Antihypertensive Effect of Angiotensin I-Converting Enzyme Inhibitory Peptides from a Sesame Protein Hydrolysate in Spontaneously Hypertensive Rats

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Sesame peptide powder (SPP) exhibited angiotensin I-converting enzyme (ACE) inhibitory activity, and significantly and temporarily decreased the systolic blood pressure (SBP) in spontaneously hypertensive rats (SHRs) by a single administration (1 and 10 mg/kg). Six peptide ACE inhibitors were isolated and identified from SPP. The representative peptides, Leu-Val-Tyr, Leu-Gln-Pro and Leu-Lys-Tyr, could competitively inhibit ACE activity at respective Ki values of 0.92 μM, 0.50 μM, and 0.48 μM. A reconstituted sesame peptide mixture of Leu-Ser-Ala, Leu-Gln-Pro and Leu-Lys-Tyr, could competitively inhibit ACE activity at respective Ki values of 0.92 μM, 0.50 μM, and 0.48 μM. A reconstituted sesame peptide mixture of Leu-Ser-Ala, Leu-Gln-Pro, Leu-Lys-Tyr, Ile-Val-Tyr, Val-Ile-Tyr, Leu-Val-Tyr, and Met-Leu-Pro-Ala-Tyr according to their content ratio in SPP showed a strong antihypertensive effect on SHR at doses of 3.63 and 36.3 μg/kg, which accounted for more than 70% of the corresponding dosage for the SPP-induced hypotensive effect. Repeated oral administration of SPP also lowered both SBP and the aortic ACE activity in SHR. These results demonstrate that SPP would be a beneficial ingredient for preventing and providing therapy against hypertension and its related diseases.

Key words: sesame peptide; angiotensin I-converting enzyme; spontaneously hypertensive rat; hypertension; oral administration

The renin-angiotensin system plays an important role in blood pressure, and in cardiac and vascular functions. Renin produces decapitare angiotensin I from angiotensinogen. The angiotensin I-converting enzyme (ACE) catalyzes the formation of vasopresor angiotensin II by cleaving dipeptide from the C-terminal of angiotensin I in the vascular wall. Therefore, the inhibition of ACE activity is a good target for antihypertension. ACE inhibitors have been prescribed for hypertensive patients throughout the world, and many clinical application data have so far been accumulated.1)

It has been reported that many ACE inhibitory peptides were obtained by protease digestion of such food materials as a thermolysin hydrolysate of dried bonito,2) an alkaline protease hydrolysate of sardine muscles,3) an alkaline protease hydrolysate of whey,4) and a pepsin hydrolysate of soy bean proteins.5) Sesame seed has been utilized as a traditional health food to prevent aging-related diseases. Sesame seed contains abundant oil (about 50% w/w), protein (about 20% w/w), and characteristic lignans. Sesame lignans has been clarified to have many beneficial effects such as antihypercholesterolemic,6) anti-inflammatory,7) and antihypertensive activity. 8) However, there is little evidence for the biological effects of sesame protein. Ochi et al.9) have isolated three peptides with ACE inhibitory activities, Met-Leu-Pro-Ala-Tyr, Val-Leu-Tyr-Arg-Asp-Gly and Ile-Val-Tyr, from a thermolysin hydrolysate of sesame proteins. Each peptide also showed an antihypertensive effect in spontaneously hypertensive rats when orally administered at the dose of 100 mg/kg.9,10)

In the present study, new peptides with high ACE inhibitory activity were isolated from a sesame protein hydrolysate and their amino acid sequences determined.
We also demonstrate the antihypertensive effects of their peptides in spontaneously hypertensive rats.

Materials and Methods

**Materials.** Sesame peptide powder (SPP), a thermo-lysin hydrolysate of sesame proteins known as GP-Powder B-20 was purchased from Kishimoto Sangyo (Osaka, Japan). The angiotensin I-converting enzyme (ACE) from rabbit lung and Val-Tyr were from Sigma-Aldrich (St. Louis, MO, USA). O-phthalaldehyde was from Nacalai Tesque (Kyoto, Japan), and Bz-Gly-His-Leu (HHL) was from Peptide Institute (Osaka, Japan). Synthetic peptides, the ACE inhibitors identified from SPP, were synthesized by Peptide Institute (Osaka, Japan). Bio-Gel P-2 gel was purchased from Bio-Rad Laboratories Japan (Tokyo, Japan). Develosil ODS-10, Develosil C30-UG-5 and Develosil Ph-UG-5 were from Nomura Chemical (Aichi, Japan), and TSK gel ODS-80Ts was from Tosoh Co. (Tokyo, Japan). All other reagents were of analytical grade.

**Purification of the ACE inhibitory peptides from SPP.** SPP was dissolved in 10% ethanol and treated in a Bio-Gel P-2 gel filtration column (15 mm ID × 820 mm) at a flow rate of 0.18 ml/min with 10% ethanol. The eluate was fractionated at intervals of 15 min, and monitored by both its absorbance at 210 nm and ACE inhibitory activity. The major active fractions were combined and lyophilized, dissolved in distilled water and subjected to reversed-phase HPLC in an ODS column (Develosil ODS-10, 20 mm ID × 250 mm) at a flow rate of 10 ml/min. Elution was by a gradient of acetonitrile containing 0.1% TFA (0–20 min, 5%; 20–80 min, 5–40%). The fractions were collected at intervals of 1 min, and the ACE inhibitory activity was measured. The active fractions were evaporated under reduced pressure and lyophilized. Each fraction was subjected to reversed-phase HPLC in a C30 column (Develosil C30-UG-5, 10 mm ID × 250 mm) with an adequate proportion of acetonitrile containing 0.1% TFA at a flow rate of 4 ml/min. The elution profile was monitored at 210 nm. Fractions were collected at intervals of 15 s, and were measured for their ACE inhibitory activity. Further purification was performed with a Ph column (Develosil Ph-UG-5, 10 mm ID × 250 mm), using isocratic elution with 6% acetonitrile containing 0.1% TFA at a flow rate of 4 ml/min, if necessary, for the amino acid sequence determination.

**Determination of the amino acid composition and amino acid sequence.** The purified peptide was subjected to amino acid, TOF-MS, and TOF-MS/MS analyses for determining the amino acid composition and amino acid sequence. For the amino acid analysis, a lyophilized peptide was hydrolyzed with 6 N HCl for 24 h at 110 °C in vacuo and then analyzed with an amino acid analyzer in a Shim pack Amino-Li column (Shimadzu Co., Japan). For the TOF-MS/MS analysis, the MS and MS/MS spectrum of the peptide was measured with Q-ToF apparatus (Waters Co., USA) in the positive-ion mode, using a cone voltage of 10 V, collision energy of 15 eV, and argon as the collision gas. Measurement of the contents of purified ACE inhibitory peptides in SPP. Leu-Val-Tyr, an ACE inhibitory peptide from SPP, was used as the standard peptide, and Val-Tyr was used as the internal standard peptide. Leu-Val-Tyr adjusted to 0.3, 3.0 or 15.0 μg/ml of 10% TFA was mixed with an equal volume of Val-Tyr (100 μg/ml of 10% TFA). The Full mass range MS/MS data were recorded for Leu-Val-Tyr and Val-Tyr at m/z 394.20 and 281.13 from their quasi-molecular ([M + H]+) ions. The most abundant daughter ions were at m/z 212.93 and m/z 182.00 for Leu-Val-Tyr and Val-Tyr, respectively. A calibration curve was established according to Leu-Val-Tyr concentration and ratio of the ion peak area at m/z 212.93 versus that at m/z 182.00. The resulting curve was linear (R² > 0.999) (data not shown).

One hundred and twenty-five mg of SPP was dissolved in 10% TFA to give 25 ml of the solution (final concentration of 5.00 mg/ml). Five hundred μl of this solution was mixed with an equal volume of the internal standard solution (100 μg/ml of Val-Tyr in 10% TFA) or in 10% TFA as a blank. SPP solutions with or without the internal standard peptide were analyzed by LC/MS and LC/MS/MS under the same conditions as those described for calibration. The relative contents of the purified ACE inhibitory peptides were determined by comparing the selected-ion-monitored areas of their quasi-molecular ions ([M + H]+) to that of Leu-Val-Tyr.

**Assay of ACE inhibitory activity.** The ACE inhibitory activity was measured by the modified method of Friedland and Silverstein.10 ACE was dissolved in a 0.1 M HEPES buffer (pH 8.3) containing 0.3 M NaCl and 0.01% Triton-X to give a 20 mM/μl solution. To measure the ACE activities, a sample (5 μl) was pre-incubated with the buffer (25 μl) and the enzyme solution (10 μl) at 37 °C for 5 min. An 8 mM HHL substrate in 20% DMSO (10 μl) was added, and the mixture was incubated at 37 °C for 30 min. After the reaction had been stopped by adding 0.1 N NaOH (40 μl), 20% of 1% o-phthalaldehyde in methanol was added, the mixture kept at room temperature for 10 min, and then 100 μl of 0.1 N HCl was added. After incubating for 30 min at 37 °C, the amount of liberated His-Leu was determined by measuring the fluorescent intensity of its adduct with o-phthalaldehyde (excitation at 355 nm and emission at 460 nm).

The SPP sample solution was adequately diluted with distilled water from the 1 mg/ml stock solution. The protein contents of the plasma and tissue extracts were measured with a Micro BCA Protein Assay reagent kit (Pierce, USA), using bovine serum albumin, fraction V (Pierce, USA), as a standard.
An ACE inhibitory assay for the synthetic peptide was similarly performed in triplicate. The purity of each peptide was determined by an amino acid analysis. A synthetic peptide was dissolved in 10% DMSO and adjusted to a net concentration of 1 mg/ml as a stock solution.

The $K_i$ value and inhibition mode of the three representative synthetic peptides, Leu-Val-Tyr, Leu-Gln-Pro and Leu-Lys-Tyr, were determined by Line-weaver–Burk plots and Dixon plots from experiments conducted at three substrate concentrations (0.8, 1.6, and 3.2 mM final concentration).

**Effect of a single oral administration of SPP and sesame peptides on SHR.** Male SHR (13 weeks old) were purchased from SLC, Inc. (Hamamatsu, Japan). The animals were provided with a standard rat feed (NMF; Oriental Yeast, Tokyo, Japan) and drinking water ad libitum for 2 weeks before experiment and housed at 24 ± 1°C and a relative humidity of 55 ± 5% under a constant 12 h light/dark cycle. The experimental protocol and animal care method in all the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences.

After the acclimatizing period, each animal (body weight of 300 to 400 g) with a systolic blood pressure (SBP) of up to 160 mm of Hg was deprived of food for 8 h before the oral administration. A single oral dose of 0.1, 1 or 10 mg/kg (0.05, 0.5 or 5 mg/ml, 2 ml/kg) of SPP was administered to each animal. The seven sesame peptides identified in this and a previous studies, Leu-Ser-Ala, Leu-Gln-Pro, Leu-Lys-Tyr, Ile-Val-Tyr, Val-Ile-Tyr, Leu-Val-Tyr, and Met-Leu-Pro-Ala-Tyr, were reconstituted according to the content ratio of each peptide in SPP. The peptide mixture was also administered at a dose of 3.63 or 36.3 mg/kg, corresponding to the respective peptide contents in 1 and 10 mg/kg of SPP.

Control rats were given the same volume of a vehicle (H$_2$O) alone. SBP was measured just before and 2, 4, 6, and 24 h after administration. The blood pressure was monitored by the tail cuff method with a pneumatic pulse transducer (BP-98A; Softron, Tokyo, Japan).

**Effect of repeated oral administration of SPP on SHR.** Male SHR were used to evaluate the effect of a chronic oral administration of SPP. Animals at 11 weeks of age (body weight of 294 ± 2 g) were separated into three groups. SPP (1 or 10 mg/kg once daily at A.M. 10:00) or the vehicle was orally administered to each animal for 2 weeks. SBP was monitored once a week by the tail cuff method just before the oral administration.

**Preparation of the tissue enzyme extract.** After the repeated oral administration of SPP (10 mg/kg/day) or the vehicle for 2 weeks, each group of three rats was anesthetized with sodium pentobarbital immediately after the SBP measurement. Blood was drawn from the abdominal aorta, and plasma was prepared immediately and stored at −40°C until being measured for ACE activities. The thoracic aorta, heart and kidney were enucleated and also stored at −40°C until preparation of the tissue enzyme extracts.

The tissue enzyme extracts of the organs were prepared by the modified method of Masuda et al. An organ was chopped into small pieces and weighed. Each chopped tissue was homogenized in 1 ml of 0.05 M HEPES (pH 7.9) containing 0.3 M NaCl with a Physcotron (Microtech-Nition). Thirty seconds of homogenization followed by 1 min of cooling on ice was conducted 2 or 3 times until the tissue was perfectly homogenized. The suspension was adjusted to 8 ml in volume with the same buffer and then centrifuged at 44,000 x g for 90 min. After the supernatant had been discarded, the pellet was resuspended in the buffer and centrifuged again under the same conditions. The obtained pellet was suspended in the buffer containing 0.5% Triton-X100 (40 ml/g for the aorta, 4 ml/g for the heart, and 4 ml/g for the kidney). Each suspension was kept for 1 h, before being centrifuged at 1,000 x g for 10 min. The resulting supernatant was used for measuring the ACE activity and protein content of the tissue enzyme extract.

**Measurement of the ACE activity of the tissue enzyme extract.** The plasma and tissue enzyme extracts were diluted with a 0.1 M HEPES buffer (pH 8.3) containing 0.3 M NaCl and 0.01% Triton-X. A diluted sample (10 μl) was mixed with the buffer (30 μl), and the ACE reaction was started by adding of the substrate (10 μl) as described for the Assay of ACE inhibitory activity. One unit (U) of ACE activity is defined as the amount of enzyme which released 1 μmol of His-Leu per min under the foregoing conditions. The specific activity of the plasma is expressed as mU/ml and that of each other tissue enzyme extract as mU/mg of protein.

**Measurement of the protein contents.** The protein contents of the plasma and tissue extracts were measured with a Micro BCA Protein Assay reagent kit (Pierce, USA), using bovine serum albumin, fraction V (Pierce, USA), as a standard.

**Statistical analysis.** Each value is expressed as the mean ± S.E.M. A one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple-comparison test were used for statistical analyses. Differences are considered significant at p < 0.05.

**Results**

**Isolation and identification of the ACE inhibitory peptides from SPP.** SPP was separated into its high-, intermediate- and low-molecular-weight fractions by Bio-Gel P-2 gel filtration column chromatography as shown in Fig. 1.
Fractions 34–39, the low-molecular-weight fraction, were collected and applied to an ODS column. The ACE inhibitory activity of each fraction and the acetonitrile concentration for HPLC are shown in Fig. 2. Five fractions, peaks A to E, showed high ACE inhibitory activities as shown in Fig. 2. Lyophilized fraction E was dissolved in 17% acetonitrile containing 0.1% TFA and then subjected to HPLC in a C30 column. The elution profile was monitored at an absorbance of 210 nm (Fig. 3). ACE inhibitory activity was detected in peak E-3 (fractions 68–74), so fraction 70 was subjected to amino acid, TOF-MS, and MS/MS analyses. The amino acid analysis showed that the fraction 70 consisted of Leu:Val:Tyr in a molar ratio of 1.00:0.83:0.82). Ion peaks at \( m/z = 394.19 \) (M + H)\(^{+}\) and 787.45 (2M + H)\(^{+}\) were observed in the MS analysis of fraction 70 (Fig. 4A), indicating that the molecular weight of fraction 70 was 393. In the MS/MS data of \( m/z = 394.21 \) as a parent ion, daughter ions at \( m/z \) 281.16, 213.15, 185.16 and 182.08 were detected as shown in Fig. 4B. These ion peaks were consistent with the theoretical daughter ion peaks that were speculated from the amino acid sequence of Leu-Val-Tyr. Finally, the amino acid sequence of the active peptide in peak E was determined to be Leu-Val-Tyr.

Peaks A, B, C and D, shown in Fig. 2, were also purified by C30 column chromatography. Peak B was further purified by HPLC in a Ph column. Peak D showed two peaks with ACE inhibitory activity by

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**Fig. 1.** Gel Filtration Chromatogram of Sesame Peptide Powder.

Eighty mg of sesame peptide powder was subjected to Bio-Gel P-2 gel filtration as described in the Materials and Methods section. ●, absorbance at 210 nm; unfilled bars, ACE inhibitory activity; ←→ fractions 34–39.

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**Fig. 2.** Reversed-Phase HPLC Chromatogram in an ODS Column of Fractions 34–39 in Fig. 1.

The chromatographic conditions are described in the Materials and Methods section. — ACE inhibitory activity of each fraction in the reversed-phase HPLC chromatogram; ——, acetonitrile concentration gradient.
HPLC in the C30 column. The amino acid sequences of the active peptides were determined by amino acid, TOF-MS, and MS/MS analyses. The amino acid sequences of the identified peptides are summarized in Table 1 with the purification procedure, amino acid composition, parent ions (m/z) by TOF-MS, and daughter ions (m/z) by TOF-MS/MS. Met-Leu-Pro-Ala-Tyr, which had previously been isolated from SPP,9) was also detected by LC/MS in this study (data not shown).

Characterization of the purified ACE inhibitory peptides

The ACE inhibitory activities (IC_{50}) of both SPP and a mixture of the seven peptides reconstituted according to the content ratio of each peptide in SPP were also measured. The contribution of each peptide to the ACE inhibition of SPP was calculated from both the content and IC_{50} data (Table 2). The sum of the contributions of the seven peptides to the ACE inhibition of SPP was 31.1%. This value is consistent with the value for the reconstituted peptide mixture (31.3%). Leu-Lys-Tyr contributed most among the identified sesame peptides in SPP.

All peptides showed potent ACE inhibitory activities as shown in Table 2. The ACE inhibitory activities (IC_{50}) of both SPP and a mixture of the seven peptides reconstituted according to the content ratio of each peptide in SPP were also measured. The contribution of each peptide to the ACE inhibition of SPP was calculated from both the content and IC_{50} data (Table 2). The sum of the contributions of the seven peptides to the ACE inhibition of SPP was 31.1%. This value is consistent with the value for the reconstituted peptide mixture (31.3%). Leu-Lys-Tyr contributed most among the identified sesame peptides in SPP.

K_i values for the three representative synthetic peptides, Leu-Val-Tyr, Leu-Gln-Pro and Leu-Lys-Tyr, were determined to be 0.92 μM, 0.50 μM and 0.48 μM by Dixon plots. Lineweaver–Burk plots also indicated that these peptides competitively inhibited ACE.

Table 1. Purification Procedure and Amino Acid Sequence Determination of the Sesame Peptides

<table>
<thead>
<tr>
<th>Fraction in Fig. 2.</th>
<th>Purification procedure and Ph-column HPLC^a</th>
<th>Amino acid composition</th>
<th>TOF-MS parent ions m/z</th>
<th>TOF-MS/MS daughter ions m/z</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak A</td>
<td>C30 column: 10%</td>
<td>Ser:Ala:Leu 1.00:1.21:0.93, 290.19</td>
<td>201.12, 177.09, 173.13</td>
<td>Leu-Ser-Ala</td>
<td></td>
</tr>
<tr>
<td>Peak B</td>
<td>C30 column: 11% Ph column: 6%</td>
<td>Glu:Pro:Leu 1.00:0.90:0.95, 357.20</td>
<td>244.13, 242.14, 214.15, 116.06</td>
<td>Leu-Gln-Pro</td>
<td></td>
</tr>
<tr>
<td>Peak C</td>
<td>C30 column: 13%</td>
<td>Leu:Tyr:Lys 1.00:0.93:0.86, 423.18</td>
<td>310.18, 242.14, 225.16, 197.13</td>
<td>Leu-Lys-Tyr</td>
<td></td>
</tr>
<tr>
<td>Peak D</td>
<td>C30 column: 14%</td>
<td>Val:Ile:Tyr 0.54:0.53:1.00, 394.24</td>
<td>281.16, 213.15, 185.16, 182.08</td>
<td>Ile-Val-Tyr</td>
<td></td>
</tr>
<tr>
<td>Peak E</td>
<td>C30 column: 17%</td>
<td>Leu:Val:Tyr 1.00:0.83:0.82, 394.21</td>
<td>281.16, 213.15, 185.16, 182.08</td>
<td>Leu-Val-Tyr</td>
<td></td>
</tr>
</tbody>
</table>

^aC30- or Ph-column HPLC for each peptide was carried out by isocratic elution with the indicated concentration of acetonitrile containing 0.1% TFA.
**Table 2.** Characterization of the Purified Sesame Peptides with ACE Inhibitory Activities

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC$_{50}$ (µM)</th>
<th>IC$_{50}$ (µg/ml)</th>
<th>Content in SPP (%)</th>
<th>Contribution Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesame peptide powder (SPP)</td>
<td>—</td>
<td>62.92</td>
<td>(100)</td>
<td>100</td>
</tr>
<tr>
<td>Leu-Val-Tyr</td>
<td>1.80</td>
<td>0.71</td>
<td>0.051</td>
<td>4.52</td>
</tr>
<tr>
<td>Leu-Ser-Ala</td>
<td>7.81</td>
<td>2.26</td>
<td>0.060</td>
<td>1.67</td>
</tr>
<tr>
<td>Leu-Gln-Pro</td>
<td>1.04</td>
<td>0.37</td>
<td>0.015</td>
<td>2.55</td>
</tr>
<tr>
<td>Leu-Lys-Tyr</td>
<td>0.78</td>
<td>0.33</td>
<td>0.087</td>
<td>16.6</td>
</tr>
<tr>
<td>Ile-Val-Tyr</td>
<td>14.74</td>
<td>5.80</td>
<td>0.059*</td>
<td>0.64</td>
</tr>
<tr>
<td>Val-Ile-Tyr</td>
<td>4.50</td>
<td>1.77</td>
<td>0.030*</td>
<td>1.07</td>
</tr>
<tr>
<td>Met-Leu-Pro-Ala-Tyr</td>
<td>1.58</td>
<td>0.94</td>
<td>0.061</td>
<td>4.08</td>
</tr>
<tr>
<td>Total</td>
<td>—</td>
<td>—</td>
<td>0.363</td>
<td>31.1</td>
</tr>
<tr>
<td>Peptide mixture*</td>
<td>—</td>
<td>0.73</td>
<td>0.363</td>
<td>31.3</td>
</tr>
</tbody>
</table>

*Data were determined from the content ratio of Ile-Val-Tyr and Val-Ile-Tyr by HPLC using a C30 column.

*The peptide mixture was reconstituted with these seven peptides according to the content of each in SPP.

*The contribution rate of each peptide was calculated as follows: (IC$_{50}$ of SPP/IC$_{50}$ of peptide) × (content in SPP).
Effect of a single oral administration of SPP on SHR

Figure 5A shows the time-course changes in SBP after an oral administration of SPP (0.1, 1 or 10 mg/kg) or vehicle to SHR. Basal SBP for each group was 177.4 ± 4.1, 180.6 ± 5.2, 182.9 ± 4.3, and 178.6 ± 3.0 mm of Hg, respectively. The administration of 0.1 mg/kg of SPP had no significant hypotensive effect. Both 1 and 10 mg/kg of SPP significantly decreased SBP 6 and 8 h after the administration, the magnitude of the reduction being similar. SBP had returned to the initial level within 24 h after the administration.

The time-course changes in SBP after an oral administration of the mixture (3.63 or 36.3 μg/kg) of the seven peptides identified from SPP, which had been prepared according to the proportion of each peptide in SPP, were also measured (Fig. 5B). Basal SBP of each mixture and the vehicle were also measured (Fig. 5B). Basal SBP of each mixture and the vehicle were also measured (Fig. 5B).

Effect of repeated oral administration of SPP on SHR

The effect of repeated oral administration of SPP on SHR was evaluated. SBP in the vehicle-treated group was continuously elevated from 158.9 ± 2.4 mm of Hg at 10 weeks of age to 184.4 ± 3.1 mm of Hg at 13 weeks of age (Fig. 6). SPP dose-dependently suppressed this SBP elevation 2 weeks after beginning the administration (172.8 ± 3.0 mm of Hg at 1 mg/kg/day of SPP, and 165.7 ± 3.2 mm of Hg at 10 mg/kg/day of SPP). During the 2 weeks of the experimental period, there was no significant difference in the body weight gain among all the experimental groups (data not shown).

The ACE activities of the plasma and tissue extracts from the rats administered with SPP (10 mg/kg/day) or the vehicle for 2 weeks are shown in Fig. 7. The ACE activities in the thoracic aorta and kidney of the rats administered with SPP were significantly lower than those administered with the vehicle, but no significant difference was apparent in either the heart or plasma.

Discussion

Several peptides derived from sour milk, a wheat germ hydrolysate, and a sardine muscle hydrolysate are well known to have ACE-inhibiting activities and to exhibit antihypertensive activities in experimental animals.15–17 The dominant ACE-inhibitory peptides are Val-Pro-Pro (IC50 of 9 μM) and Ile-Pro-Pro (IC50 of 5 μM) from sour milk,18 Ile-Val-Tyr (IC50 of 0.48 μM) from the wheat germ hydrolysate,19 and Val-Tyr (IC50 of 5.2 μM) from the sardine muscle hydrolysate.20 We
have demonstrated here the antihypertensive effects of a thermolysin digest of sesame protein on SHR. Among the six peptides identified in this study, three have been reported as ACE inhibitors from other origins, i.e., Leu-Gln-Pro from α-zein,20) Leu-Lys-Tyr from Antarctic krill21) and Ile-Pro-Pro from sour milk: 0.6 and 0.3 mg/kg, respectively21), in spite of their similar IC50 values. The reason for the effectiveness of the mixture of reconstituted peptides cannot be explained from the results of the present experiment.

It is known that small peptides, such as di- or tripeptides, are easily absorbed in their intact forms from the intestines,23,24) suggesting that the digestion and absorption of SPP-derived ACE inhibitory peptides are not markedly different from such other peptides as Val-Pro-Pro, Ile-Pro-Pro, and Val-Tyr. It is possible that the efficiency of the SPP-derived ACE inhibitory peptides may be related to the accumulation of these peptides in the vascular tissues. Alternatively, it cannot be dismissed that the SPP-derived ACE inhibitory peptides had other biological activity which could modulate the blood pressure (e.g., calcium antagonistic, direct vasodilatory, α-adrenergic receptor antagonistic, or central antihypertensive effects). A recent study has indicated that a small peptide such as carnosine (β-alanyl-L-histidine) exhibited a relaxant response in an isolated rat aorta,25) but we could not identify such an activity of SPP and the mixture of seven peptides in the same preparation (unpublished observation). Further studies should therefore be conducted to clarify the mechanism for the potent antihypertensive effect of the SPP-derived ACE inhibitory peptides.

Takai et al.26) have reported that treatment with an ACE inhibitor significantly reduced the blood pressure in SHR, and that a significant correlation was observed between SBP and ACE activity in the aorta, but not in the plasma or other tissues such as the heart and brain. Murakami et al.27) have demonstrated that a treatment with captopril lowered both the blood pressure and aortic ACE activity in stroke-prone SHR, whereas a treatment with hydralazine lowered the blood pressure without affecting ACE activity. This suggests that the lowering effect on the aortic ACE activity may not have resulted from the blood pressure reduction, and that the inhibition of aortic ACE activity may be most important for the hypotensive effects of ACE inhibitors on hypertensive rats.

The repeated oral administration of SPP in the present study lowered both the blood pressure and aortic ACE activity in SHR, indicating that repeated administration of SPP suppressed the blood pressure elevation in SHR through its inhibitory effect of ACE, especially inhibiting the aortic ACE activity.

In conclusion, we have demonstrated that both single and repeated oral administrations of SPP produced potent antihypertensive effects in vivo, at least in part, via the ACE inhibitory activity in the aorta. It seems likely that SPP would be a beneficial ingredient for a prophylactic and therapeutic treatment against hypertension and its related diseases.

Fig. 7. ACE Activities of the Plasma, Heart, Thoracic Aorta and Kidney from SHR Rats Administered with SPP (10 mg/kg/day) or the Vehicle for Two Weeks.

Each value represents the mean ± S.E.M. *p < 0.05, **p < 0.01; compared with the vehicle group.
References


