the hypothalamus were immediately weighed and stored at −80 °C. The GABA content of the hypothalamus was determined by HPLC using the ninhydrin reaction of the amino acid.26)

RSNA. In expt. 1, the RSNA in the rats was measured as described by Shokoji et al.27) The renal sympathetic nerve branch of the left kidney was isolated near the aortic renal arterial junction, and a Teflon-coated stainless steel bipolar electrode was placed on it. A stabilization period of 20 min was allowed after the placement of the nerve electrodes. Measurement of the RSNA was made for 5 min. The renal nerve discharge was amplified using a differential amplifier (Nihon Kohden, Tsukuba, Japan) with a band-pass filter. The output from the amplifier was counted in spikes/s, and the recorded RSNA was integrated using PowerLab Chart 5. The spikes/s of the RSNA and the integrated RSNA values were expressed as percentages relative to the values (100%) for the control group.

Sodium and potassium balance. In expt. 2, the daily Na intake and daily K intake were calculated as the product of the 24-h food intake and the Na or K concentration respectively of the food. The daily urinary Na and daily K excretion were also calculated as the product of the 24-h urinary volume and the urinary concentration of Na or K respectively. The daily Na and daily K balance were calculated by subtracting the daily urinary Na and K excretion from the daily Na and K intake respectively, as described by Zhou et al.28)

Morphometric analysis. In expt. 1, all the available intramyocardial coronary arterioles (n = 1–2/rat) from the left ventricle, the thoracic aorta (n = 1/rat), and the interlobular artery (n = 2–3/rat) were analyzed using an Olympus SP 500F image analyzer (Olympus Optical, Tokyo). The outer and inner medial circumferences of these vessels were traced, and the thicknesses of the media, luminal cross-sectional areas, and media:lumen ratios were calculated as described by Ibrahim et al.29)

Statistical analysis. One-way ANOVA followed by Tukey’s test was used to evaluate the significance of differences among the groups.

Results

Hypotensive effect of a single oral administration of GABA-rich soy sauce on SHRs

The effects of a single oral administration of GABA-rich soy sauce or GABA on SBP in the SHRs are shown in Fig. 1. SBP was significantly lower than in the controls (distilled water) at 8 h after administration of GABA-rich soy sauce at a dose of 0.033 ml/kg body wt, and was also significantly lower than that in the controls at 1, 6, and 8 h after administration of the soy sauce at
a dose of 0.33 ml/kg body wt. Furthermore, SBP was significantly lower than that in the controls at 8 h after administration of GABA alone at a dose of 0.33 mg/kg body wt, and also significantly lower than that in the control group at 3, 6, and 8 h after administration of GABA alone at a dose of 3.3 mg/kg body wt. At 8 h after administration of GABA-rich soy sauce at doses of 0.033 ml/kg body wt and 0.33 ml/kg body wt (equivalent to GABA doses of 0.33 mg/kg body wt and 3.3 mg/kg body wt respectively), SBP decreased significantly, by 25.2±8.0 mmHg (P < 0.01) and 21.3±7.2 mmHg (P < 0.01) respectively, as compared with the control group. SBP returned to the baseline at 24 h after administration. SBP also decreased, significantly by 14.5±2.4 mmHg (P < 0.05) and 24.0±5.6 mmHg (P < 0.01) respectively, at 8 h after administration of GABA alone at doses of 0.33 mg/kg body wt and 3.3 mg/kg body wt. There were no significant differences in SBP between SHRs administered GABA-rich soy sauce at 0.033 and 0.33 ml/kg body wt and SHRs administered the corresponding doses of GABA alone (0.33 and 3.33 mg/kg body wt respectively) at any time-point time of measurement. In contrast, no changes in blood pressure were observed in the SHRs administered soy sauce (0.33 ml/kg body wt) or K-rich soy sauce (0.33 ml/kg body wt). There were no significant changes in HR in any of the groups (data not shown).

Mean intakes of soy sauce, K-rich soy sauce, and GABA-rich soy sauce (expts. 1–3)
The mean body weights and food intakes were almost the same among the animal groups in expts. 1–3 (data not shown). In expt. 1, the mean intakes of soy sauce, K-rich soy sauce, and GABA-rich soy sauce were 2.94 ml, 2.87 ml, and 2.87 ml/kg body wt/d. The respective intakes were almost the same in the animals in expts. 2 and 3 (2.87-3.27 ml/kg body wt/d). Also, the mean GABA intakes in the GABA-rich soy sauce group were almost the same in expts. 1–3 (28.7–32.7 mg/kg body wt/d).

Hypotensive effect and inhibitory effects of chronic oral administration of GABA-rich soy sauce in SHRs on RSNA and vascular hypertrophy (expt. 1)
The time-course of changes in SBP during the administration period is shown in Fig. 2. SBP increased
gradually with age in the control group. The SBP in the salt group tended to be higher than that in the control group at the 1st, 2nd, 5th, and 6th weeks, and that in the soy sauce group also tended to be higher than that in the control group at the 5th and 6th weeks. SBP in the GABA-rich soy sauce group was significantly lower than in the salt group and the soy sauce groups at the 4th and 5th weeks ($P < 0.05$), and maintained lower values at the 3rd and 6th weeks. SBP in the K-rich soy sauce group tended to be lower than the values in the salt and soy sauce groups at the 5th and 6th weeks, although the differences were not significant. SBP in the GABA-rich soy sauce group was lower than in the soy sauce group or the K-rich soy sauce group from the 3rd to the 6th week. A hypotensive effect of GABA-rich soy sauce was also observed in expts. 2 and 3 (data not shown).

BUN, plasma renin activity, plasma aldosterone concentrations, and plasma norepinephrine concentrations were almost the same among the five groups in expt. 1 (data not shown).

Table 1. Renal Sympathetic Nerve Activity (RSNA) Expressed in Percentage Changes from the Activity of the Control Group

<table>
<thead>
<tr>
<th>Group</th>
<th>RSNA (%)</th>
<th>Spikes/s</th>
<th>Integrated RSNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td>100 ± 33</td>
<td>100 ± 8$^{ab}$</td>
</tr>
<tr>
<td>Salt group</td>
<td></td>
<td>115 ± 37</td>
<td>122 ± 29$^{b}$</td>
</tr>
<tr>
<td>Soy sauce group</td>
<td></td>
<td>123 ± 31</td>
<td>126 ± 19$^{b}$</td>
</tr>
<tr>
<td>GABA-rich soy sauce group</td>
<td></td>
<td>100 ± 33</td>
<td>80 ± 11$^{a}$</td>
</tr>
<tr>
<td>K-rich soy sauce group</td>
<td></td>
<td>86 ± 42</td>
<td>93 ± 13$^{ab}$</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D., $n = 7$/group. Values in a column without a common letter differ, $P < 0.05$. Spikes/s of RSNA and integrated RSNA values are expressed as percentages relative to the values (100%) for the control group.

Inhibitory effect of chronic oral administration of GABA-rich soy sauce on Na retention in SHRs (expt. 2)

As shown in Table 2, water intake and urinary volume in the K-rich soy sauce and GABA-rich soy sauce groups were greater than in the other groups. Although the Na intake and urinary Na output in the salt, soy sauce, K-rich soy sauce, and GABA-rich soy sauce groups were greater than in the control group, the urinary Na output was highest in the GABA-rich soy sauce group among all the groups (Table 2). The positive sodium balance in the salt, soy sauce, K-rich soy sauce, and GABA-rich soy sauce groups was more pronounced than that in the control group, whereas the sodium balance tended to be less positive in the K-rich soy sauce and GABA-rich soy sauce groups than in the salt and soy sauce groups (Table 2), indicating that soy sauce containing K or GABA enhanced sodium excretion and reduced sodium retention in the SHRs. K intake and urinary K output were greater and the K balance more pronounced in the K-rich soy sauce group than in the other groups, although the differences were not
Discussion

GABA content in SHRs (expt. 3) were more pronounced in the GABA-rich soy sauce group. These inhibitory effects on vascular hypertrophy were observed in the control group (Table 3). The parameters tended to be higher than those in the salt and soy sauce groups. The GABA-rich soy sauce attenuated salt-induced acceleration of hypertension, and furthermore, our studies showed that less-sodium soy sauce containing GABA (GABA-rich soy sauce) containing 8.4 g/100 ml of NaCl and 1.0 g/100 ml of GABA was used.

In our chronic experiments (expts. 1 and 2), the diet containing GABA-rich soy sauce attenuated salt-induced acceleration of hypertension, and furthermore, attenuated the rise in blood pressure normally observed with increasing age in SHRs (Fig. 2). We confirmed that a diet containing regular soy sauce with added GABA, which was produced by the addition of GABA to regular soy sauce, attenuated salt-induced hypertension in SHRs (data not shown). The diet containing GABA-rich soy sauce also attenuated the salt-induced rise in RSNA and restored the positive Na balance in the salt-fed SHRs (Fig. 3, Tables 1 and 2). The urinary Na output tended to increase in the SHR fed a diet containing GABA-rich soy sauce (Table 2). The hypotensive effect and RSNA-inhibitory effect of GABA-rich soy sauce were stronger than those of K-rich soy sauce (Fig. 2, Table 1). Increased RSNA is known to be a factor capable of decreasing renal excretory function. 

Inhibitory effect of chronic oral administration of GABA-rich soy sauce on vascular hypertrophy in SHRs (expt. 1)

The cross-sectional area of the media and the media:lumen cross-sectional ratios of the thoracic aorta and the coronary and renal interlobular arteries in the salt and soy sauce groups tended to be higher than those in the control group (Table 3). The parameters tended to decrease in the GABA-rich soy sauce group as compared with the values in the salt group or the soy sauce group. These inhibitory effects on vascular hypertrophy were more pronounced in the GABA-rich soy sauce group than in the K-rich soy sauce group (Table 3).

Influence of GABA-rich soy sauce on hypothalamic GABA content in SHRs (expt. 3)

The GABA content in the hypothalamus of the control group of SHRs (3.12 ± 0.30 μmol/g) tended to be lower than that in the WKY group (3.84 ± 0.43 μmol/g). In SHRs, GABA content in the hypothalamus of the GABA-rich soy sauce group (3.11 ± 0.54 μmol/g) was almost the same as that in the control group.

Discussion

GABA is known as a dietary factor that exhibits antihypertensive effect, but the mechanism underlying the antihypertensive effect of GABA observed after oral administration of the agent has not been fully elucidated. In this connection, we developed a procedure for the preparation of GABA-rich soy sauce by fermentation with lactic acid bacteria. Regular soy sauces contain about 16.0–16.6 g/100 ml of NaCl and no GABA. In our studies, less-sodium soy sauce containing GABA (GABA-rich soy sauce) containing 8.4 g/100 ml of NaCl and 1.0 g/100 ml of GABA was used.

In our chronic experiments (expts. 1 and 2), the diet containing GABA-rich soy sauce attenuated salt-induced acceleration of hypertension, and furthermore, attenuated the rise in blood pressure normally observed with increasing age in SHRs (Fig. 2). We confirmed that a diet containing regular soy sauce with added GABA, which was produced by the addition of GABA to regular soy sauce, attenuated salt-induced acceleration of hypertension in SHRs (data not shown). The diet containing GABA-rich soy sauce also attenuated the salt-induced rise in RSNA and restored the positive Na balance in the salt-fed SHRs (Fig. 3, Tables 1 and 2). The urinary Na output tended to increase in the SHR fed a diet containing GABA-rich soy sauce (Table 2). The hypotensive effect and RSNA-inhibitory effect of GABA-rich soy sauce were stronger than those of K-rich soy sauce (Fig. 2, Table 1). Increased RSNA is known to be a factor capable of decreasing renal excretory function. 

The increased RSNA induced an increase in renal tubular sodium reabsorption, leading to renal sodium retention, and decreased renal blood flow and the glomerular filtration rate with renal vasoconstriction and increased renal vascular resistance. On the other hand, Hayakawa et al. reported that chronic oral administration of GABA significantly decreased blood pressure in sham-operated SHRs, but not in renal-sympathetic-denervated SHRs.22) Fujimura et al. reported

### Table 2. Water Intake, Urinary Volume, Na Intake, Na Output, and Na Balance of SHR Fed Diet with GABA-Rich Soy Sauce for 6 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Water intake (ml/100 g body wt/d)</th>
<th>Urinary volume (ml/100 g body wt/d)</th>
<th>Na intake (mEq/100 g body wt/d)</th>
<th>Urinary Na output (mEq/100 g body wt/d)</th>
<th>Na balance (mEq/100 g body wt/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>9.85 ± 2.09</td>
<td>2.91 ± 0.23</td>
<td>0.587 ± 0.014</td>
<td>0.332 ± 0.024</td>
<td>0.255 ± 0.030</td>
</tr>
<tr>
<td>Salt group</td>
<td>10.54 ± 1.99</td>
<td>3.44 ± 0.52</td>
<td>0.966 ± 0.030</td>
<td>0.683 ± 0.030</td>
<td>0.283 ± 0.043</td>
</tr>
<tr>
<td>Soy sauce group</td>
<td>10.83 ± 1.85</td>
<td>3.34 ± 0.12</td>
<td>0.962 ± 0.037</td>
<td>0.679 ± 0.017</td>
<td>0.283 ± 0.045</td>
</tr>
<tr>
<td>GABA-rich soy sauce group</td>
<td>11.90 ± 1.02</td>
<td>3.91 ± 0.60</td>
<td>0.966 ± 0.023</td>
<td>0.697 ± 0.030</td>
<td>0.269 ± 0.04</td>
</tr>
<tr>
<td>K-rich soy sauce group</td>
<td>12.55 ± 4.32</td>
<td>3.86 ± 0.96</td>
<td>0.909 ± 0.032</td>
<td>0.670 ± 0.056</td>
<td>0.256 ± 0.055</td>
</tr>
</tbody>
</table>

Each value is mean ± S.D., n = 6/group. Values in a column without a common letter differ, P < 0.05.

### Table 3. Inhibitory Effect of Vascular Hypertrophy of GABA-Rich Soy Sauce in SHRs

<table>
<thead>
<tr>
<th></th>
<th>Thoracic aorta</th>
<th>Media:lumen cross-sectional ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Thoracic aorta</td>
<td>Media:lumen</td>
</tr>
<tr>
<td></td>
<td>Imca</td>
<td>Renal interlobular artery</td>
</tr>
<tr>
<td>Control group</td>
<td>1.060 ± 0.100</td>
<td>0.0122 ± 0.0041</td>
</tr>
<tr>
<td>Salt group</td>
<td>1.194 ± 0.115</td>
<td>0.0162 ± 0.0088</td>
</tr>
<tr>
<td>Soy sauce group</td>
<td>1.215 ± 0.185</td>
<td>0.0139 ± 0.0038</td>
</tr>
<tr>
<td>GABA-rich soy sauce group</td>
<td>1.063 ± 0.234</td>
<td>0.0099 ± 0.0040</td>
</tr>
<tr>
<td>K-rich soy sauce group</td>
<td>1.147 ± 0.334</td>
<td>0.0111 ± 0.0055</td>
</tr>
</tbody>
</table>

Imca, intramyocardial coronary arteriole. Each value is mean ± S.D., n = 7/group in thoracic aorta, n = 10–11/group in imca, and n = 17–18/group in renal interlobular artery.
that GABA attenuated electrical renal nerve stimulation-induced increase in renal perfusion pressure and renal noradrenaline efflux in isolated perfused rat kidney.30 These findings taken together suggest that inhibition of Na retention by natriuresis, as a result of inhibition of RSNA by GABA present in soy sauce, contributed to the antihypertensive effect of GABA in SHRs.

In our studies, the long-term hypotensive effect of GABA-rich soy sauce was weaker than its short-term hypotensive effect (Fig. 1 and 2). Three mechanisms may be proposed to account for this difference in effect. First, the mechanisms underlying the short-term and the long-term hypotensive effects of GABA might differ. Hayakawa et al. reported that the short-term hypotensive effect of GABA might be due to arterial vasodilation resulting from inhibition of peripheral sympathetic nerve activity by the agent, and that its long-term hypotensive effect might be mediated by its inhibition of renal sympathetic nerve activity in SHRs.22) Secondly, the effect of GABA-rich soy sauce might become weaker with repeated administration. Abe et al. reported that the hypotensive effect of GABA gradually became weaker when it was given to SHRs three times by intravenous administration.31) Thirdly, the absorption and plasma level of GABA might be lower in SHRs receiving chronic administration of a diet containing GABA-rich soy sauce as compared with that in SHR direct single oral administration of the soy sauce. Further investigation is necessary to determine the mechanism underlying this difference in effect.

The Na balance was less positive and the urinary Na excretion was greater in the GABA-rich soy sauce group than in the salt and soy sauce groups, although the differences among the groups were not statistically significant (Table 2). Boer et al. reported that urinary sodium excretion in SHRs was slightly lower than that in the WKYs, but that even slight sodium retention contributed to the development of hypertension in SHRs.32) K supplementation slightly decreased the Na space (extracellular fluid volume) in Na-loaded SHRs, and that even this slight change might contribute to the antihypertensive effect of K supplementation in Na-loaded SHRs.33) In our study, inhibition of sodium retention might have contributed to the antihypertensive effect in GABA-rich soy sauce-fed SHR.

The hypothalamus may be an important region of the central nervous system for blood pressure control. It contains high concentrations of GABA and its synthetic enzyme, and controls sympathetic outflow.34) Intracerebroventricular injection of GABA (50–200 μg) reduced sympathetic nervous activity, blood pressure, and HR in SHRs, and these results suggest that by depressing hypothalamic function, centrally injected GABA depressed hypothalamic function, decreasing sympathetic nervous activity and thereby lowering blood pressure and HR in SHRs.35) Elevation of GABA concentration in the brain by inhibition of GABA transaminase resulted in a reduction in blood pressure.36) However, the hypothalamic GABA content in the GABA-rich soy sauce group was almost the same as that in the control group in our study. Since the blood-brain barrier is impermeable to GABA,37) it is speculated that the antihypertensive effects observed in GABA-rich soy sauce-fed SHRs were not due to centrally exerted effects, and were more likely to be due to the peripherally exerted effects of GABA.

In our study, RSNA in the GABA-rich soy sauce group was significantly attenuated as compared with that in the salt group or the soy sauce group, but the plasma norepinephrine concentrations were almost the same in these groups. The changes in adrenergic activity probably reflect changes in RSNA more accurately than those in plasma norepinephrine concentrations, because changes in plasma norepinephrine concentrations can also be affected by alterations in norepinephrine clearance.13,16,38)

In our studies, a single oral administration of K-rich soy sauce did not decrease SBP in SHRs, but chronic oral administration of K-rich soy sauce attenuated the salt-induced acceleration of hypertension in SHRs. These results, taken together with the results of other studies32,39) suggest that only chronic oral administration of K is associated with an antihypertensive effect.

The Na intake/d of SHRs in expt. 1 in our study was about 10 times as great as the mean estimated Na intake/d of the Japanese population in 2003. Although the SHRs were fed large amounts of Na, vascular hypertrophy was prevented in GABA-rich soy sauce group. This inhibitory effect on vascular hypertrophy and the antihypertensive effect in the GABA-rich soy sauce group were more pronounced than those in the K-rich soy sauce group. This inhibitory effect on vascular hypertrophy might be related to the antihypertensive effect observed in the SHRs fed GABA-rich soy sauce. It has been suggested that vascular hypertrophy is associated with reduced vascular compliance and thus causes increased cardiovascular risk.40) Intake of less-sodium soy sauce containing GABA might help to reduce overall cardiovascular risk.

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References

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