

Effect of a γ -aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar–Kyoto rats

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We investigated the blood-pressure-lowering effects of γ -aminobutyric acid (GABA) and a GABA-enriched fermented milk product (FMG) by low-dose oral administration to spontaneously hypertensive (SHR/Izm) and normotensive Wistar–Kyoto (WKY/Izm) rats. FMG was a non-fat fermented milk product produced by lactic acid bacteria, and the GABA contained in FMG was made from the protein of the milk during fermentation. A single oral dose of GABA or FMG (5 ml/kg; 0.5 mg GABA/kg) significantly ($P < 0.05$) decreased the blood pressure of SHR/Izm from 4 to 8 h after administration, but did not increase that of WKY/Izm rats. The hypotensive activity of GABA was dose-dependent from 0.05 to 5.00 mg/kg in SHR/Izm. During the chronic administration of experimental diets to SHR/Izm, a significantly slower increase in blood pressure with respect to the control group was observed at 1 or 2 weeks after the start of feeding with the GABA or FMG diet respectively ($P < 0.05$) and this difference was maintained throughout the period of feeding. The time profile of blood-pressure change due to administration of FMG was similar to that of GABA. FMG did not inhibit angiotensin 1-converting enzyme. Furthermore, an FMG peptide-containing fraction from reverse-phase chromatography lacked a hypotensive effect in SHR/Izm rats. The present results suggest that low-dose oral GABA has a hypotensive effect in SHR/Izm and that the hypotensive effect of FMG is due to GABA.

γ -Aminobutyric acid: Fermented milk: Blood pressure: Spontaneously hypertensive rat

High blood pressure is the leading risk factor for CVD, which remains one of the most important public health problems in the world (Guidelines Subcommittee of the World Health Organization–International Society of Hypertension, 1999; International Society of Hypertension Working Group, 2003). The development of hypertension is caused by several factors, including diet and lifestyle. It is well known that nutritional factors influence blood pressure; for example, excessive salt and alcohol intake may lead to an increase in blood pressure (Suter *et al.* 2002). Studies using the Dietary Approaches to Stop Hypertension (DASH) diet demonstrate that a diet rich in fruits, vegetables, low-fat dairy products, fibre and minerals (Ca, K and Mg) significantly lowers blood pressure and is effective as the first-line therapy (Moore *et al.* 2001; Whelton *et al.* 2002; Akita *et al.* 2003). Many studies have addressed the effect of minerals on blood pressure, but few have assessed the influence of a small quantity of an amino acid as a dietary supplement.

γ -Aminobutyric acid (GABA) is an amino acid that has long been reported to lower blood pressure by intravenous administration in experimental animals (Takahashi *et al.* 1955; Stanton, 1963; Lacerda *et al.* 2003) and in human subjects (Elliott & Hobbiger, 1959). However, the effect of dietary GABA has attracted little attention as a factor that may influence blood pressure, and it is not known whether low-dose oral administration of GABA affects blood pressure. GABA is present in many vegetables and fruits but not in dairy products. We developed a fermented low-fat milk product containing GABA (FMG) by using two types of lactic acid bacteria. Although GABA is not normally detectable in milk, it was made from the protein of the milk by the metabolic action of the lactic acid bacteria.

Our previous study showed that FMG had a blood-pressure-lowering effect in mildly hypertensive patients (Inoue *et al.* 2003). Although various fermented milk and cheese products have stronger hypotensive effects, owing to

Abbreviations: ACE, angiotensin I-converting enzyme; FMG, γ -aminobutyric acid-enriched fermented milk; GABA, γ -aminobutyric acid; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; WKY, Wistar–Kyoto.

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inhibition of angiotensin I-converting enzyme (ACE), it is not clear whether the effect of FMG depends on GABA or other components.

Therefore, in the present study we compared the effects of single-dose or chronic oral administration of GABA or FMG on the blood pressure of spontaneously hypertensive rats (SHR). We determined the ACE-inhibiting activity of FMG to clarify the hypotensive mechanism of this product; we then determined the minimum effective dose and the dose–response curve for the hypotensive action of GABA.

Materials and methods

Chemicals and reagents

GABA and other reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Preparation of fermented milk

FMG (Table 1) was produced by fermenting skimmed cows' milk with two starters, *Lactobacillus casei* strain Shirota and *Lactococcus lactis* YIT 2027 (Kimura *et al.* 2002a). The concentration of GABA in FMG was 1 mmol/l.

Animals and diets

SHR (SHR/Izm) and normotensive Wistar–Kyoto rats (WKY/Izm) (male, 14 weeks old, weighing 310–370 g

for the single oral administration experiment (Expt 1); 6 weeks old, weighing 145–160 g at the beginning of the chronic administration experiment (Expt 2)) were purchased from Japan SLC, Inc. (Shizuoka, Japan), the breeder for the Disease Model Cooperative Research Association (Kyoto, Japan). Izm is the original strain type of SHR established by Okamoto & Aoki (1963). The animals were divided into four groups of six animals each in Expt 1 and three groups of ten animals each in Expt 2. The diets were standard laboratory diet (MF diet; Oriental Yeast Co. Ltd, Tokyo, Japan) in Expt 1 and modified AIN-93G diet (Table 2; Oriental Yeast Co. Ltd) in Expt 2. All animals received tap water *ad libitum* and were housed under a 12 h light–dark cycle (lights on 08.30 to 20.30 hours). An automatic air conditioning system (Air Handling Unit TUC-244BH, Toyo Engineering Works Ltd, Tokyo, Japan) controlled the temperature at $24 \pm 2^\circ\text{C}$ and the humidity at $60 \pm 5\%$. Rats were acclimated to the housing conditions for at least 1 week before the start of the experiments. The present study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted by the Committees for Animal Experiments of the Yakult Central Institute.

Dosage

Expt 1. FMG (5 ml/kg body weight), a fraction containing the peptides from FMG (5 ml/kg), a suspension (60 ml/l) of skimmed milk powder (5 ml/kg body weight), GABA (0.05, 0.50 or 5.0 mg/kg body weight) or captopril (10 mg/kg body weight) were given orally to SHR/Izm and WKY/Izm rats (six per group) by stomach tube between 09.30 and 10.00 hours. GABA, skimmed milk powder and captopril were dissolved in physiological saline (9 g NaCl/l). The dose of captopril (a typical ACE inhibitor) as a positive control was chosen so as to have a similar magnitude of blood-pressure-lowering effect as the level of FMG found in SHR/Izm.

Table 1. Composition of γ -aminobutyric acid (GABA)-enriched fermented milk

Ingredient	Amount
Moisture (g/kg)	888
Protein (g/kg)	22
Fat (g/kg)	nd
Ash (g/kg)	5
Carbohydrate (g/kg)	81
Fibre (g/kg)	4
Na (mg/kg)	381
Ca (mg/kg)	742
Free amino acids (mg/kg)	
GABA	102
Asp	50
Thr	28
Ser	9
Glu	14
Pro	83
Gly	19
Ala	35
Cys	0
Val	29
Met	6
Ile	15
Leu	26
Tyr	4
Phe	9
Lys	30
His	11
Arg	6
Energy (kJ/kg)	1340

nd, not detected.

Table 2. Composition (g/kg) of the experimental diets

Diet...	Control	GABA	FMG
Maize starch	392	392	392
Casein	195	195	155
Dextrinized maize starch	132	132	132
Sucrose	100	100	40
Soyabean oil	70	70	70
Cellulose powder	50	50	50
Mineral mixture*	35	35	35
Vitamin mixture†	10	10	10
L-Cystine	3	3	3
Choline bitartrate	2.5	2.5	25
<i>tert</i> -Butylhydroquinone	0.014	0.014	0.014
NaCl	10	10	10
GABA	–	0.1	–
FMG‡	–	–	100

GABA, γ -aminobutyric acid; FMG, γ -aminobutyric acid-enriched fermented milk.

* AIN-93G-MX; Oriental Yeast Co., Tokyo, Japan.

† AIN-93G-VX; Oriental Yeast Co.

‡ Freeze-dried FMG (GABA-enriched fermented milk; FMG was modified so that carbohydrate and moisture were 30 and 939 g/kg respectively. The remaining composition was not altered).

Expt 2. The basal diet was modified AIN-93G containing NaCl (10 g/kg; Table 2). For 3 weeks, SHR/Izm rats were fed a basal diet (control, group 1), or a diet containing freeze-dried FMG (100 g/kg) (final concentration of GABA 0.1 g/kg, group 2) or GABA (0.1 g/kg, group 3). The animals were maintained on the basal diet before the experiment.

Measurement of blood pressure and heart rate

At 0, 4, 8, and 24 h after a single oral administration of the sample solutions, individual rats were placed in a constant-temperature box at 37°C for a few minutes, and then systolic blood pressure (SBP) and heart rate were measured by the tail-cuff method using a blood pressure analyser (BP-98A; Softron Co. Ltd, Tokyo, Japan) connected to a personal computer (Mebius series; Sharp Co., Tokyo, Japan). To assess the hypotensive effects of different doses of GABA alone, SBP and heart rate were measured 0 and 4 h after a single administration. During chronic administration (Expt 2), SBP and HR were measured once per week.

Measurement of angiotensin I-converting enzyme inhibitory activity

ACE inhibitory activity was measured by the method of Cushman & Cheung (1971), with modifications. Each test sample was centrifuged at 6000 g for 10 min, and the supernatant fraction was adjusted to pH 8.3 by adding 5 M-NaOH. The supernatant fraction was used for the assay. A sample (80 µl) of treated sample supernatant fraction was mixed with 0.1 U ACE solution (20 µl/ml) and 20 µl 0.2 M-borate buffer (pH 8.3) containing 5 mM-hippur-L-histidyl-L-leucine (Sigma, St Louis, MO, USA) and 0.3 M-NaCl. After incubation at 37°C for 30 min, the reaction was stopped by adding 1 M-HCl (250 µl). The hippuric acid liberated from hippur-L-histidyl-L-leucine by the enzymic action of ACE was extracted with ethyl acetate and was determined photometrically at 228 nm. The coefficient of ACE inhibitory activity (IDR₅₀) was defined as the dilution ratio of the sample needed to inhibit the ACE activity by 50%.

Preparation of peptides fraction from γ -aminobutyric acid-enriched fermented milk

FMG was centrifuged at 6000 g for 10 min, and 10 ml supernatant fraction was loaded onto a Bond-Elute C₁₈ chromatography cartridge (Varian Inc., Harbor City, CA, USA). The solution that flowed through the chromatograph was collected as the unadsorbed fraction of FMG. After the cartridge had been washed with water, peptides were eluted with 60% acetonitrile. The eluted fraction was evaporated to dryness, and the residual material was dissolved in 10 ml purified water and was used for ACE inhibitory assays as the peptide fraction of FMG.

Statistical analyses

Statistical analyses were performed using SAS Systems version 6.12 (SAS Institute Inc., Cary, NC, USA). Initial body weight and resting blood pressure data were analysed by Student's *t* test to compare the SHR and WKY groups.

One-way ANOVA was used to compare the ACE inhibitory activity, dose-response, body weight and food intake among the three diet groups during chronic administration. When the variance ratio (*F*) was significant, mean values were compared by Dunnett's test or the William's test. Two-way ANOVA was used to compare the result profiles over time with respect to blood pressure in single- and chronic-administration experiments. When the variance ratio (*F*) was significant among groups, mean values were compared by Fisher's least significant difference test. All results are reported as mean values with their standard errors. All statistical analyses were two-tailed, and statistical significance was established at *P* < 0.05.

Results

Effect of a single oral dose of γ -aminobutyric acid-enriched fermented milk on blood pressure in spontaneously hypertensive/Izm and Wistar-Kyoto/Izm rats

Before dosing, the average body weight and resting SBP in SHR/Izm and WKY/Izm rats were as shown in Table 3. There was a significant difference in SBP between SHR/Izm and WKY/Izm (*P* < 0.01), but not in body weight. The effects of a single oral dose of FMG (5 ml/kg; equivalent to 0.5 mg GABA/kg), GABA (0.5 mg/kg) or skimmed milk solution (5 ml/kg; GABA-free) on SBP are shown in Fig. 1. In SHR/Izm rats (Fig. 1(A)), two-way ANOVA showed independent effects of group (*P* < 0.05) and time (*P* < 0.01): SBP (mmHg) 4 h after administration were 191.7 (SEM 3.5) for the control group (saline), 177.8 (SEM 4.7) for the FMG group (*P* < 0.05) and 177.7 (SEM 3.2) for the GABA group (*P* < 0.05); SBP (mmHg) 8 h after administration were 191.3 (SEM 2.1), 177.2 (SEM 4.4) (*P* < 0.05) and 178.5 (SEM 3.4) (*P* < 0.05) respectively. SBP returned to the baseline value by 24 h after dosing. In comparison, FMG and GABA did not change the SBP in WKY/Izm rats (Fig. 1(B)). Skimmed milk solution did not affect SBP in either strain of rats. There were no significant changes in heart rate after administration of FMG, GABA or skimmed milk solution in any animals (results not shown).

Dose-responsive hypotensive effect of single administration of γ -aminobutyric acid

Fig. 2 shows the hypotensive effects of different doses of GABA at 4 h after a single oral dose. Administration of GABA doses of 0.50 and 5.00 mg/kg (but not 0.05 mg/kg)

Table 3. Body weight and resting blood pressure in spontaneously hypertensive (SHR/Izm) and normotensive Wistar-Kyoto (WKY/Izm) rats (14 weeks old) at the beginning of Expt 1† (Mean values with their standard errors for six rats per group)

	SHR/Izm		WKY/Izm	
	Mean	SEM	Mean	SEM
Body weight (g)	333.8	6.0	353.3	6.1
Resting SBP (mmHg)	195.3**	2.4	123.1	3.0

Mean value was significantly different from that for WKY/Izm rats: ***P* < 0.01. † For details of diets and procedures, see Table 1 and pp. 412–413.

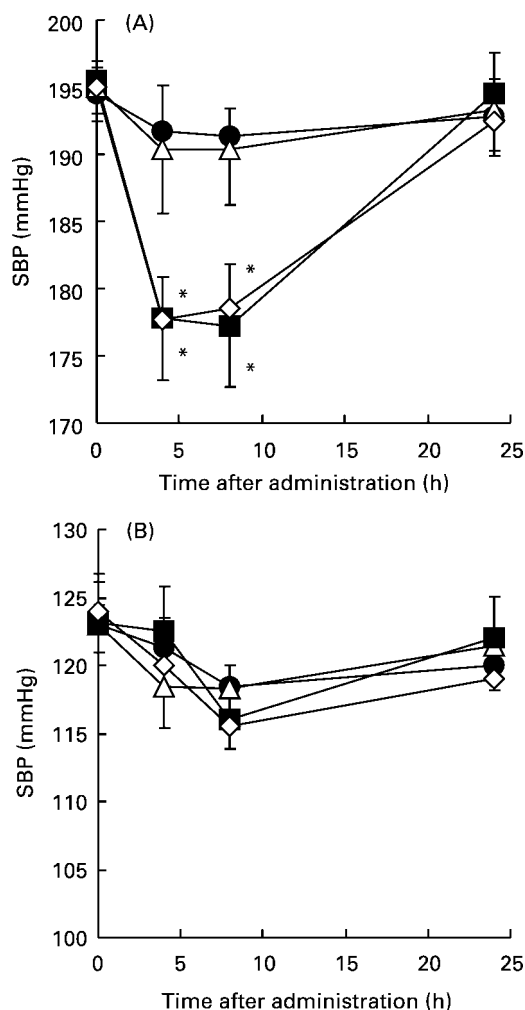


Fig. 1. Effect of a single oral administration of γ -aminobutyric acid-enriched fermented milk (FMG) or γ -aminobutyric acid (GABA) on systolic blood pressure (SBP) in spontaneously hypertensive/Izm rats (A) and Wistar-Kyoto/Izm rats (B). The doses of saline (9 g NaCl/l; ●), skimmed milk solution (60 ml/l; Δ) and FMG (■) were 5 ml/kg body weight, and for GABA (\diamond) the dose was 0.5 mg/kg. For details of diets and procedures, see Table 1 and p. 412. Values are means with their standard errors for six animals per group. Mean values were significantly different from that of the control (saline) value at the same time point: * $P < 0.05$.

significantly ($P < 0.05$) decreased SBP. The hypotensive effect of GABA was dose-dependent from 0.05 to 5.00 mg/kg (r 0.972).

Angiotensin I-converting enzyme inhibitory activity and effect on systolic blood pressure of γ -aminobutyric acid-enriched fermented milk and its peptide-containing fraction given as a single dose

Table 4 shows the ACE inhibitory activities and blood-pressure-lowering effects in SHR/Izm of FMG and its peptide-containing fraction. Captopril (an ACE inhibitor) as a positive control showed a strong ACE inhibitory effect and lowered the SBP significantly. In contrast, FMG, both its fractions, and GABA did not inhibit ACE activity, but SBP was significantly decreased compared with the saline control at 4 h after administration of FMG, the unadsorbed

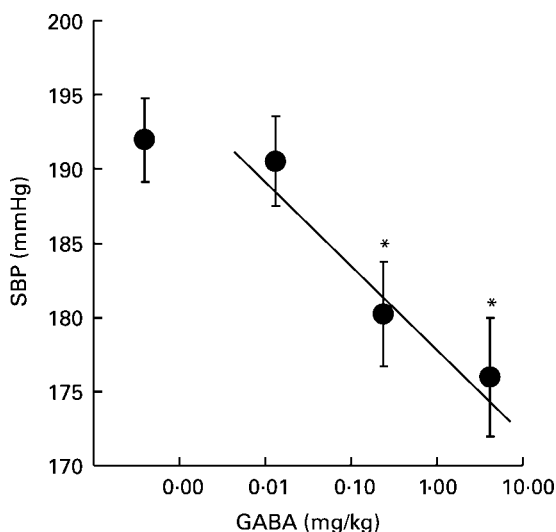


Fig. 2. Dose response of antihypertensive effect of γ -aminobutyric acid (GABA; non-linear scale) in spontaneously hypertensive/Izm rats. SBP, systolic blood pressure measured 4 h after administration of a single oral dose (5 ml/kg body weight). For details of diets and procedures, see Table 1 and pp. 412–413. Values are means with their standard errors for six animals per group. Mean values were significantly different from that of the control (saline, 9 g NaCl/l (0.00 mg GABA/kg)) value: * $P < 0.05$.

Table 4. Angiotensin-I converting enzyme (ACE) inhibitory activity and antihypertensive effects of γ -aminobutyric acid-enriched fermented milk (FMG) and its fractions† (Mean values with their standard errors for six rats per group)

	IDR ₅₀ of ACE inhibition	SBP (mmHg)‡	
		Mean	SEM
FMG			
Whole	0.1	178.2*	5.2
Peptide fraction	< 0.1	191.8	3.2
Unadsorbed fraction§	< 0.1	176.5*	2.9
GABA (1 mmol/l)	< 0.1	178.0*	3.3
Captopril (0.5 mmol/l)	> 1000	178.3*	3.9
Saline (9 g NaCl/l)	< 0.1	191.9	2.9

IDR₅₀, dilution ratio of sample needed to inhibit ACE activity by 50%; SBP, systolic blood pressure; GABA, γ -aminobutyric acid.

Mean values were significantly different from that of the saline group (control): * $P < 0.05$.

† For details of procedures, see Table 1 and p. 412.

‡ The SBP of spontaneously hypertensive/Izm rats was measured 4 h after a single oral dose (5 ml/kg body weight; at time 0 h, SBP 195.3 (SEM 2.4) mmHg).

§ The fraction of FMG that was not adsorbed on reverse-phase chromatography and that included 1 mmol GABA/l.

fraction of FMG, GABA or captopril ($P < 0.05$). The peptide fraction of FMG showed neither ACE inhibitory activity nor blood-pressure-lowering effects. The profile of blood pressure change due to administration of the non-adsorbed fraction of FMG was very similar to that for FMG in SHR/Izm (Fig. 3).

Chronic administration of γ -aminobutyric acid-enriched fermented milk or γ -aminobutyric acid

The body weight and food intake of SHR/Izm in Expt 2 are shown in Table 5. There were no significant differences in

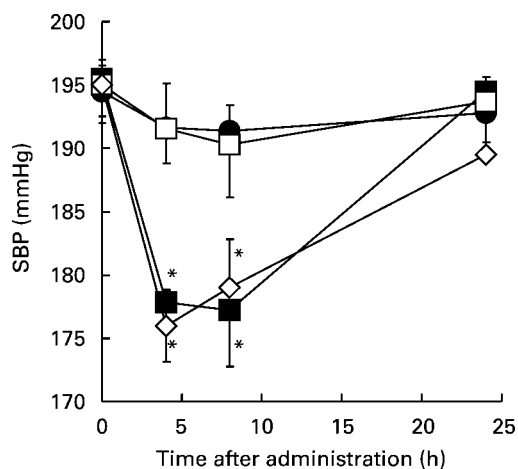


Fig. 3. Effect of a single oral administration of fractions of γ -aminobutyric acid-enriched fermented milk (FMG) on systolic blood pressure (SBP) in spontaneously hypertensive (SHR)/Izm rats. ●, Saline (9 g NaCl/l); □, FMG (peptide fraction); ◇, FMG (without peptide fraction); ■, FMG (whole). The dose was 5 ml/kg body weight. For details of diets and procedures, see Table 1 and pp. 412–413. Values are means with their standard errors for six animals per group. Mean values were significantly different from that of the control (saline) value at the same time point: * $P < 0.05$.

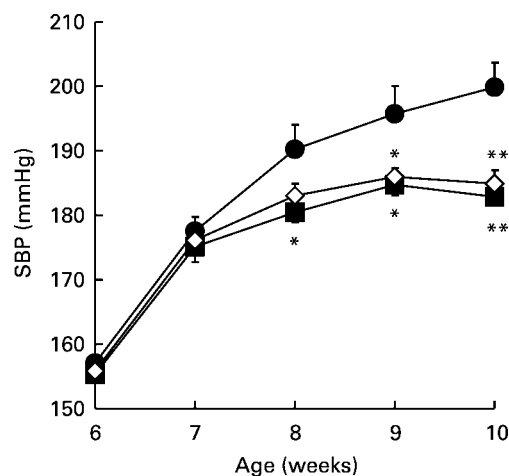


Fig. 4. Effect of chronic administration of γ -aminobutyric acid-enriched fermented milk (FMG) or γ -aminobutyric acid (GABA) on systolic blood pressure (SBP) in spontaneously hypertensive/Izm rats. ●, Control; ■, FMG; ◇, GABA. Test diets were fed from 7 to 10 weeks of age. For details of diets and procedures, see Tables 1 and 2 and pp. 412–413. Values are means with their standard errors for ten animals per group. Mean values were significantly different from that of the control value at the same time point: * $P < 0.05$.

these values between the three groups. The time course of SBP during the experiment is shown in Fig. 4. The SBP increased gradually with age and reached >200 mmHg at 10 weeks of age in the control group. A significantly slower increase in SBP compared with the control group was observed 1 or 2 weeks after the start (at 7 weeks of age) of feeding with the GABA or FMG diet respectively ($P < 0.05$), and this difference was maintained throughout the period of feeding. After 3 weeks of feeding the experimental diets, SBP values (mmHg) in the control, GABA and FMG groups were 199.8 (SEM 3.8), 184.9 (SEM 1.4) ($P < 0.01$ v. control group) and 182.8 (SEM 2.4) ($P < 0.01$ v. control group) respectively. There were no significant changes in heart rate in the control, FMG or GABA groups (results not shown).

Discussion

FMG and the identical amount of reagent GABA had a similar magnitude of hypotensive effect by single-dose and chronic administration to SHR/Izm. We also previously reported that FMG has a hypotensive effect in patients having mild hypertension (Inoue *et al.* 2003), although it was not clear whether the effect depended on

GABA. However, it seems reasonable to conclude that the hypotensive effect of FMG was caused by GABA. After a single oral dose of GABA (about 7 mg/kg) was given to rats (body weight 350 g), the plasma GABA concentration peaked (1.4 μ g/ml) after 1 h, then decreased gradually to 0.8 μ g/ml after 6 h, and disappeared after 24 h (Nakamura *et al.* 2000). In our present experiment, the hypotensive effect of GABA continued until 8 h after oral administration, and the effect disappeared after 24 h. There was good correlation between the plasma GABA level and the hypotensive effect (Fig. 1).

Many studies have linked increased long-term consumption of milk and dairy products with decreased blood pressure and reduced risk of hypertension. For example, a study of 5000 people found that those with normal blood pressure reported higher consumption of milk than did hypertensive subjects (Ackley *et al.* 1983). The authors suggested that some component of milk, probably Ca, exerted a protective effect against hypertension. In a recent review, the Dietary Approaches to Stop Hypertension (DASH) diet (a diet high in low-fat dairy foods, fruits and vegetables) reduced blood pressure (Appel *et al.* 1997; Miller *et al.* 2000). However, the hypotensive effect of milk is small (Allender *et al.* 1996), and a single oral dose of skimmed

Table 5. Body weight and food intake of rats in Expt 2* (Mean values with their standard errors for ten rats per group)

Diet... Variable	Control		GABA		FMG		Statistical significance of effect (one-way ANOVA): <i>P</i>
	Mean	SEM	Mean	SEM	Mean	SEM	
Body weight (g)							
7 weeks old	184.4	1.9	182.5	2.1	180.9	2.0	0.482
10 weeks old	264.7	5.4	271.0	2.5	268.9	2.5	0.545
Food intake (g per rat in 3 weeks)	350.7	19.5	362.5	16.2	361.5	18.2	0.805

GABA, γ -aminobutyric acid; FMG, γ -aminobutyric acid-enriched fermented milk.
* For details of diets and procedures, see Tables 1 and 2 and p. 412.

milk had no blood-pressure-lowering effect in SHR/Izm in our present study.

Some studies reported that in both single-dose and long-term regimens, various fermented milk or cheese products had stronger hypotensive effects than milk (Nakamura *et al.* 1995; Yamamoto *et al.* 1999; Saito *et al.* 2000; Seppo *et al.* 2003). Some lactic acid bacteria produce peptides that are released from the protein of the milk by proteolysis and inhibit ACE, which is instrumental in inducing hypertension (Nakamura *et al.* 1995; Yamamoto *et al.* 1999). It seemed reasonable to think that the hypotensive effect of FMG was related to inhibition of ACE. However, we found that neither FMG nor its peptide fraction showed any substantial inhibition of ACE activity. Nor did the peptides of FMG have a hypotensive effect after their oral administration to SHR/Izm rats. Furthermore, the two starters used to produce FMG, *Lactobacillus casei* strain Shirota and *Lactococcus lactis* YIT 2027, did not generate any hypotensive peptides from the protein of the milk. These and our other results indicate that the hypotensive effect of FMG is closely related to the presence of GABA.

During fermentation to produce FMG, GABA is made from the protein of the milk by *Lactococcus lactis* YIT 2027, which has strong glutamate decarboxylase activity. From the point of view of GABA production, acidic fermented milk is beneficial, because the glutamate decarboxylase activity of *Lactococcus lactis* YIT 2027 is optimal at about pH 5.3 (K Hayakawa *et al.*, unpublished results); its GABA transaminase (optimal pH 8–10; Shelp *et al.* 1999), which catalyses the transformation of GABA to succinic semialdehyde, is poorly active under acidic conditions. Therefore, GABA accumulates during fermentation.

Although the mechanism of the hypotensive action of systemically administered GABA has not been fully elucidated, several hypotheses have been postulated. Because GABA barely passes the blood–brain barrier (Gelder & Elliott 1958; Kuriyama & Sze 1971), it is reasonable to consider that, at low doses, GABA cannot act centrally but only peripherally. In practice, GABA inhibits nor-adrenaline release from sympathetic nerve terminals in the mesenteric arterial bed in SHR/Izm rats (Hayakawa *et al.* 2002). It has been suggested that this effect is mediated through peripheral activation of the GABA_B receptor, because the hypotensive effect is reduced by pre-administration of the GABA_B receptor antagonist saclofen.

Several recent studies have reported that various GABA-containing dietary materials or products decrease blood pressure in SHR and hypertensive patients. Gabaron tea (anaerobically fermented tea; Oomori *et al.* 1987), extracts of beni-koji (Tsuji *et al.* 1992) and a particular defatted rice germ (Onoda *et al.* 1998) are included among these supplements. However, these dietary materials and products contain many ingredients besides GABA, whose effects were not ruled out, although it was presumed that the hypotensive effect was due to the presence of GABA.

In a previous study, we investigated the safety of FMG in healthy adults with normal blood pressure (Kimura *et al.* 2002a). Administration of 100 ml FMG/d for 8 weeks and 300 ml/d for 2 weeks failed to induce any changes in normal blood pressure and heart rate. In

addition, we observed no remarkable changes in haematological and biochemical indices. It is important that FMG has no effect on normal blood pressure in human subjects, as it is undesirable to reduce blood pressure further than the level needed. In the present animal study, we observed a similar effect, in that FMG did not affect blood pressure in normotensive WKY/Izm rats; FMG exhibited a hypotensive effect only in the SHR/Izm.

In conclusion, our present results clearly show that our novel GABA-containing fermented milk product significantly reduced blood pressure in SHR/Izm rats. We consider that the hypotensive effect of FMG depends on GABA. Therefore, our FMG will probably be a beneficial addition to the diets of people with essential hypertension. The low incidence of hypertension among vegetarians may be partly attributable to their ingestion of GABA via these foods; however, further studies are required to confirm this theory.

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