Original Article

Green Coffee Bean Extract and Its Metabolites Have a Hypotensive Effect in Spontaneously Hypertensive Rats

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The effects of a water-soluble green coffee bean extract (GCE) on blood pressure were investigated using spontaneously hypertensive rats (SHR). There was a dose-dependent reduction in blood pressure after a single ingestion (180 to 720 mg/kg, p.o.) or long-term ingestion (0.25 to 1% diet for 6 weeks) of GCE. A single oral ingestion (50 to 200 mg/kg) of 5-caffeoylquinic acid (5-CQA), the major component of GCE, dose-dependently decreased blood pressure, suggesting that 5-CQA is involved in the hypotensive effect of GCE in SHR. Because significant increases in caffeic acid (CA) or ferulic acid (FA) were detected in plasma after oral ingestion of 5-CQA in SHR, these acids (2.5, 5, 10 μ mol/kg) were intravenously injected into SHR under anesthesia and the carotid arterial pressure was measured. Of the two components, FA had a stronger depressor effect than CA. The depressor effect of FA (50 mg/kg, p.o.) was attenuated by the concurrent injection of atropine sulfate (5 mg/kg, s.c.), suggesting that the hypotensive effect of FA in SHR might be mediated via the muscarinic acetylcholine receptors. These findings indicate that oral ingestion of GCE or 5-CQA decreases blood pressure in SHR, and that FA, which is a metabolite of 5-CQA, is a candidate hypotensive component. (*Hypertens Res* 2002; 25: 99–107)

Key Words: blood pressure, coffee, inbred SHR, chlorogenic acid, ferulic acid

Introduction

Both lifestyles and hereditary factors are known to be related to the development of hypertension. Control of lifestyle is important for the prevention and improvement of hypertension (1–3), and improvement in eating habits has received great attention in this regard. Epidemiologic studies suggest that higher intakes of potassium, calcium, magnesium (4, 5), peptides from fish or milk proteins (6–9), antioxidants (10–16), polyunsaturated fatty acids (17, 18), and food components (19–24) are beneficial for preventing hypertension. However, the depressor effects or hypotensive mechanisms of these components are not fully understood. In the present study, we found that green coffee bean extract (GCE) had a depressor effect in spontaneously hypertensive rats (SHR), and we investigated the hypotensive mechanism in this model. The results suggest that GCE might be considered as a

novel antihypertensive food.

The GCE used in this report was a hot-water extract of green coffee beans, the main components of which were chlorogenic acids. Human oral ingestion of chlorogenic acids stimulates stomach secretion *via* an enhancement of hydrochloric acid production (25). Many investigators report that chlorogenic acids have antioxidant activities (26–28) and inhibitory effects on chemical-induced carcinogenesis *in vitro* and *in vivo* (29–32). However, there have been no studies of the effects of chlorogenic acids on blood pressure in animals or humans.

The aim of the present study was to investigate the hypotensive effects of GCE and 5-caffeoylquinic acid, the major component of chlorogenic acids in GCE, in detail in SHR.

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Table 1. Components of Green Coffee Bean Extract

Component	Content (wt %)
Chlorogenic acids	28
Caffeine	6
Ethanol	5
Water	50
Others	11

Methods

Materials

GCE was a hot-water extract of green coffee beans that was subjected to ion-exchange chromatography (Flavor Holder RC; T. Hasegawa Co., Ltd., Tokyo, Japan). The components of GCE are shown in Table 1. 5-Caffeoylquinic acid (5-CQA), caffeic acid (CA), ferulic acid (FA), captopril, nicardipine, and prazosin were purchased from Sigma Chemical Co. (St. Louis, USA). Quinic acid (QA), atropine sulfate, and reagents for high performance liquid chromatography were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and Blood Pressure Measurement

Male SHR (SHR/NCrj) and Wistar Kyoto (WKY) rats (WKY/NCrj) purchased from Charles River Japan, Inc. (Tokyo, Japan) were used. All rats were maintained at a temperature of $25 \pm 1^\circ\text{C}$, $55 \pm 10\%$ humidity, and 12-h on/off light cycle (7:00 AM–7:00 PM). Blood pressure and heart rate were measured using the tail-cuff method in conscious SHR and WKY rats (33). After warming in a warmer at 37°C for 15 min, the rat was placed in a holder, and the blood pressure and heart rate of the tail artery were measured using an automatic blood pressure monitoring system (BP-98A; Softron Co., Ltd., Tokyo, Japan). The Animal Care and Use Committee of the Kao Tochigi Institute approved the present study. All experiments strictly followed the guidelines of that committee, which adhere completely to governmental legislation in Japan.

Experimental Protocols

Effects of a Single Oral Administration of GCE in SHR and WKY rats

GCE (180, 360, 720 mg/kg body weight) dissolved in physiologic saline was orally administered to SHR ($n = 6$, 15 week old) using a stomach probe. GCE (720 mg/kg) was orally administered to WKY rats ($n = 5$, 15 week old). Because a preliminary ingestion of GCE (360 mg/kg) decreased blood pressure in SHR, the doses of GCE were 180, 360, and 720 mg/kg. Physiologic saline was orally administered to the control group. Systolic blood pressure (SBP) and heart

rate were measured 3, 6, 9, 12, 24, and 48 h after oral administration in SHR using the tail-cuff method. In WKY rats, the SBP and heart rate were measured 3, 6, 9, 12, and 24 h after oral administration.

Long-Term Effects of GCE in SHR and WKY rats

A moderate fat (MF) diet (Oriental Bio-Service Kanto Inc., Tsukuba, Japan) was used as the control diet. The components of the MF diet were protein (23.8%), fats (5.1%), ash (6.1%), fiber (3.2%), soluble non-nitrogenous organisms (54.0%), and water (7.8%). The MF diet was combined with 0.25, 0.5, or 1.0% GCE as the test diet. SHR ($n = 8$, 7 week old at initiation of the experiment) were given the four types of diet, and WKY rats ($n = 8$, 7 week old at initiation of the experiment) were given the control diet or a 1.0% GCE diet for 6 week *ad libitum*. Daily food intake and body weight were measured weekly for 6 weeks. In the 6th week after initiation of the experiment, daily urine was collected using a metabolic cage (Natsume Seisakusho, Co., Ltd., Tokyo, Japan), and the urine volume was measured. The SBP and heart rate were measured using the tail-cuff method in conscious SHR and WKY rats before and during the 6th week of the experiment. Blood was collected from SHR at the end of the 6-week experimental period. After serum and plasma were separated, the total cholesterol, triglyceride, insulin, sodium ion, potassium ion, epinephrine, norepinephrine, dopamine, and plasma renin activity were measured. Measurements were performed by Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan).

Effects of a Single Oral Administration of 5-CQA in SHR

5-CQA was dissolved in physiologic saline, and orally administered at 50, 100, or 200 mg/kg to SHR ($n = 6$ each group; male, 15 week old) using a stomach probe. Because the content of chlorogenic acid was 28% in GCE, the doses of 5-CQA were 50, 100, and 200 mg/kg. Physiologic saline was orally administered to the control group ($n = 6$). SBP was measured using the tail-cuff method 3, 6, 9, 12, and 24 h after oral administration in SHR.

Plasma Phenolic Compounds in SHR after Oral Administration of 5-CQA

5-CQA was dissolved in physiologic saline, and orally administered at 200 mg/kg to SHR (male, 15 week old). From the jugular vein, 0.3 ml/animal of blood was collected 3, 6, 9, 12, and 24 h after oral administration, and the plasma was separated. Phenolic compounds were extracted from the plasma and quantified according to a modification of the method reported by Tsai *et al.* (34). After adding 0.5 ml of 0.5 M perchloric acid solution (containing 0.1 mM EDTA-2Na and 100 ng/ml dihydroxybenzylamine hydrobromide as an internal standard) to 0.1 ml of plasma, the mixture was centrifuged ($20,000 \times g$, 15 min) and the supernatant was adjusted to pH 3 with 1 M sodium acetate. The supernatant was filtered using a $0.45\text{-}\mu\text{m}$ filter, and $20\text{ }\mu\text{l}$ was applied to

Fig. 1. Effects of a single oral administration of GCE in conscious SHR and WKY rats. Change in SBP is expressed as the difference in SBP before and after administration. GCE, green coffee bean extract; SBP, systolic blood pressure. $\circ$, saline; $\bullet$, GCE (180 mg/kg); $\blacktriangle$, GCE (360 mg/kg); $\blacksquare$, GCE (720 mg/kg). Each value represents the mean $\pm$ SE ($n = 6$). * $p < 0.05$ and ** $p < 0.01$ vs. controls.

a high performance liquid chromatograph with an electrochemical detector (ECD-HPLC; Eicom Co., Tokyo, Japan). The HPLC analytical conditions were as follows: Column, EICOMPAK^{TM} CA-50DS 4.6 mm $\phi \times$ 150 nm; mobile phase, 0.1 M phosphate buffer (1% methanol, sodium 1-octanesulfonate 400 mg/l, EDTA-2Na 50 mg/ml); flow rate, 1 ml/min; column temperature, 25°C; detector, Eicom ECD-100 (action electrode WE-3G); voltage, +550 mV vs. Ag/AgCl. The detection limits of 5-CQA, CA, or FA were 24, 6, or 12 ng/ml, respectively.

Changes in Carotid Arterial Blood Pressure after Intravenous Injection of Phenolic Compound in SHR

SHR (male, 15 week old) were anesthetized by intraperitoneal injection of 0.7 ml/100 g body weight α -chloralose (1.5%)/urethane (7%). As reported previously (35), a cannula was inserted into the carotid artery, and blood pressure was recorded using a Polygraph System^{TM} (Nihon Kohden, Co., Tokyo, Japan). CA, QA, or FA, 0.1 ml/100 g body weight, was infused into the femoral vein. The doses were 2.5, 5, and 10 μ mol/kg.

Combined Effects of FA and Drugs on Blood Pressure in SHR

FA was dissolved in saline and 50 mg/kg was orally administered to SHR. Captopril (10 mg/kg, angiotensin-converting enzyme inhibitor), nicardipine (10 mg/kg, calcium channel blocker), or prazosin (1 mg/kg, adrenergic α receptor antagonist) was administered alone or concurrently with FA. Atropine sulfate (5 mg/kg, muscarinic acetylcholine receptor antagonist) was subcutaneously injected into the neck 20 min before oral administration of FA. The control group received oral administration of physiologic saline. Each

group consisted of five or six animals. SBP and heart rate were measured 1 h after oral administration of FA using the tail-cuff method. The dosage of atropine was the same as those reported previously (36, 37).

Statistical Analysis

All values were expressed as the means $\pm$ SE. Data were initially analyzed using the analysis of variance for each group. When a significant F -value ($p < 0.05$) was obtained, Duncan's multiple test was performed for *post hoc* analysis.

Results

Effects of a Single Oral Administration of GCE in SHR and WKY Rats

Changes in SBP after oral administration of GCE in SHR are shown in Fig. 1A. The initial SBP values were 216 ± 4 mmHg in the physiologic saline group, and 212 ± 3 , 224 ± 3 , and 226 ± 6 mmHg in the 180, 360, and 720 mg GCE groups, respectively. There were no significant changes in SBP in the physiologic saline group throughout the experimental period. GCE significantly decreased blood pressure in a dose-dependent manner, and the changes in SBP 12 h after administration were -6.8, -8.9, and -12.5% in the 180, 360, and 720 mg GCE groups, respectively. The reduction in blood pressure persisted for 24 h after administration and the changes were -5.7 and -11.1% in the 360 and 720 mg/kg GCE groups, respectively. SBP did not differ significantly from the initial value 48 h after administration in any group (data not shown). The initial heart rates were 412 ± 19 (beats/min) in the physiologic saline group, and 421 ± 13 ,

Table 2. Body Weights, Urinary Volume, Heart Rate, and Systolic Blood Pressure during the 6-Week Experimental Period in SHR or WKY Rats

Group	BW (g)	UV (ml/100 g/day)	HR (beats/min)	SBP (mmHg)
SHR control	307±6	5.3±0.8	406±7	211±3
SHR GCE (0.25%)	302±5	4.9±0.8	403±13	199±2*
SHR GCE (0.5%)	294±8	5.6±1.0	395±5	186±3**
SHR GCE (1%)	311±6	4.7±0.8	387±14	179±4**
WKY control	335±5	4.4±0.8	302±11	126±4
WKY GCE (1%)	335±13	4.4±0.4	306±10	123±2

Each value represents the mean±SE ($n=8$). BW, body weight; UV, urinary volume; HR, heart rate; SBP, systolic blood pressure; GCE, green coffee bean extract. * $p < 0.01$ SHR control vs. SHR GCE; ** $p < 0.001$ SHR control vs. SHR GCE.

Table 3. Fasting Serum Cholesterol, Triglyceride, Sodium or Potassium Ions, Insulin, Plasma Epinephrine, Norepinephrine, Dopamine, and Plasma Renin Activity in SHR^a

	Control	GCE (% of diet)		
		0.25	0.5	1.0
Total cholesterol (mg/dl)	46.3±1.2	43.9±1.2	45.0±1.6	46.6±1.0
Triglyceride (mg/dl)	24.4±4.5	22.6±0.8	21.3±1.3	23.2±1.1
Insulin (μ U/ml)	7.63±0.75	7.88±0.90	6.86±0.67	7.25±0.65
Na ⁺ (mEq/l)	139.6±0.6	139.1±0.6	140.0±0.4	139.3±0.5
K ⁺ (mEq/l)	5.38±0.25	5.53±0.28	5.26±0.25	5.44±0.27
Epinephrine (ng/ml)	2.19±0.11	2.29±0.25	2.33±0.19	2.09±0.19
Norepinephrine (ng/ml)	0.819±0.046	0.833±0.110	0.810±0.032	0.768±0.060
Dopamine (ng/ml)	0.074±0.008	0.083±0.018	0.077±0.008	0.075±0.012
PRA (ng/ml/h)	11.7±1.7	12.3±1.5	11.2±1.6	11.2±1.6

Each value represents the mean±SE ($n=8$). ^a Rats were sacrificed after 6 weeks of feeding with each diet. On the final day of experiments, blood was collected after 16 h of food deprivation. GCE, green coffee bean extract; PRA, plasma renin activity.

408±13, and 411±26 (beats/min) in the 180, 360, and 720 mg GCE groups, respectively. After 12 h, they were 369±6, 379±23, 367±13, and 380±10 (beats/min) in the physiologic saline group and the 180, 360, and 720 mg GCE groups. These data suggested that oral ingestion by SHR of GCE had no influence on heart rate in this experiment.

GCE was orally administered to normotensive WKY rats at a dose of 720 mg/kg, the dose that had a marked hypotensive effect in SHR, and SBP was measured for 24 h after administration (Fig. 1B). The mean initial SBP was 131±2 mmHg. Because almost no changes were observed in blood pressure after GCE administration, a single administration of GCE at this concentration might not affect blood pressure in WKY rats.

Longer-Term Effects of GCE in SHR and WKY Rats

During the 6-week study period, there were no significant differences in daily food intake, body weight, or urinary volume between the test diet and control diet groups in either SHR or WKY rats (Table 2). There were no changes in general health and there were no differences in the weights of

the liver, kidneys, spleen, or testes among the groups during the study period.

Table 2 shows the SBP and heart rate after 6 weeks. Tail arterial SBP before ingestion in the control diet group and the 0.25, 0.5, and 1% GCE diet groups were 157±5, 158±4, 159±4, and 159±3 mmHg, respectively. In the control diet group, blood pressure gradually increased with age and reached 211±3 mmHg in the 6th week after initiation (at age 13 weeks). In the GCE diet groups, the increase in blood pressure was significantly inhibited compared to that in the control diet group, and inhibition of the blood pressure elevation was proportional to the amount of GCE ingested. There were no significant differences in heart rate between the control diet and GCE diet groups in SHR.

At the initiation of the study, SBP was 110±5 mmHg in WKY rats (7 week old). The control diet or 1% GCE diet was given for 6 weeks, and there were no significant changes in SBP in either group, nor were there significant differences in heart rate between the groups during the ingestion period in WKY rats (Table 2).

The results of serum and plasma component analyses after 6 weeks in SHR are shown in Table 3. There were no effects

Fig. 2. Effects of a single oral administration of 5-CQA in SHR. Change in SBP is expressed as the difference in SBP before and after administration. 5-CQA, 5-caffeoylquinic acid; SBP, systolic blood pressure. $\circ$, saline; $\blacksquare$, 5-CQA (50 mg/kg); $\blacktriangle$, 5-CQA (100 mg/kg); $\blacklozenge$, 5-CQA (200 mg/kg). Each value represents the mean $\pm$ SE (n = 6). * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs. controls.

of GCE ingestion on any item tested at any dose examined.

Effects of a Single Oral Administration of 5-CQA in SHR

Changes in SBP after oral administration of the GCE component 5-CQA are shown in Fig. 2. The initial SBP value was 216 ± 4 mmHg in the physiologic saline group, and changed to 209 ± 5 mmHg 9 h after administration (change: - 2.2%). The initial SBP values were 214 ± 4 , 213 ± 3 , and 214 ± 2 mmHg in the 50, 100, and 200 mg/kg 5-CQA groups, respectively. When 5-CQA was administered, a dose-dependent hypotensive effect was observed, and the SBP values 9 h after administration were 197 ± 7 , 188 ± 6 , and 180 ± 5 mmHg in the 50, 100 and 200 mg/kg treatment groups, respectively (change: - 7.3, - 13.1, - 15.7%). The SBP value 24 h after administration was almost the same as the initial value. These findings confirmed the hypotensive effect of a single oral administration of 5-CQA in SHR. The initial heart rate values were 376 ± 13 , 377 ± 15 , 370 ± 20 , and 383 ± 14 (beats/min) in the physiologic saline group and the 180, 360, and 720 mg/kg 5-CQA groups, respectively. After 9 h of the oral ingestion, they were 368 ± 17 , 359 ± 9 , 361 ± 24 , and 367 ± 9 (beats/min), suggesting that oral ingestion by SHR of 5-CQA had no influence on heart rate.

Plasma Phenolic Compounds after Oral Administration of 5-CQA in SHR

Because the hypotensive effect of 5-CQA was confirmed, we analyzed the blood metabolites after a single oral administration of 5-CQA. 5-CQA, CA, and FA were analyzed. Fig-

Fig. 3. Phenolic compounds in plasma after oral ingestion of 5-CQA in SHR. Each value represents the mean $\pm$ SE (n = 6). 5-CQA, 5-caffeoylquinic acid; $\blacktriangle$, caffeic acid; $\blacklozenge$, ferulic acid.

ure 3 shows the amounts of the three components in SHR plasma after administration of 5-CQA. Plasma component analysis by ECD-HPLC indicated that the blood concentration of 5-CQA after oral administration of 200 mg/kg 5-CQA was below the limit of detection. In contrast, the plasma concentration of CA, a decomposition compound of 5-CQA, peaked at 179 ng/ml 6 h after administration, then disappeared from the plasma by 24 h. The blood FA concentration peaked at 174 ng/ml 9 h after administration. Similar to CA, most plasma FA had disappeared by 24 h.

Changes in Blood Pressure after Intravenous Injection of Phenolic Compounds in SHR

Because CA and FA were the main phenolic compounds detected after oral administration of 5-CQA, these compounds were intravenously injected and changes in carotid arterial pressure were observed. Figure 4 shows the representative results. When 5 and 10 μ mol/kg of CA were administered, blood pressure decreased slightly after administration, and the SBP decreased by 3.2 and 10.0%, respectively. When 2.5 and 5 μ mol/kg of FA were administered, the SBP decreased by 15.7 and 29.6%, respectively. When 5 μ mol/kg QA was administered, the SBP decreased by 1.7%. Therefore, of CA, FA, and QA, FA had the strongest hypotensive effect within the dose range examined in the present study.

Combined Effects of FA and Drugs on Blood Pressure in SHR

The results are shown in Table 4. FA (50 mg/kg) was orally administered to SHR, and blood pressure was measured over time using the tail-cuff method. Blood pressure reached the lowest value (decrease: - 11.0%) 1 h after administration, and returned to the initial value 6 h after administration (data not shown). Heart rates before and 1 h after FA admin-

Fig. 4. Carotid arterial pressure in anesthetized SHR. Carotid arterial pressure after intravenous injection of caffeic acid (A) or ferulic acid (B). Data are representative of six rats.

Table 4. Combined Effects of Ferulic Acid and Drugs on Blood Pressure in SHR

Group	Blocker	Ferulic acid (50 mg/kg, p.o.)	Change in SBP (%)
1	None	-	-0.3 ± 1.1
2		+	-11.0 ± 1.1*
3	Captopril	-	-10.3 ± 0.4*
4	(10 mg/kg, p.o.)	+	-14.1 ± 1.5*,**
5	Nicardipine	-	-43.1 ± 1.0*
6	(10 mg/kg, p.o.)	+	-52.4 ± 1.3*,†
7	Prazosin	-	-16.9 ± 0.8*
8	(1 mg/kg, p.o.)	+	-32.7 ± 2.5*,††
9	Atropine	-	-1.0 ± 0.2
10	(5 mg/kg, s.c.)	+	-2.0 ± 0.8‡

Each value represents the mean ± SE ($n=5$ or 6). SBP, systolic blood pressure; p.o., personal administration; s.c., subcutaneous injection. * $p < 0.001$ vs. group 1, ** $p < 0.05$ vs. group 3, † $p < 0.05$ vs. group 5, †† $p < 0.01$ vs. group 7, ‡ $p < 0.001$ vs. group 2.

istration were 404 ± 15 and 409 ± 11 (beats/min), respectively, showing no significant change from the initial value. When captopril, nicardipine, or prazosin was orally administered alone, blood pressure decreased significantly after 1 h. When these drugs were administered concurrently with FA, the reduction of blood pressure was enhanced. SBP did not change when atropine (5 mg/kg) was subcutaneously administered, but the heart rate markedly increased from 375 ± 25

before administration to 536 ± 4 (beats/min), consistent with the atropine-induced blockade of the muscarinic acetylcholine receptors. When FA was orally administered after atropine, there was almost no decrease in SBP after 1 h, suggesting that the hypotensive effect of FA was affected by pretreatment with atropine in SHR.

Discussion

We investigated the effects of GCE on blood pressure in SHR and WKY rats. GCE had a hypotensive effect and inhibited the increase in blood pressure in SHR. A single oral ingestion of 5-CQA, the major component of GCE, lowered the tail blood pressure, and its putative metabolites had hypotensive effects on the carotid arterial pressure in SHR.

There have been no previous physiologic studies on the effects of GCE. The present study demonstrated that this extract has an antihypertensive effect in SHR. There are many epidemiologic studies regarding the effects of roasted coffee extract, *i.e.*, coffee beverages (38–45). These studies indicated that roasted coffee does not affect blood pressure in hypertensive individuals. We have also observed that a single oral ingestion of roasted coffee extract had little effect on blood pressure in SHR, and that the hypotensive effect of GCE in SHR was not affected by an addition of caffeine (data not shown). These results suggested that the differences in the hypotensive effects of GCE and roasted coffee extract might be explained by differences in the structural changes in chlorogenic acids, or in by-products from the roasting of

green coffee beans. Clifford (46) suggested that there may be marked changes in the chemical structures of chlorogenic acids, such as the migration of phenolic groups, hydrolysis or fragmentation, or polymerization during the roasting of coffee beans. In addition, the loss of free amino acids, marked decreases in free saccharides (particularly sucrose) and trigonelline, the formation of brown pigment melanoidine by the Maillard reaction of amino acids and sucrose, and hydrogen peroxide production are characteristic of roasted coffee beans (25, 46–49). The different hypotensive effects of roast coffee beans and GCE are considered to be attributable to the differences in their components. The pressor effects of the components in roasted coffee beans require further investigation.

In the present study, 5-CQA decreased blood pressure in SHR. This finding suggests that the reduction of blood pressure after a single ingestion of GCE in SHR was at least partially due to 5-CQA. While the hypotensive effect persisted 24 h after a single ingestion of GCE, the effect of 5-CQA returned to the pretreatment level 24 h after administration. This difference might be due to a component other than 5-CQA or to a delay in intestinal absorption of 5-CQA, although the precise reasons for the differences remain unclear.

We orally administered 5-CQA at a hypotensive dose to SHR and measured the blood phenolic compounds by ECD-HPLC. 5-CQA was not transferred into the circulation, but its metabolites CA and FA were detected, suggesting that 5-CQA was hydrolyzed to CA and QA, and intestinally absorbed CA was further metabolized to FA in the circulation in SHR. The present results are consistent with the results of previous studies in rats and humans (50, 51). The concentrations of these compounds reached maximal levels 6 to 9 h after oral administration. This time course is consistent with that of the hypotensive effects after oral administration of 5-CQA shown in Fig. 2, suggesting that the hypotensive effect observed in SHR was due to the metabolites CA and/or FA.

In the present study, a cannula was inserted into the carotid artery under anesthesia in SHR, and the blood pressure was measured *via* the cannula using a pressure transducer. CA and FA were then injected into the femoral vein. FA had a marked hypotensive effect. Based on this finding, we speculated that the hypotensive effect of orally administered 5-CQA shown in Fig. 3 was due mainly to the blood FA. While intravenously injected CA slightly decreased arterial pressure in SHR, CA was less likely to contribute to the hypotensive effect of orally administered 5-CQA than FA. FA is also a precursor of lignin, which forms the cell walls of plants, and is an intermediate in the phenylpropanoid pathway in which sinapic acid is produced from phenylalanine. Various studies indicate that FA has a protective effect against β -amyloid peptide toxicity (52), a photoprotective effect (53), an antioxidative effect (54), and anti-tumor activities (55–57). There are no previous reports regarding the effects of FA on hypertensive animals or humans, and this is

the first report to state that FA reduces blood pressure in SHR.

The hypotensive effect of FA (50 mg/kg, p.o.) in SHR was not affected by concurrent administration of captopril, nicardipine, or prazosin. The result suggested that the use of these drugs had little influence on the depressor effect of FA in SHR. In contrast, pretreatment with atropine markedly decreased the hypotensive effect of FA. Atropine is a nonspecific antagonist that acts *via* muscarinic acetylcholine receptors. In blood vessels, the stimulation of the receptors induces endothelium-derived relaxing factors, e.g., nitric oxide (NO), and vasodilation. Recently, many investigators have reported that the mesenteric arterioles of SHR show increased superoxide anion production (58), and that these superoxide anions react with NO, thereby effectively depleting NO in vascular endothelial cells (59). Because FA is reported to scavenge superoxide anions (60), it might be that FA improves the bioavailability of NO in blood vessels in SHR.

In summary, our results showed that oral ingestion of GCE and 5-CQA contained in GCE decreased blood pressure in SHR, and that FA, which is considered to be a metabolite of 5-CQA, is a candidate hypotensive component. These findings suggest that the hypotensive effect of FA in SHR is mediated *via* the muscarinic acetylcholine receptors. The hypotensive mechanism of FA *in vitro* and *in vivo* is now being investigated.

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