JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY ARTICLE

J. Agric. Food Chem. 2010, 58, 615–619 615
DOI:10.1021/jf903008t

Antihypertensive Effect of a γ-Aminobutyric Acid Rich Tomato Cultivar 'DG03-9' in Spontaneously Hypertensive Rats

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This study aimed to investigate the effects of a γ-aminobutyric acid (GABA) rich tomato (*Solanum lycopersicum* L.) cultivar 'DG03-9' in comparison with 'Momotaro', a commonly consumed tomato cultivar in Japan, on systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR). In a single administration study, treatment with the GABA-rich cultivar elicited a significant decrease in SBP compared to the control group. In a chronic administration study, SHR were fed diets containing one of the tomato cultivars for 4 weeks. Both cultivars significantly reduced the increase in SBP compared to the control. The antihypertensive effect of the GABA-rich cultivar was higher than that of the commonly consumed cultivar in both the single- and chronic-administration studies. Treatment with a comparable amount of GABA elicited a similar response to treatment with the GABA-rich cultivar. These results suggest that the GABA-rich cultivar 'DG03-9' is a potent antihypertensive food and may be useful for treating hypertension effectively.

KEYWORDS: γ-Aminobutyric acid; tomato; hypertension; spontaneously hypertensive rats

INTRODUCTION

Hypertension, which is one of the most common lifestyle-related diseases, has become a significant problem especially in developed countries in recent years. Epidemiological studies show that both arteriosclerosis and essential hypertension rank among the most common causes of cerebrovascular, cardiac, and renal pathology. Presently, much effort is being invested in the detection of bioactive components in foods for the treatment and prevention of hypertension (*1*).

γ-Aminobutyric acid (GABA) is a depressive neurotransmitter in the sympathetic nervous system (*2*) and also has physiological functions to depress the elevation of systolic blood pressure (SBP) (*3*), improve discrimination learning (*4*), and induce mental relaxation (*5*). GABA is common in various foods including vegetables, fruits, and yogurt. Feeding with GABA-enriched foods such as green tea (*6*), fermented milk products (*7*), and soy sauce (*8*) has been reported to depress the elevation of SBP in spontaneously hypertensive rats (SHR). Hayakawa and co-workers (*7*) showed GABA and fermented milk products significantly decreased SBP from 4 to 8 h after single oral administration, and the antihypertensive effect of GABA was dose-dependent in SHR. The antihypertensive effect of GABA has also been reported in humans (*9*).

Many studies have shown that the consumption of tomato (*Solanum lycopersicum* L.) and tomato-based foods reduce the risk of chronic diseases including cancer and cardiovascular disease (*10*, *11*). It is generally assumed that lycopene in tomatoes accounts for their beneficial effects (*12*, *13*). Although the effect of lycopene is biologically relevant, tomatoes are also an excellent source of nutrients including potassium, vitamin C, and various carotenoids and phytochemicals such as polyphenols. Additionally, tomato contains significant quantities of GABA. 'DG03-9' has been recently identified as a GABA-rich tomato cultivar (*14*), and the biochemical mechanisms of higher GABA accumulation in this cultivar have been elucidated (*15*). In this study, we report the effect of the GABA-rich tomato cultivar 'DG03-9' on blood pressure in SHR.

MATERIALS AND METHODS

Amino Acids Assay. We used two cultivars of tomato, 'DG03-9', a GABA-rich tomato cultivar, and 'Momotaro', a commonly consumed fresh tomato cultivar in Japan. Both cultivars were grown by the University of Tsukuba (Tsukuba, Japan). Tomato fruits at the ripe stage were used for the present experiments. They were lyophilized using a BFED-500 freeze-dryer (Nihon Freezer Co., Tokyo, Japan) and pulverized into fine powder using a laboratory blender. These powders were stored at 4 °C until use. We analyzed the concentrations of GABA and other amino acids using a Hitachi L8500 amino acid analyzer (Hitachi Co., Tokyo, Japan). Amino acid composition was determined for every lot of tomatoes used in this study.

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Published on Web 11/09/2009

Table 1. Composition of the Experimental Diets (%)

dietary group	control (AIN-93G)	common tomato 1.6%	common tomato 8.0%	GABA-rich tomato 1.6%	GABA-rich tomato 8.0%
casein	20.0	20.0	20.0	20.0	20.0
L-cystine	0.3	0.3	0.3	0.3	0.3
cornstarch	39.75	39.66	39.29	39.54	38.73
α-cornstarch	13.2	13.2	13.2	13.2	13.2
sucrose	10.0	10.0	10.0	10.0	10.0
soy oil	7.0	7.0	7.0	7.0	7.0
cellulose	5.0	5.0	5.0	5.0	5.0
mineral mixture (AIN93G)	3.5	3.5	3.5	3.5	3.5
vitamin mixture (AIN93)	1.0	1.0	1.0	1.0	1.0
choline bitartrate	0.25	0.25	0.25	0.25	0.25
tert-butylhydroquinone	0.0014	0.0014	0.0014	0.0014	0.0014
'Momotaro' tomato dried powder		0.093	0.464		
'DG03-9' tomato dried powder				0.205	1.024

Animals. Male SHR/Izumo strain rats (4 or 14 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). They were caged individually in a room under the following conditions: temperature 23 ± 3 °C, humidity 55 ± 15%, a ventilation frequency of at least 12 times per h by an all-fresh-air system, and lighting for 12 h (lights on at 6:00). For the single administration study, 14-week-old rats were used; 4-week-old rats were used for the chronic administration study. All rats were raised on a AIN-93G refined diet (Oriental Yeast Co., Kanagawa, Japan), with water ad libitum. They were used for the experiments after a 1-week quarantine period. All animal procedures were performed according to the Basic Guidelines for the Use of Experimental Animals in Institutions under the Jurisdiction of Japanese Ministry of Health, Labour and Welfare.

Blood Pressure and Heart Rate Measurements. SBP, diastolic blood pressure (DBP), and heart rate were measured in unanesthetized animals using the tail-cuff method with a BP-98A automated noninvasive blood-pressure meter (Softron Co., Tokyo, Japan). Each of these parameters was measured three times in each animal, and the mean of the three readings was regarded as the value of each parameter in each animal. To raise the body temperature of the animals, the animals were placed in a heated box (38 °C) for about 5 min before measurement. Blood pressure was measured in a soundproof booth.

Single Administration Study. Study 1: Male SHR (15 weeks old, 313−349 g) were used for the single administration study. They were divided into 5 groups of 8 rats: control, 2 g/kg (fresh tomato weight/body weight) common tomato, 10 g/kg common tomato, 2 g/kg GABA-rich tomato, and 10 g/kg GABA-rich tomato. These samples were dissolved in 0.5% methyl cellulose (Shin-etsu Chemical Co., Tokyo, Japan) and given orally (5 mL/kg body weight) to the animals. Blood pressure and heart rate were measured before administration of the samples and at 2, 4, 6, 8, and 24 h after administration. Study 2: Male SHR (15 weeks old, 330−369 g) were used for the single administration study. They were divided into 3 groups of 10 rats: control, 10 g/kg (fresh tomato weight/body weight, containing 17.9 mg/kg of GABA) GABA-rich tomato, and 17.9 mg/kg GABA (Kyowa Hakko Bio Co., Tokyo, Japan). The sample administration and measurement of blood pressure and heart rate were conducted as described above.

Chronic Administration Study. Male SHR (5 weeks old, 104−151 g) were used for the chronic administration experiment. They were divided into the following 5 groups of 10 rats: (1) control group fed an AIN-93G refined diet, (2) 1.6% common tomato group fed a diet containing 1.6% (fresh weight) common tomato, (3) 8% common tomato group fed a diet containing 8% common tomato, (4) 1.6% GABA-rich tomato group fed a diet containing 1.6% GABA-rich tomato, and (5) 8% GABA-rich tomato group fed a diet containing 8% GABA-rich tomato. The composition of the AIN-93G-based experimental diets is shown in **Table 1**. The animals were allowed free access to the diets and drinking water for 4 weeks. Food and water intake was recorded once a week. Body weight, blood pressure, and heart rate were measured once a week at a consistent time of day.

Statistical Analysis. Each value is expressed as the mean and its standard error. Changes from baseline values to postadministration values were analyzed by Dunnett's test. Two-way ANOVA was used for comparisons among the various groups. Where the variance ratio (F) was significant among groups, mean values of blood pressure or other parameters were compared by Dunnett's test. All statistical analyses were 2-tailed, and statistical significance was established at $p < 0.05$. Statistical analysis was performed by Student's paired t-test for comparisons of amino acid concentrations between the two tomato cultivars. For the comparisons of body weight and food intake of rats in chronic study, statistical analysis was performed by the Kruskal−Wallis test and the Mann−Whitney test with modified significance level. Statistical analyses were performed using SPSS Systems (SPSS Inc., Chicago, IL).

Table 2. Amino Acid Concentration[a] in Two Cultivars of Tomato (mg/100 g Fresh Weight)

	common tomato	GABA-rich tomato
γ-aminobutyric acid	15.8 ± 1.0	179.4 ± 33.8*
aspartic acid	17.9 ± 1.1	26.3 ± 6.5*
threonine	26.2 ± 4.2	66.7 ± 4.4*
serine	1.0 ± 1.0	16.7 ± 0.9*
glutamic acid	136.4 ± 8.2	150.1 ± 34.2
glycine	0.3 ± 0.1	2.6 ± 0.4*
alanine	4.7 ± 0.6	15.3 ± 3.9*
cystine	ND[b]	ND
valine	0.7 ± 0.1	7.5 ± 2.2*
methionine	0.4 ± 0.1	1.7 ± 0.2*
isoleucine	0.4 ± 0.1	7.4 ± 1.3*
leucine	0.7 ± 0.2	5.6 ± 0.6*
tyrosine	ND	3.0 ± 0.7
phenylalanine	1.1 ± 0.1	16.6 ± 1.3*
ornithine	ND	0.1 ± 0.1
lysine	1.7 ± 0.2	10.3 ± 1.0*
histidine	1.6 ± 0.1	6.9 ± 0.9*
arginine	1.0 ± 0.3	6.1 ± 1.4*
proline	77.2 ± 2.7	32.6 ± 11.7

[a] Values are given as means ± SEM. Mean values were significantly different between the two cultivars: *$p < 0.05$. [b] ND: not detected.

RESULTS AND DISUCUSSION

Acute Antihypertensive Effects of Tomato Powder in SHR. We investigated whether GABA-rich tomato cultivar has an antihypertensive effect after oral administration to SHR. The content of amino acids in the tomato cultivars used in this study is shown in **Table 2**. The concentration of GABA in the GABA-rich tomato cultivar is about 10 times higher than that in the common tomato cultivar. The effects of the both cultivars on SBP are shown in **Figure 1A**. In the control group, SBP significantly declined time-dependently from 2 h to 8 h after administration and returned to nearly baseline by 24 h after administration. The mean value of SBP at 8 h after administration was 11 mmHg lower than the pretreatment ($p < 0.01$).

Both GABA-rich and common tomato cultivars decreased SBP dose-dependently. Treatment with 10 g/kg GABA-rich tomato cultivar (containing 17.9 mg/kg GABA) had a significant antihypertensive effect at 4 h, 6 h, and 8 h after administration compared to the control group ($p < 0.01$). Treatment with 2 g/kg GABA-rich tomato cultivar (containing 3.6 mg/kg GABA) also had a significant antihypertensive effect at 6 h and 8 h after administration compared to the control group ($p < 0.01$). Both dosages of the common tomato cultivar (2 g/kg common tomato containing 0.3 mg/kg GABA, 10 g/kg containing 1.6 mg/kg GABA) showed a tendency to decrease on SBP, but it was not significant. In each group, the maximal decrease in SBP occurred at 8 h after administration. SBP (mmHg) value at 8 h after administration were 180.9 ± 0.5 for the control group, 176.9 ± 3.4 for the 2 g/kg common tomato group, 170.5 ± 3.9 for the 10 g/kg common tomato group, 167.0 ± 3.5 for the 2 g/kg GABA-rich

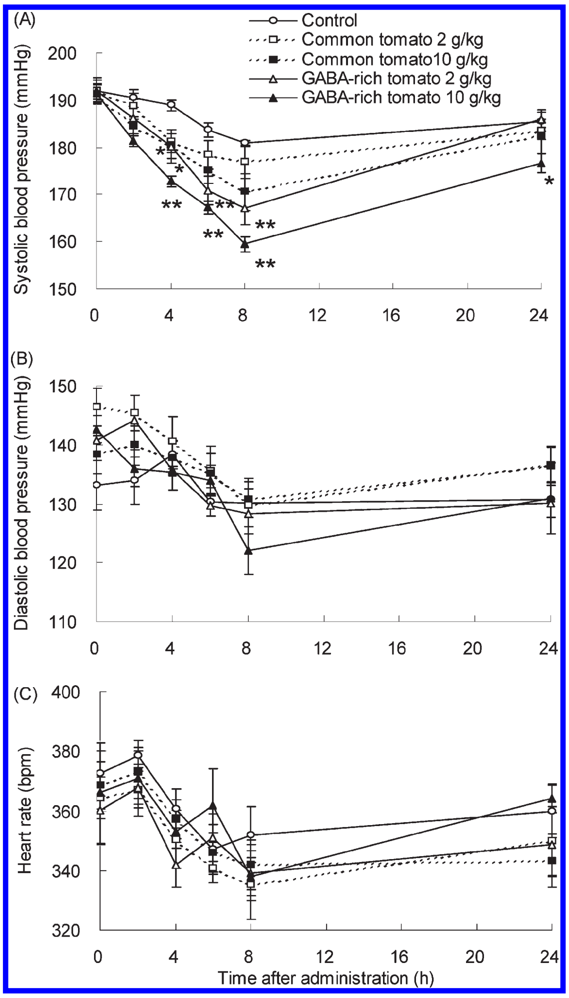

Figure 1. Effects of single oral administration of GABA-rich tomato cultivar and common tomato cultivar on systolic blood pressure (**A**), diastolic blood pressure (**B**), and heart rate (**C**) in SHR. Each point represents the mean ± SEM (n = 8). Mean values were significantly different from the control value at the same time point: *$p < 0.05$, **$p < 0.01$.

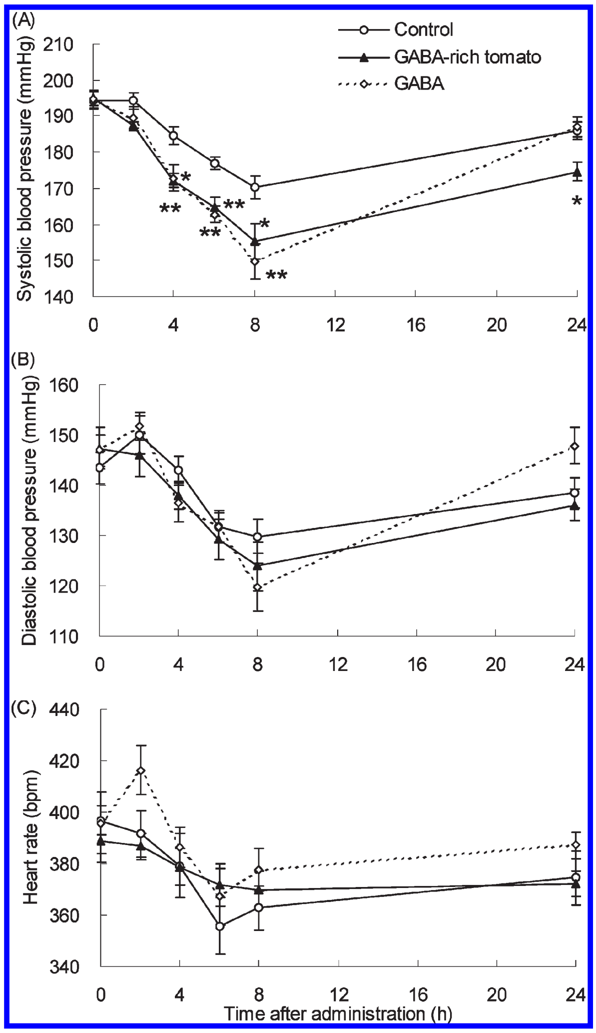

Figure 2. Effects of single oral administration of GABA-rich tomato cultivar and GABA on systolic blood pressure (**A**), diastolic blood pressure (**B**), and heart rate (**C**) in SHR. Each point represents the mean ± SEM (n = 10). Mean values were significantly different from the control value at the same time point: *$p < 0.05$, **$p < 0.01$.

tomato group ($p < 0.01$), and 159.5 ± 1.7 for the 10 g/kg GABA-rich tomato group ($p < 0.01$).

Figure 1B shows the results of DBP measurement. We divided the SHR according to the initial value of SBP and weight. The initial values of DBP and heart rate were not same among the 5 groups, but there were no significant differences among the groups. In the control group, the mean value of DBP showed no significant change over the 24 h test period. In the 2 g/kg common tomato group, a significant decrease in DBP occurred at 6 h, 8 h, and 24 h after administration compared to the pretreatment value ($p < 0.01$). In the 10 g/kg GABA-rich tomato group, a significant decrease in DBP was observed at 6 and 8 h after administration compared to the pretreatment ($p < 0.01$). There was no significant difference in DBP across the 5 groups, and there were no significant changes in heart rate across or within the groups (**Figure 1C**).

In the single administration study, the GABA-rich tomato cultivar had a dose-dependent antihypertensive effect on SBP. Similarly, the common tomato cultivar showed a tendency to decrease SBP, but it was not significant.

Contribution of GABA to the Antihypertensive Effect of the GABA-Rich Tomato. To confirm the antihypertensive effect caused by GABA, we compared the antihypertensive effect of the GABA-rich tomato cultivar and the equivalent amount of a purified GABA upon single administration to SHR. The effects of the GABA-rich tomato cultivar and GABA alone on SBP are shown in **Figure 2A**. In the control group, the SBP significantly declined time-dependently over the first 8 h after administration, and then began to increase back to the preadministration level.

Compared to the control group, the GABA-rich tomato group showed significant antihypertensive effect at 4 h, 6 h, 8 h, and 24 h after administration ($p < 0.05$), and GABA also showed a significant antihypertensive effect at 4 h, 6 h, and 8 h after administration ($p < 0.05$). Each group showed the maximal decrease in SBP at 8 h after administration. SBP (mmHg) values at 8 h after administration were 170.2 ± 3.1 for the control group, 155.4 ± 4.9 for the GABA-rich tomato group, and 149.8 ± 4.9 for the GABA group. There were no significant changes in DBP (**Figure 2B**) or heart rate among the groups (**Figure 2C**).

The antihypertensive effect of the GABA-rich tomato and the same dosage of GABA were highly similar, suggesting that GABA is an important component of the antihypertensive effect of the GABA-rich tomato cultivar in this study.

Chronic Antihypertensive Effects of Tomato Powder in SHR. We then investigated the antihypertensive effect of GABA-rich tomato cultivar upon chronic administration. There were no significant differences in the initial values obtained for body

Table 3. Body Weight and Food Intake of Rats in Chronic Study[a]

	control	common tomato		GABA-rich tomato	
		1.6%	8.0%	1.6%	8.0%
initial body weight (g) (at 5 weeks of age)	123.8 ± 3.4	124.0 ± 2.3	123.0 ± 3.0	128.0 ± 2.4	127.0 ± 3.0
final body weight (g) (at 9 weeks of age)	269.9 ± 4.7	270.0 ± 4.3	275.0 ± 2.5	272.0 ± 3.2	281.0 ± 3.0
food intake (g/day)	17.7 ± 0.3	17.9 ± 0.3	18.3 ± 0.3	17.9 ± 0.3	18.1 ± 0.3
GABA intake (mg/day)	ND[b] a	0.12 ± 0.00 b	0.40 ± 0.01 c	0.47 ± 0.01 d	2.21 ± 0.04 e
water intake (mL/day)	27.3 ± 0.7	27.2 ± 0.8	28.2 ± 0.6	31.0 ± 0.8	28.0 ± 0.7

[a] Values are given as means ± SEM. GABA intake was calculated from the amount of food consumed. The different letters showed significant difference among the groups: $p < 0.05$. [b] ND: not detected.

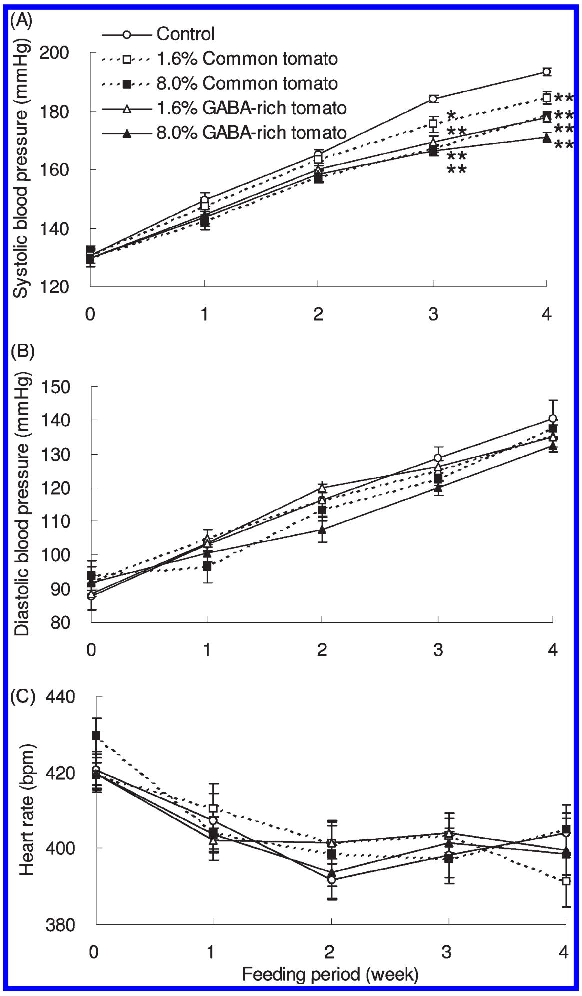

Figure 3. Effects of chronic administration of GABA-rich tomato cultivar and common tomato cultivar on systolic blood pressure (**A**), diastolic blood pressure (**B**), and heart rate (**C**) in SHR. Each point represents the mean ± SEM ($n = 10$). Mean values were significantly different from the control value at the same time point: *$p < 0.05$, **$p < 0.01$.

weight, blood pressure, or heart rate among the 5 groups of SHR. The rates of increase in body weight and food and water consumption were not significantly different across the groups (**Table 3**).

The time course of the change in SBP is shown in **Figure 3A**. In the control group, the SBP (mmHg) was 130.7 ± 2.8 before the start of feeding, and it increased gradually with age and reached 193.2 ± 1.2 after 4 weeks of test period ($p < 0.01$). Both the GABA-rich tomato and the common tomato groups showed significantly slower increases in SBP after 3 weeks of feeding on the test diets ($p < 0.05$), and these differences were maintained throughout the rest of the feeding period. After 4 weeks of feeding on the test diets, SBP values (mmHg) were 193.2 ± 1.2 in the control group, 184.5 ± 2.1 in the 1.6% common tomato group, 178.5 ± 1.3 in the 8% common tomato group, 177.6 ± 1.8 in the 1.6% GABA-rich tomato group, and 170.8 ± 1.7 in the 8% GABA-rich tomato group. Both the GABA-rich and the common tomato cultivars had dose-dependent antihypertensive effects on SBP. At the end of the experimental period, the mean SBP values of the two groups treated with GABA-rich tomato cultivar were significantly lower than the groups treated with the same doses of common tomato cultivar (1.6%, $p < 0.05$; 8%, $p < 0.01$). **Figure 3B** shows the results of measurement of DBP. In the control group, the mean value of DBP (mmHg) was 87.8 ± 4.0 before the start of feeding, but it increased to 140.4 ± 5.7 after 4 weeks of test period ($p < 0.01$). Compared to the control group, no significant change in DBP was observed in the other groups. There were also no significant changes in heart rate among all the tested groups (**Figure 3C**).

The average daily GABA intakes are shown in **Table 3**. The tomato diets showed the antihypertensive effect on SBP in a dose-dependent manner by GABA. It seemed that the concentration of GABA influences the antihypertensive effect of tomatoes.

In recent studies of various GABA-containing dietary supplements and other products, regular ingestion of GABA for several weeks yielded an antihypertensive effect in SHR (*6*, *16*). Though the largest GABA intake in this study (2.2 mg/rat daily in the 8% GABA-rich tomato diet) was less than that of GABA-rich tea (4 mg/rat daily) (*6*) or fermented milk (9 mg/rat daily) (*16*), the GABA-rich tomato cultivar had a significant effect in this study. The tomato is also a good source of potassium, fiber, vitamin C, carotenoids, and amino acids. Engelhard and co-workers (*17*) showed that tomato extract, containing carotenoids such as lycopene, β-carotene, and vitamin E, reduced blood pressure in patients with grade-1 hypertension. In observational studies, a significant inverse association between blood pressure and a vegetarian diet rich in fiber, magnesium, potassium, calcium, and protein has been reported (*18*). And several amino acids, such as tyrosine (*19*) and phenylalanine (*20*), showed antihypertensive effects. Therefore, other components of tomato, such as lycopene, potassium, fiber, and amino acids, might also be factors involved in the decrease in SBP observed in the chronic study.

Several mechanisms underlying the antihypertensive action of GABA in the peripheral vasculature have been postulated, including ganglionic blockade (*3*), activation of GABAergic receptors (*3*, *21*), direct action on vasculature (*22*), and inhibition of transmitter release from sympathetic nerve terminals (*23*, *24*). GABA has been shown to inhibit noradrenaline release from sympathetic nerve terminals within the mesenteric arterial bed of SHR (*25*). This effect may be mediated through the peripheral activation of $GABA_B$ receptors, because the resulting antihypertensive effect is reduced by preadministration of the $GABA_B$-receptor antagonist saclofen (*26*). In chronic dietary administration, GABA inhibited the development of hypertension in sham-operated SHR but not in renal sympathetic-denervated SHR (*16*), indicating that the antihypertensive effect of GABA is also influenced by its impact on renin release.

In this study, our present results show that the GABA-rich tomato cultivar, 'DG03-9', significantly reduced SBP in SHR. 'DG03-9' is one of GABA-rich tomato cultivar. In addition to the effort of the screening for genetic resources, we pursue the development of a new cultivation method and/or postharvest treatment to promote further GABA accumulation in 'DG03-9' fruit (*27*). Tomatoes are one of the most commonly consumed fresh and canned vegetables in the world. The frequency and the extent of consumption of GABA-rich tomato such as cultivar 'DG03-9' could be beneficial for people with essential hypertension. Further work is required to study the antihypertensive effect of GABA-rich tomato in clinical study.

ABBREVIATIONS USED

GABA, γ-aminobutyric acid; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; DBP, diastolic blood pressure; SEM, standard error of the mean.

ACKNOWLEDGMENT

We acknowledge the staff members of the Nihon Bioresearch Inc. for their technical assistance.

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Received for review August 26, 2009. Revised manuscript received October 20, 2009. Accepted October 23, 2009. This work was supported by the Research and Development Program for New Bioindustry Initiatives (BRAIN).