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Green Asparagus (*Asparagus officinalis*) Prevented Hypertension by an Inhibitory Effect on Angiotensin-Converting Enzyme Activity in the Kidney of Spontaneously Hypertensive Rats

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ABSTRACT: Green asparagus (*Asparagus officinalis*) is known to be rich in functional components. In the present study, spontaneously hypertensive rats (SHR) were used to clarify whether green asparagus prevents hypertension by inhibition of angiotensin-converting enzyme (ACE) activity. Six-week-old male SHR were fed a diet with (AD group) or without (ND group) 5% asparagus for 10 weeks. Systolic blood pressure (SBP) (AD: 159 ± 4.8 mmHg, ND: 192 ± 14.7 mmHg), urinary protein excretion/creatinine excretion, and ACE activity in the kidney were significantly lower in the AD group compared with the ND group. Creatinine clearance was significantly higher in the AD group compared with the ND group. In addition, ACE inhibitory activity was observed in a boiling water extract of asparagus. The ACE inhibitor purified and isolated from asparagus was identified as 2″-hydroxynicotianamine. In conclusion, 2″-hydroxynicotianamine in asparagus may be one of the factors inhibiting ACE activity in the kidney, thus preventing hypertension and preserving renal function.

KEYWORDS: *green asparagus, ACE inhibitor, hypertension, SHR, 2″-hydroxynicotianamine*

INTRODUCTION

Hypertension induces chronic renal failure, which aggravates renal function and structure, and is a risk factor for cardiovascular disease.[1,2] The renin−angiotensin system (RAS) plays a major role in the pathology of hypertension.[3] Angiotensin-converting enzyme (ACE) constricts arterial vessels and acts to raise blood pressure in the regulation of the RAS.[4] ACE inhibitors, such as captopril, are used in the pharmacotherapy for hypertension. Recently, food protein sources, such as casein and tuna, have been identified as containing ACE inhibitory peptides.[5] It has been reported that nonpeptidyl ACE inhibitors, such as nicotianamine, are contained in many plant foods.[6,7] Izawa et al. measured ACE inhibitory activity and nicotianamine content in hot water fractions of eighty kinds of vegetables, including asparagus. [8] High ACE inhibition was observed in asparagus even though it contains only small amounts of nicotianamine.[8]

Asparagus is known to be a healthy and nutritious vegetable that contains functional components. Hafizur et al. reported that asparagus extract exerts antidiabetic effects by improving insulin secretion and β-cell function in streptozotocin-induced type 2 diabetic rats.[9] The objective of this study was to investigate the effects of asparagus on blood pressure and on ACE activity in spontaneously hypertensive rats (SHR) and to identify the active substance. As far as we know, the antihypertensive effect of green asparagus in SHR and the ACE inhibitory activity of compounds from green asparagus have not been investigated.

MATERIALS AND METHODS

Reagents and Chemicals. The hippuryl-histidyl-leucine (HHL) substrate was obtained from the Peptide Institute, Inc. (Osaka, Japan). Rabbit lung ACE was obtained from Sigma-Aldrich Japan Co. (Tokyo, Japan).

Samples. For the experimental diets used in this study, green asparagus spears grown in Australia were obtained from a local market in Toshima-ku. The asparagus spears were steamed, freeze-dried, and chopped in a food processor.

To isolate the ACE inhibitors, green asparagus spears grown in Nagasaki Prefecture were purchased at a fruit and vegetable shop in Sakado-shi. The asparagus spears used for quantifying the isolated active compound and nicotianamine were purchased at a department store or at a supermarket in Ikebukuro, Tokyo. Two of the samples were from Australia, and one was from New Zealand. The healthy obstacle by the excessive intake of asparagus has not been reported.

Animals. Five-week-old male SHR from the Izumo colony and Wistar Kyoto (WKY) rats, used as normotensive controls, were purchased from Japan SLC, Inc. (Hamamatsu, Japan). All animals were treated in accordance with the guidelines for the care and use of laboratory animals. The experimental protocols were approved by the Laboratory Animal Care Committee of Kagawa Nutrition University.

Experimental Diets. To prepare the diet containing asparagus (AD), freeze-dried and chopped asparagus spears were added to the normal diet (ND), at 5% and mixed. Dietary fiber was considered to be effective in lowering blood pressure. To remove the influence, the same amount of dietary fiber (cellulose) was added to the ND diets (Table 1).

Effects of Asparagus on Blood Pressure and Renal Function in SHR. SHR and WKY rats were individually housed in cages and allowed free access to food and tap water. Animals were maintained at our specified pathogen-free facilities, where the temperature (22 ± 2 °C), humidity (55 ± 5%), and lighting (6:00−18:00 h) were

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Table 1. Composition of Experimental Diets[a]

ingredient	ND	AD
corn starch (g/kg)	560	536
casein (g/kg)	237	221
DL-methionine (g/kg)	3	3
sucrose (g/kg)	80	80
corn oil (g/kg)	50	50
vitamin mixture (g/kg)	10	10
mineral mixture (g/kg)	40	40
powdered cellulose (g/kg)	20	10
asparagus (g/kg)		50
energy (kcal/kg)	3710	3700

[a]ND, diet without asparagus; AD, diet with asparagus.

controlled. At 6 weeks of age, SHR and WKY rats were each assigned to two groups, receiving either ND or AD. Eight rats were examined in each group. The body weight of the rats was measured once weekly, and daily food intake was determined twice weekly. Systolic blood pressure (SBP) was measured under no anesthesia every 2 weeks using a non-preheating, noninvasive blood pressure monitor for mice and rats (MK-2000; Muromachi Kikai Co., Ltd., Tokyo, Japan) using the tail cuff method. Urine collection (24-h) was performed using metabolic cages every 2 weeks. Urinary protein excretion was measured using the Biuret method, and urinary creatinine excretion was measured using the Jaffe method. Urinary protein excretion/urinary creatinine excretion was calculated from the above measurements. At 16 weeks of age, after 24 h of fasting, rats were subcutaneously anesthetized with nembutal (50 mg/kg body weight). Blood samples were obtained by heart puncture, and rats were then sacrificed. To measure ACE activity, both kidneys and lungs were perfused with phosphate buffered saline (pH 7.2), then removed and weighed. Creatinine clearance was measured and estimated as the glomerular filtration rate.

Measurement of ACE Activity in the Blood and Organs of SHR. Blood samples were added to EDTA-2Na. Plasma was centrifuged at 1000*g* for 15 min at 4 °C.

Organs were chopped into small pieces and homogenized in 0.2 mol/L borate buffer (pH 8.3) containing 0.5% nonidet P-40 using a polytron homogenizer (PT 1035; Kinematica AG, Luzern, Switzerland). The suspension was centrifuged at 4000*g* for 20 min at 4 °C. The supernatants and plasma were used as the enzyme extract for measuring ACE. The reaction was initiated by adding 30 μL of the extract to a mixture of 500 μL of 7 mmol/L HHL solution, 400 μL of 2 mol/L NaCl, and 70 μL of water and then incubating for 30 min at 37 °C. The reaction was stopped by adding 500 μL of 3% metaphosphoric acid. ACE activity was determined as hippuric acid formed from HHL using high-performance liquid chromatography (HPLC) assays. HPLC was performed with a Shimadzu 10A Series system (Shimadzu Co., Kyoto, Japan). HPLC analysis parameters were as follows: analytical column, Myghtysil RP-18(H)GP (4.6 mm i.d. × 250 mm) (Kanto Chemical Co., Inc., Tokyo, Japan); mobile phase, methanol; 10 mM potassium dihydrogen phosphate (pH 3.0) at 1:1; flow rate, 0.5 mL/min; column temperature, 40 °C; sample size, 20 μL; UV detection at 228 nm. One unit of ACE activity was defined as the amount that formed 1 mmol of hippuric acid from HHL in 1 min at 37 °C. Specific activity was expressed in terms of units/mg protein. Protein in all organs was measured by Bio-Rad DC-protein assay.

Measurement of ACE Inhibitory Activity in Asparagus. ACE inhibitory activity in asparagus was measured using a modification of the Cushman and Cheung method.[10]

Identification of ACE Inhibitor in Asparagus. To purify and isolate ACE inhibitors from asparagus, a total of 27.5 L of ethanol was added to 12.75 kg of asparagus spears, such that ethanol comprised 70% of the total volume. This mixture was homogenized in a blender and left overnight to settle. The juice was extracted using cotton filter bags and was then passed through a paper filter to obtain a clear extract. The residual pulp in the cotton filter bag was mixed with 10 L of 70% ethanol and homogenized, and this was then filtered to obtain an extract, as described above. This procedure was repeated twice. All extracts were collected and concentrated to approximately 1 L using a rotary evaporator. The resulting concentrated extract was transferred into a separatory funnel, and approximately 500 mL of diethyl ether was added to remove the fat. This procedure was repeated twice. The remaining defatted extract was concentrated into a total volume of approximately 500 mL.

The defatted extract was loaded onto a cationic exchange resin column (Amberlite IR-120 (H^+); 8 cm i.d. × 50 cm) (Organon K.K.,Tokyo, Japan) and an anion exchange resin column (Amberlite IRA-45 (OH^-); 5 cm i.d. × 50 cm), which were connected in series. Ten liters of water were passed through the connected columns and collected as the nonionic fraction. Connected columns were subsequently separated, and 14 L of 2 mol/L and 5 L of 2 mol/L ammonia were passed through the Amberlite IR-120 (H^+) and IRA-45 (OH^-) columns, respectively, in order to obtain the cation and anion fractions. The resulting fractions were concentrated and desiccated, and water was added to give a final volume of 500 mL for measurement of ACE inhibitory activity.

As ACE inhibitory activity was localized in the cation fraction, this fraction was concentrated to approximately 100 mL and was then further purified by gel filtration chromatography using a Sephadex G-15 column (3 cm i.d. × 100 cm) (GE Healthcare Japan, Tokyo, Japan) equilibrated with 0.3 mol/L acetic acid. A 20 mL extract sample was loaded onto the column and eluted with this solution to yield 5 mL fractions. Ninhydrin reactivity and ACE inhibitory activity were measured in each fraction, and chromatograms were obtained (Figure 1). Chromatograms indicated that the majority of ACE inhibitory activity was present in the weak ninhydrin-positive fractions, and these were eluted more quickly than the strong ninhydrin-positive fractions, which appeared to be amino acids. Subsequently, the remaining cation fractions were repeatedly chromatographed and all fractions showing ACE inhibition were collected.

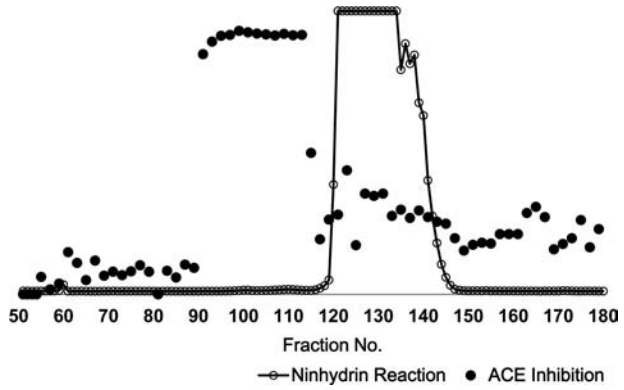

Figure 1. Sephadex G-15 chromatogram of the cationic fraction. Chromatograms indicated that the majority of ACE inhibitory activity was present in the weak ninhydrin-positive fractions. These were eluted more quickly than the strong ninhydrin-positive fractions.

The ACE inhibitory fractions concentrated by gel filtration chromatography were then passed through a Dowex 1 × 4 (acetate form) anion exchange resin column (The Dow Chemical Japan Co., Tokyo, Japan). After equilibrating this column (3 cm i.d. × 100 cm) with sufficient purified water, the active fraction, adjusted to pH 8, was loaded and then eluted with a linear gradient of acetic acid (1.5 L of water and 1.5 L of 1 mol/L acetic acid). The eluate was separated into 10 mL fractions, which were chromatographed by measuring ninhydrin reactivity and ACE inhibition. Subsequently, a ninhydrin-positive compound indicating strong ACE inhibition was eluted in fractions 125−135 (Figure 2). When the fractions were concentrated using a rotary evaporator, a clear, crystallized precipitate was obtained. The crystallized precipitate was dissolved in a small amount of water,

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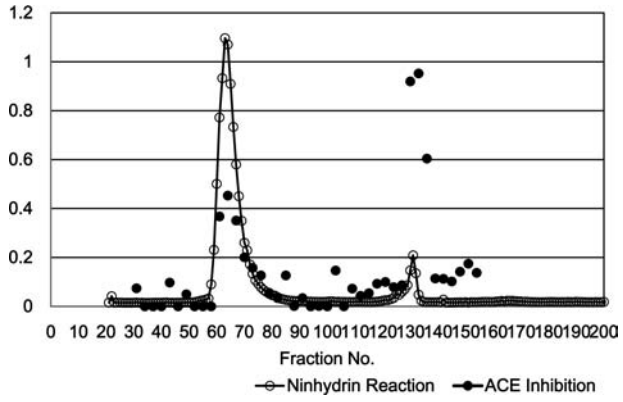

Figure 2. Dowex 1 × 4 chromatogram of ACE inhibiting fraction purified by Sephadex G-15 chromatography. The ninhydrin-positive compound in fractions 125−135 indicated strong ACE inhibitiory activity was eluted in these fractions.

and heated ethanol was added until the solution became cloudy; this was left to stand until crystals appeared. This recrystallization procedure was repeated twice.

To identify the ACE inhibitor, the crystallized compound (I) from asparagus was identified using high-performance liquid chromatography−time-of-flight mass spectrometry (HPLC-TOF MS), nuclear magnetic resonance (NMR), and infrared absorption (IR). HPLC-TOF MS was performed with a Waters 2695 HPLC (Nihon Waters K.K., Tokyo, Japan) equipped with an LCT Premier (TOF MS detector; Nihon Waters K.K., Tokyo, Japan). HPLC analysis parameters were as follows: SieLC Primesep 100 column (2.2 mm i.d. × 100 mm) (SIELC Technologies Inc., USA); mobile phase, 0.125% TFA in 30% acetonitrile; flow rate, 0.2 mL/min; column temperature, 40 °C; and sample size, 10 μL. TOF MS was performed in the positive ion detection mode using an electrospray-ionization (ESI) source. Analysis was conducted under the following conditions: cone voltage, 50 V; source temperature, 100 °C; and desolvation temperature, 300 °C. For NMR, the sample was dissolved in D_2O and 3-trimethylsilylpropionate-d_4 (TMSP) was used as an internal standard. ^1H and ^{13}C NMR spectrum measurements were performed on a JEOL EX-270 spectrometer (JEOL Ltd., Tokyo, Japan). Signal assignment of each spectrum was performed using various two-dimensional NMR techniques. The IR spectrum was measured using the Fourier transform infrared spectrophotometer IRAffinity-1 (Shimadzu Co., Kyoto, Japan).

To quantify nicotianamine and 2″-hydroxynicotianamine in asparagus, a 2 g sample of freeze-dried powder was rehydrated by adding 50 mL of hot water and heating under reflux for 30 min in a 100 °C water bath. After cooling and centrifugal separation, the supernatant was collected. Cold water was added to the extraction residue twice, which was then separated by centrifugation. To prepare the sample solution, supernatant was adjusted to pH 12 with 1 mmol/L NaOH. This sample solution was passed through a column (2 cm i.d. × 10 cm) of Dowex 1 × 4 (acetate form) resin and, following a wash with 100 mL of 0.2 mmol/L pyridine, was eluted with 200 mL of 0.5 mmol/L acetic acid solution. After the eluate was condensed and desiccated, the residue was dissolved in 5 mL of 0.05 mmol/L SDS solution, passed through a membrane filter (0.20 μm), and analyzed by HPLC. HPLC analysis by ion-pair chromatography was performed on a Shimadzu Class-VP (Shimadzu Co., Kyoto, Japan) under the following conditions: TSK gel ODS-80Ts (4.6 mm i.d. × 250 mm) column (Tosoh Co., Tokyo, Japan); mobile phase was prepared by mixing 0.05 mmol/L sodium lauryl sulfate and acetonitrile in a 6.5:3.5, v/v, ratio adjusted to pH 2.4 at a flow rate of 0.75 mL/min and column temperature of 40 °C. The color reagent, *o*-phthalaldehyde (OPA)/2-mercaptoethanol (0.2 mmol/L borate buffer, pH 9.9), was combined at a flow rate of 1 mL/min. Upon termination of the reaction at 50 °C, fluorescent detection was performed using the postcolumn method at an excitation wavelength of 365 nm and an emission wavelength of 455 nm. As standard reference materials, nicotianamine and 2″-hydroxynicotianamine were prepared from soy milk and buckwheat flour, respectively, and peak areas were used for quantitative measurements.

Statistical Analysis. A statistical software package (SPSS 17.0J; SPSS Japan Inc., Tokyo, Japan) was used for all statistical analyses. Results are expressed as means ± SD. Student's *t* test was used to compare SHR and WKY rats. Bonferroni's multiple comparison was used to compare between the diet groups. Differences with a value of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effects of Asparagus on Blood Pressure and Renal Function in SHR. Body weights were significantly lower in SHR than in WKY rats for the experimental term. In SHR and WKY rats, there were no significant differences in body weight between the AD and ND groups (Figure 3). Food intake was

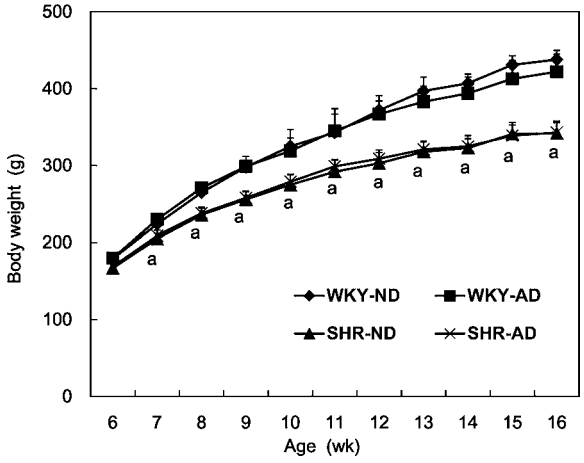

Figure 3. Time course of body weight. WKY rats and SHR received ND (diet without asparagus) or AD (diet with asparagus) from 6 to 16 weeks of age. Body weights in SHR were lower when compared with WKY rats. There were no significant differences between the ND and AD groups in either the SHR or WKY rats. WKY-ND, WKY rats fed on ND (without asparagus); WKY-AD, WKY rats fed on AD (with asparagus); SHR-ND, SHR fed on ND (without asparagus); SHR-AD, SHR fed on AD (with asparagus). Numbers of WKY rats and SHR were 8. a: $p < 0.05$, SHR vs WKY.

unchanged in SHR and WKY rats fed either AD or ND for the experimental term (data not shown). SBP was significantly higher in SHR than in WKY rats for the experimental term. In SHR, SBP was significantly lower in the AD group than in the ND group at 10 weeks of age. There were no significant differences between the AD and ND groups in WKY rats for the experimental term (Figure 4). Urinary protein excretion/creatinine excretion was higher in SHR than in WKY rats except at 10 and 12 weeks of age. In SHR, urinary protein excretion/creatinine excretion was lower in the AD group than in the ND group after 8 weeks of age. At 16 weeks of age, urinary protein excretion/creatinine excretion was significantly lower in the AD group than in the ND group and there were no significant differences between the AD and ND groups in WKY rats for the experimental term (Figure 5). Creatinine clearance was significantly lower in SHR than in WKY rats. In SHR, creatinine clearance was significantly higher in the AD group than in the ND group. There were no significant differences in creatinine clearance between the AD and ND groups in WKY rats (Figure 6). ACE activity in the plasma, lung, and kidney

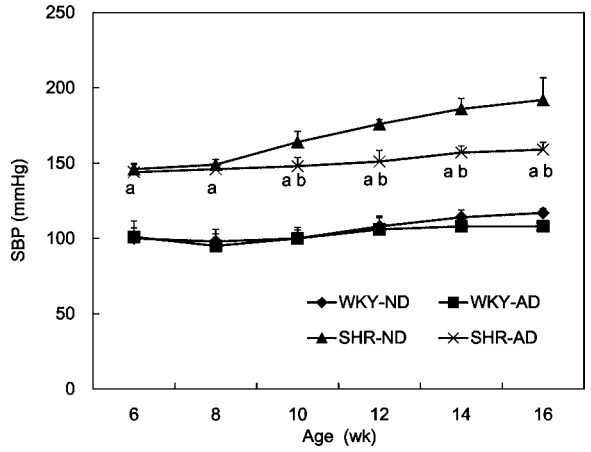

Figure 4. Time course of SBP. WKY rats and SHR received ND (diet without asparagus) or AD (diet with asparagus) from 6 to 16 weeks of age. SBP in SHR was higher when compared with WKY rats. In SHR, SBP in the AD group was significantly lower than that in the ND group after 10 weeks of age. There were no significant differences between the ND and AD groups in WKY rats. WKY-ND, WKY rats fed on ND (without asparagus); WKY-AD, WKY rats fed on AD (with asparagus); SHR-ND, SHR fed on ND (without asparagus); SHR-AD, SHR fed on AD (with asparagus). Numbers of WKY rats and SHR were 8. a: $p < 0.05$, SHR vs WKY; b: $p < 0.05$, SHR-AD vs SHR-ND.

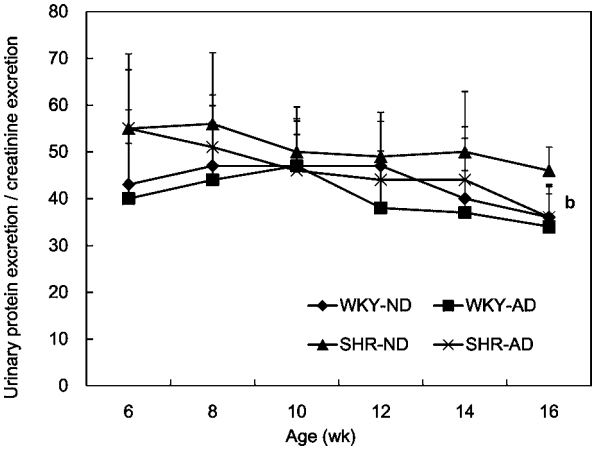

Figure 5. Time course of urinary protein excretion/creatinine excretion. WKY rats and SHR received ND (diet without asparagus) or AD (diet with asparagus) from 6 to 16 weeks of age. In SHR, urinary protein excretion/creatinine excretion in the AD group was significantly lower than that in the ND group at 16 weeks of age. There were no significant differences between the ND and AD groups in WKY rats. WKY-ND, WKY rats fed on ND (diet without asparagus); WKY-AD, WKY rats fed on AD (diet with asparagus); SHR-ND, SHR fed on ND (diet without asparagus); SHR-AD, SHR fed on AD (diet with asparagus). Numbers of WKY rats and SHR were 8. b: $p < 0.05$, SHR-AD vs SHR-ND.

were significantly higher in SHR than in WKY rats. In SHR, ACE activity in kidney extracts from the AD group was lower when compared with the ND group, but there were no significant differences in ACE activity in the plasma and lung between the AD and ND groups. In WKY rats, there were no significant differences in ACE activity in the plasma, lung, or kidney between the AD and ND groups (Table 2).

In the present study, we examined whether the ACE inhibitor in asparagus affected blood pressure in SHR. We found that asparagus prevented elevation of blood pressure in

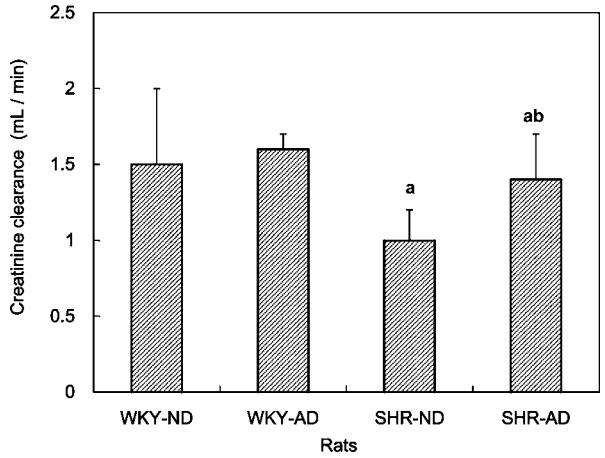

Figure 6. Creatinine clearance in WKY rats and SHR. WKY rats and SHR received ND (diet without asparagus) or AD (diet with asparagus) from 6 to 16 weeks of age. In SHR, creatinine clearance in the AD group was significantly higher than that in the ND group. There were no significant differences between the ND and AD groups in WKY rats. WKY-ND, WKY rats fed on ND (diet without asparagus); WKY-AD, WKY rats fed on AD (diet with asparagus); SHR-ND, SHR fed on ND (diet without asparagus); SHR-AD, SHR fed on AD (diet with asparagus). Numbers of WKY rats and SHR were 8. a: $p < 0.05$, SHR vs WKY; b: $p < 0.05$, SHR-AD vs SHR-ND.

SHR. In human studies, obesity induces hypertension, and it has been recognized that reducing weight by energy restriction induces decreases in blood pressure.[11] However, there were no significant differences in body weight or food intake between the AD and ND groups, suggesting that asparagus has an SBP-lowering effect. The RAS is thought to be important in the regulation of blood pressure. ACE, a component of the RAS, is involved in the development of hypertension. ACE is expressed in plasma and various tissues, including the brain, heart, aorta, lungs, and kidney.[12−16] Kreutz et al. reported that the ACE present in plasma is not involved in the regulation of blood pressure.[17] Murakami et al. indicated that inhibition of ACE activity in the aorta reduced blood pressure in SHR.[18] Some reports suggested that inhibition of ACE activity in the kidney reduced blood pressure, thereby inhibiting proteinuria and preserving renal function.[19−23] Wolfgang et al. indicated that long-term ACE inhibition protected against hypertension-induced renal injury in SHR.[24] We measured ACE activity in plasma, lungs and kidney in order to examine the underlying mechanism of the antihypertensive effects of asparagus. ACE in the kidney was significantly lower in the AD group than in the ND group in SHR. A significant positive correlation was observed between SBP and ACE activity in the kidney, but not in plasma or lungs. ACE activity in the kidney was inversely correlated with creatinine clearance. These results suggest that inhibition of ACE in the kidney prevents the development of high blood pressure, exerts beneficial effects on renal function, and reduces urinary protein excretion in SHR. This study demonstrated that the ACE inhibitor in asparagus inhibits ACE activity in the kidney, exerting antihypertensive effects and preventing the progression of renal injury in SHR.

Identification of ACE Inhibitor in Asparagus. A colorless crystallized compound (I) (33 mg) from 12.75 kg of asparagus spears was purified and isolated as an ACE inhibitor. On silica gel thin-layer chromatography (Merck GF 60) (Merck Ltd., Tokyo, Japan), this crystallized compound exhibited single ninhydrin-positive spots at R_f 0.03 and R_f 0.06,

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Table 2. Angiotensin-Converting Enzyme Activity[a]

	WKY-ND	WKY-AD	SHR-ND	SHR-AD
plasma (munit/mL)	1.40 ± 0.70	1.20 ± 0.30	10.0 ± 3.00 a	12.0 ± 3.00 a
lung (munit/mgprotein)	5.01 ± 0.81	4.68 ± 0.70	10.7 ± 2.78 a	11.1 ± 2.31 a
kidney (munit/mgprotein)	1.10 ± 0.30	1.20 ± 0.30	2.40 ± 0.20 a	1.90 ± 0.20 ab

[a]WKY rats and SHR received ND (diet without asparagus) or AD (diet with asparagus) from 6 to 16 weeks of age. Angiotensin-converting enzyme activity was measured at 16 weeks of age. Numbers of WKY rats and SHR were 8. Data values are expressed as means ± SD. a: $p < 0.05$, SHR vs WKY. b: $p < 0.05$, SHR-AD vs SHR-ND.

using the following developing solvents: *n*-butanol, acetic acid, and water in a 4:1:2 ratio, and *n*-propanol and ammonia in a 7:3 ratio, respectively.

The total ion chromatogram of the ACE inhibitor crystal (I) by HPLC-TOF MS showed a single peak at 2.86 min, and its MS spectrum showed a $[M + H]^+$ ion peak at m/z 320.1521. The elemental composition of this ion was calculated to be $C_{12}H_{22}N_3O_7$ (theoretical value of m/z 320.1458), and the predicted molecular formula was $C_{12}H_{21}N_3O_7$. The ^{1}H NMR (D_2O) spectrum revealed signals at δ 2.05−2.3 (2H, m, H-2′), 2.45−2.80 (2H, m, H-3), 3.20−3.53 (4H, m, H-1″ and H-1′), 3.80−3.86 (1H, m, H-3′), 3.90−4.15 (2H, m, H-4), 4.01 (1H, d, 3.3 Hz, H-3″), 4.40−4.48 (1H, m, H-2″), and 4.78 (1H, t, 9.57 Hz, H-2). The ^{13}C NMR (D_2O) spectrum showed signals at δ 23.9 (C-3, CH_2), 27.7 (C-2′, CH_2), 51.1 (C-1″, CH_2), 53.3 (C-4, CH_2), 54.1(C-1′, CH_2), 60.5 (C-3″, CH), 62.4 (C-3′, CH), 68.4 (C-2″, CH), 69.6 (C-2, CH), 173.3 (C-4″, CO), 175.1 (C-4′, CO), and 176.0 (C-1, CO). These data confirmed that this compound was identical to the ACE inhibitor, 2″-hydroxynicotianamine, in buckwheat flour, which we previously reported. The IR spectrum showed absorption peaks at ν^{KBr}_{max} (cm^{-1}): 3500−3400, 1633, 1614, 1593, 1558, 1404, 1313, 1298, 1230, 1188, 1072, and 820, which precisely matched the spectrum for 2″-hydroxynicotianamine. On the basis of these data, we concluded that the compound was 2″-hydroxynicotianamine.

The nicotianamine and 2″-hydroxynicotianamine contents in the three types of green asparagus spears were measured. 2″-Hydroxynicotianamine was present at 4.7−5.4 mg per 100 g fresh weight, which amounts to 66−80 mg on a dry matter basis. Nicotianamine was found at less than or equal to one-tenth of this amount (or 1.6−4.4 mg per 100 g dry matter).

Nicotianamine was reported to have antihypertensive effects due to direct inhibition of ACE in vascular smooth muscle and activation of adrenergic receptors,[25] and it has been identified in angelica (*Angelica keiskei*) and mulukhiyya (*Corchorus olitorius L*).[26,27] Izawa et al. previously screened for ACE inhibitory activity and nicotianamine contents in a number of hot water extracts of plant foods.[8] Many plants which are used as food have been shown to contain considerable quantities of nicotianamine and high ACE inhibitory activity. Asparagus has high ACE inhibitory activity despite having a low nicotianamine content.[8] The purpose of our study was to identify the active compound producing such high ACE inhibitory activity. We identified 2″-hydroxynicotianamine as the active compound, which was the same ACE inhibitor that we structurally elucidated after its isolation from buckwheat flour extract. We have also identified and reported the presence of this compound in various other polygonaceous plants.[28] Maejima et al. demonstrated that 2″-hydroxynicotianamine was a safe functional food compound in safety test.[29] Recently, chemical synthesis of the compound revealed that its three-dimensional structure was (2″*S*)-hydroxynicotianamine.[30] Asparagus belongs to the lily family (*Liliaceae*); therefore, this report is the first to demonstrate the presence of 2″-hydroxynicotianamine in non-polygonaceous plants. The asparagus spears contained 66−80 mg of this compound on a dry matter basis, which is the highest concentration of all the plant foods previously reported, including buckwheat flour (30 mg), buckwheat shoots (48 mg), and young buds of *Polygonum hydropiper L* (52 mg). We believe consumption of this compound, together with nicotianamine, is an important contributing factor in stabilizing blood pressure. Asparagus may be a valuable addition to the ACE inhibitory group. The discovery of 2″-hydroxynicotianamine in a nonpolygonaceous plant demonstrates the need for further examination of the distribution and role of this compound in the plant kingdom.

Hayashi et al. reported that nicotianamine (1 mg/20 g body weight) decreased blood pressure and inhibited ACE activity in Tsukuba hypertensive mice after a single oral administration. One hour after administration, the plasma concentration of nicotianamine was 2.9 μg/mL.[31] Takenaka et al. reported on the effects of test diets containing 20, 100, and 200 mg of nicotianamine/kg on SHR for 8 weeks. All of the tested diets produced antihypertensive effects. Nicotianamine was not detected in the blood of SHR given the 20 mg and 100 mg/kg diets; however, it was detected in blood from SHR given the 200 mg/kg diet.[32] In our study, a test diet containing 40 mg of 2″-hydroxynicotianamine/kg was given to SHR for 10 weeks, administered as 4.3 mg of 2″-hydroxynicotianamine/kg body weight/day. We considered this to be enough 2″-hydroxynicotianamine to produce an antihypertensive effect, based on nicotianamine research. Further research is required into the influence of 2″-hydroxynicotianamine on blood pressure, ACE activity, and progressive renal injury.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

SHR, spontaneously hypertensive rats; ACE, angiotensin-converting enzyme activity; SBP, systolic blood pressure; HHL, hippuryl-histidyl-leucine

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