ELSEVIER

European Journal of Pharmacology 304 (1996) 93–98

ejp

Antihypertensive effect of angiotensin I-converting enzyme inhibitory peptides derived from hemoglobin

Kazue Mito [a], Mikio Fujii [a], Masayoshi Kuwahara [b], Nobuyasu Matsumura [a], Toshio Shimizu [a], Shigeru Sugano [b], Hideaki Karaki [b,*]

[a] *Foods Research Group, Asahi Chemical Industry Co. Ltd., Fuji-shi, Shizuoka 416, Japan*

[b] *Department of Veterinary Medical Science, Graduate School of Agriculture and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

Received 4 January 1996; revised 13 February 1996; accepted 16 February 1996

Abstract

From proteolytic digest of swine hemoglobin, we isolated four peptides, E-1 (Phe-Gln-Lys-Val-Val-Ala), E-2 (Phe-Gln-Lys-Val-Val-Ala-Gly), peptide 30–3 (Phe-Gln-Lys-Val-Val-Ala-Lys) and H-1 (Gly-Lys-Lys-Val-Leu-Gln). These peptides inhibited angiotensin I-converting enzyme activity with an IC_{50} of 5.8, 7.4, 2.1 and 1.9 μM, respectively. Oral administration of 50 mg/kg E-1 and 50 mg/kg H-1 decreased blood pressure in spontaneously hypertensive rats. In normotensive rats, oral administration of 500 mg/kg E-1 and 500 mg/kg H-1 inhibited the pressor effect of i.v. administrated 300 ng/kg angiotensin I, possibly by inhibiting its conversion to angiotensin II. These results suggest that these peptides are orally effective inhibitors of angiotensin I-converting enzyme that have a hypotensive effect.

Keywords: Angiotensin I-converting enzyme; Angiotensin I-converting enzyme inhibitor; Peptide; Hemoglobin; (Swine)

1. Introduction

It has been recognized that angiotensin I-converting enzyme (petidyldipeptide hydrolase, EC 3.4.15.1) plays a key role in regulating blood pressure (Skeggs et al., 1956). This concept has been confirmed by the fact that angiotensin I-converting enzyme inhibitors have a potent antihypertensive effect in both humans and animals (Ondetti et al., 1977). Recently, various peptides derived from food proteins were found to have angiotensin I-converting enzyme inhibitory activity. These foods include milk casein (Maruyama and Suzuki, 1982; Maruyama et al., 1987), fish (Kohama et al., 1988; Yokoyama et al., 1992; Karaki et al., 1993; Matsumura et al., 1993a,b), gelatin (Ohshima et al., 1979), baker's yeast (Kohama et al., 1990) and corn (Maruyama et al., 1989; Miyoshi et al., 1991a). Some of these angiotensin I-converting enzyme inhibitory peptides, given orally, inhibit the pressor effect of angiotensin I (Karaki et al., 1990, 1993) and decrease blood pressure in spontaneously hypertensive rats (Karaki et al., 1990, 1993; Miyoshi et al., 1991b), as summarized in Table 1. These results suggest that these peptides are absorbed from the digestive tract, inhibit endogenous angiotensin I-converting enzyme activity and decrease blood pressure. These results imply that the peptides inhibiting the angiotensin I-converting enzyme are produced from foods in the digestive tract and may play a physiological role. Thus, the importance of food proteins for cardiovascular function may be not only to support maintenance of the blood vessel walls but also to inhibit angiotensin I-converting enzyme activity and maintain normal blood pressure.

Hemoglobin is digested by proteolytic enzymes to make the heme-iron-enriched substance and globin peptides. The former is utilized as foodstuff and in pharmaceuticals containing easily absorbable iron (Eriksson, 1982) although the physiological effect of the latter is not understood. Here we report that globin peptides have angiotensin I-converting enzyme inhibitory activity and antihypertensive effects.

* Corresponding author. Department of Veterinary Medical Science, Graduate School of Agriculture and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan. Fax: (81) (3) 5802-2959; e-mail: ahkrki@hongo.ecc.u-tokyo.ac.jp

PII S0014-2999(96)00146-X

2. Materials and methods

2.1. Preparation of angiotensin I-converting enzyme inhibitory peptides

Hemoglobin was prepared from swine red blood cells according to the method of DeLoach et al. (1986) with slight modifications. Frozen red blood cells obtained from local abattoir were thawed and diluted with the same amount of water to hemolyze the cells. The pH of the hemolysate was adjusted to 5.2 with 1 M citric acid and the residual cell membrane was removed by filtration through a microfilter PSP-113 (0.1 μm; Asahi Chemical Industry, Tokyo, Japan). The filtrate (20 kg) was dissolved in 30 l water and adjusted to pH 11.0 with 5 N NaOH to denature hemoglobin. After 1 h at room temperature, the solution was adjusted to pH 9.0 with 1 M citric acid and 124 g Alcalase 0.6L (Novo Nordisk, Denmark) was added at 50°C. The pH was kept at 9.0 with 5 N NaOH throughout the reaction. The degree of hydrolysis was calculated from the amount of NaOH added to neutralize the reaction (Alder-Nissen, 1986). The degree of hydrolysis reached 15% after about 2 h and then 10 l of water was added and the mixture was filtered through an ultrafilter SIP-1013 (Asahi Chemical; 6000 Da cut off). The ultrafiltrate (40 l) containing 3.9% peptides was used as a source of the angiotensin I-converting enzyme inhibitory peptides.

Conditions for high-performance liquid chromatography were similar to those described previously (Matsumura et al., 1993a,b) unless otherwise stated. Amino-acid sequences were determined as described in the previous reports (Matsumura et al., 1993a,b).

2.2. Estimation of IC_{50} values

The angiotensin I-converting enzyme inhibitory activities were measured for synthetic peptides. They were synthesized by Fujiya (Tokyo, Japan) using a solid-phase method. The purity of the peptide was confirmed to be at least 99% by reverse-phase high-performance liquid chromatography. Peptides were hydrolyzed with hydrochloric acid and analysed by an amino-acid analyser to estimate the precise concentration of the peptide in a solution. Captopril was purchased from Research Biochemical (Natick, MA). For the estimation of IC_{50} values, inhibition of angiotensin I-converting enzyme activity was measured using at least seven different concentrations of the inhibitor to be tested. For each concentration, two series of experiments were performed. The IC_{50} values were calculated by a least-squares method and are shown as means and the 95% confidence limits.

2.3. Measurements of blood pressure

Male spontaneously hypertensive rats were purchased from Charles River (Japan). Rats were kept at 21 ± 2°C and 55 ± 5% humidity under 12-h light and 12-h dark conditions. 12–13-week-old spontaneously hypertensive rats (260–290 g body weight) having a systolic blood pressure of 186–205 mm Hg were used. Blood pressure was measured with a tail-cuff apparatus (BP-98; Softron, Tokyo, Japan).

Male Sprague-Dawley rats (8–10-week-old and about 300 g) were anesthetized with urethane (1.25 g/kg i.p.). Polyethylene cannulas were inserted into the abdominal aorta from the femoral artery for blood pressure recording, and into the femoral vein for i.v. injection of test agents, as described by Weeks and Jones (1960). The increase in arterial blood pressure in response to i.v. injection of 300 ng/kg angiotensin I was measured with a pressure transducer (Nihon Kohden TP-400T or TP-200T, Tokyo, Japan) connected to the arterial cannula. Peptides were given orally with 1 ml distilled water. Control rats were given the same volume of distilled water.

Results of the experiments are given as means ± S.E.M. Statistical analysis of the results was done by using Student's *t*-test.

3. Results

3.1. Extraction and purification of peptides

The ultrafiltrate (0.8 ml) containing 3.9% peptides (see Materials and methods) was loaded onto a Sep-Pak Plus C_{18} cartridge (Waters, Milford, MA) and washed with distilled water. Peptides were eluted with 15 (v/v) % acetonitrile followed by 30 (v/v) % acetonitrile. Nine other series of Sep-Pak treatments were performed to make total peptide content of 310 mg, and eluates were mixed together. The substances eluted from Sep-Pak cartridges with 15 (v/v) % acetonitrile were concentrated and subjected to ion-exchange high-performance liquid chromatography on a SP-5PW column (21.5 mm i.d. × 150 mm L; Tosoh, Tokyo, Japan), as shown in Fig. 1. The angiotensin I-converting enzyme inhibition assay indicated that fractions E and H had high inhibitory activities (Fig. 1).

Peptides in fraction H were purified by reverse-phase high-performance liquid chromatography with RP-18(e) columns. By this procedure, a fraction with high angiotensin I-converting enzyme inhibitory activity was obtained. This fraction contained about 143 μg of single peptide (H-1) and the amino-acid sequence was determined as Gly-Lys-Lys-Val-Leu-Gln.

Peptides in fraction E were purified with the same reverse-phase high-performance liquid chromatography protocol, followed by gel-permeation high-performance liquid chromatography (Asahipak GS-220). Five fractions with high angiotensin I-converting enzyme inhibitory activity were obtained by gel-permeation high-performance liquid chromatography. Two of them were sequenced as

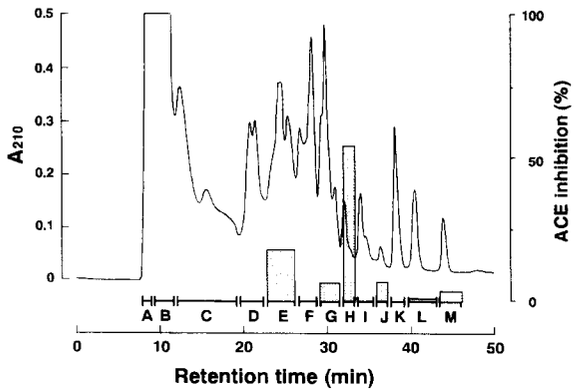

Fig. 1. Fractionation of peptides with an ion-exchange high performance liquid chromatography column. Peptides were eluted with a linear gradient of Na_2SO_4 (0–0.5 M) in 20 mM sodium acetate buffer (pH 5.5) at a flow rate of 4 ml/min. UV-absorbing peaks (left ordinate) were fractionated and desalted using Sep-Pak Plus C_{18} cartridges by washing with water and eluted with 50 (v/v) % acetonitrile solution. Angiotensin I-converting enzyme inhibitory activity of 5 mg of peptides in each fraction (right ordinate) was measured in a reaction volume of 76 μl.

Phe-Gln-Lys-Val-Val-Ala (E-1) and Phe-Gln-Lys-Val-Val-Ala-Gly (E-2). Two of the other three fractions contained 2.6 μg and 2.7 μg of peptide, respectively. Both peptides comprised seven-amino-acid residues and were found to have the same six-amino-acid sequence as E-1, Phe-Gln-Lys-Val-Val-Ala, at the N-terminal. The C-terminal amino acids of these two peptides were not determined by the Edman degradation. Approximately 10 μg of peptide existed in the last fraction. However, its amino-acid sequence was not determined since the fraction contained more than two peptides. Further purification of this fraction was not attempted.

Substances eluted with 30 (v/v) % acetonitrile from the Sep-Pak cartridge were purified on RP-18(e) columns twice followed by high-performance liquid chromatography on a GS-220 column. Some fractions contained a single peptide with high angiotensin I-converting enzyme inhibitory activity. Amino-acid sequence analyses indicated that one of the fractions contained a unique peptide, Phe-Gln-Lys-Val-Val-Ala-Lys (peptide 30–3). The amino-acid sequences of peptides in the other fractions were the same as that of either E-1, E-2 or H-1.

The amounts and potencies of the angiotensin I-converting enzyme inhibitory peptides isolated in the present experiments are summarized in Table 2. It is shown that the amino-acid sequences of E-1, E-2 and peptide 30–3 were identical except for their C-terminal amino-acid residues. However, the IC_{50} values were almost identical (5.8 μM for E-1, 7.4 μM for E-2, 2.1 μM for peptide 30–3 and 1.9 μM for H-1). The concentrations of these peptides needed to induce 20% inhibition and 80% inhibition were 1.3 and 21 μM for E-1, 2.0 and 50 μM for E-2, 1.0 and 7.9 μM for peptide 30–3 and 0.5 and 8.4 μM for H-1, respectively. The IC_{50} value for captopril was 10 nM (9–11 nM) under the present assay conditions (Table 1).

3.2. Antihypertensive effects

Peptides E-1 and H-1 were given orally to spontaneously hypertensive rats. The hypotensive effects were compared with that with captopril. As shown in Fig. 2, oral administration of 50 mg/kg E-1 or H-1 decreased blood pressure by approximately 30 mm Hg. Further increases in dose to 100 mg/kg did not have an additional effect. E-1 or H-1 at 25 mg/kg was ineffective. Oral administration of 10 mg/kg captopril decreased the blood pressure of spontaneously hypertensive rats from 172 ± 5 mm Hg to 150 ± 6 mm Hg in 30 min ($n = 4$).

In Sprague-Dawley rats, 300 ng/kg angiotensin I was i.v. administered every 30 min. Each administration produced a transient increase in blood pressure of a mean

Table 1
Effects of captopril and peptides to inhibit the angiotensin I-converting enzyme activity and to decrease blood pressure in spontaneously hypertensive rats

Source	Sequence [a]	IC_{50} (μM) [b]	Decrease in SBP mm Hg (at mg/kg p.o.)	References
Casein	FFVAPFPEVFGK	77	34 (100)	Karaki et al. (1990) Maruyama et al. (1987)
Casein	AVPYPQ	15	10 (100)	Karaki et al. (1990) Maruyama et al. (1987)
Casein	TTMPLW	16	14 (100)	Karaki et al. (1990) Maruyama et al. (1987)
Bonito bowels	GVYPHK	1.6	29 (200)	Karaki et al. (1993) Matsumura et al. (1993a)
Bonito bowels	IRPVQ	1.4	19 (100)	Karaki et al. (1993) Matsumura et al. (1993a)
Corn	LRP, LSP, LQP	0.27–1.9	12 (5000) [c]	Miyoshi et al. (1991a,b)
Hemoglobin	GKKVLQ	1.9	28 (50)	Present result
Hemoglobin	FQKVVA	5.8	32 (100)	Present result
Captopril		0.025	50 (10)	Ondetti et al. (1977)
		0.010	22 (10)	Present result

[a] Amino-acid sequence is shown by the single-letter notation. [b] Conditions for IC_{50} estimation are not identical. [c] Effect of crude product.

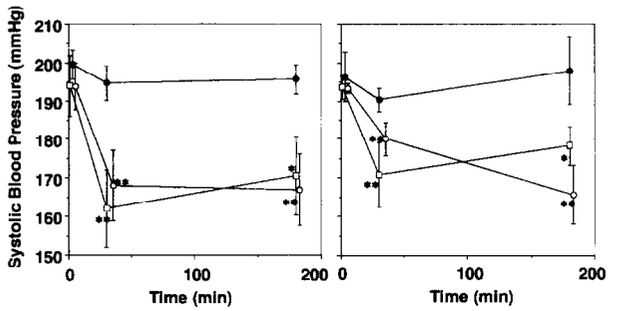

Fig. 2. Hypotensive effects of oral administration of angiotensin I-converting enzyme inhibitory peptides E-1 (left) and H-1 (right) in spontaneously hypertensive rats. Rats were orally given the peptide at 0 min. Systolic blood pressure was measured before and 30 min and 180 min after the peptide administration. ●, 25 mg/kg; ○, 50 mg/kg; □, 100 mg/kg. Each point represents mean of four rats and S.E.M. is shown by vertical bars. * and ** indicate significant difference from the value before the peptide administration with $P < 0.05$ and 0.01, respectively.

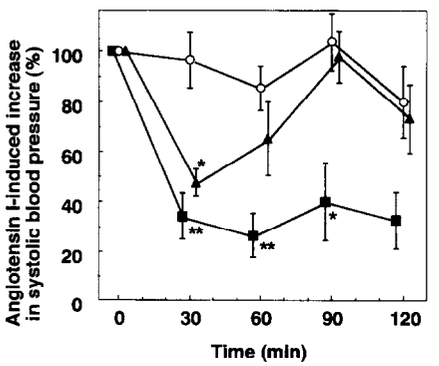

Fig. 3. Inhibitory effects of oral administration of angiotensin I-converting enzyme inhibitory peptides, E-1 and H-1, on angiotensin-I-induced pressor response. ○, control; ■, E-1; ▲, H-1. Pressor response due to 300 ng/kg i.v. injection of angiotensin I, measured before the peptide administration, was taken as 100%. Distilled water without (control, $n = 4$) or with E-1 (500 mg/kg, $n = 3$) or H-1 (500 mg/kg, $n = 3$) was orally given at time 0. Each point represents mean value and S.E.M. is shown by vertical bars. * and ** indicate significant difference from the value before the peptide administration with $P < 0.05$ and 0.01, respectively.

21.9 ± 3.3 mm Hg ($n = 10$). The pressor effect of angiotensin I was significantly inhibited 30, 60 and 90 min after the oral administration of E-1, and 30 min after the oral administration of H-1 (Fig. 3). The maximum inhibition was 73.6% at 60 min for E-1 and 52.3% at 30 min for H-1.

4. Discussion

In the present experiments, we isolated four peptides, E-1 (Phe-Gln-Lys-Val-Val-Ala), E-2 (Phe-Gln-Lys-Val-Val-Ala-Gly), peptide 30-3 (Phe-Gln-Lys-Val-Val-Ala-Lys) and H-1 (Gly-Lys-Lys-Val-Leu-Gln), from the proteolytic digest of swine hemoglobin (Table 2). The amino-acid sequences of E-1, E-2 and H-1 were found to be identical to the amino-acid residues 130–135, 130–136 and 64–69, of swine hemoglobin β-chain, respectively (Braunitzer et al., 1978). The amino-acid sequence of peptide 30–3 did not exist in the swine hemoglobin chains. Furthermore, no protein that contains the amino-acid sequence of peptide 30–3 was found in the protein-sequence database (Protein Information Resource, Release 40.00, National Biomedical Research Foundation, Washington, DC). One of the possibilities is that the origin of peptide 30–3 is a mutant globin chain. However, the amino-acid substitution of lysine for glycine does not result in a single-nucleotide substitution, insertion or deletion in the hemoglobin gene. Since the hemoglobin used in the present experiment was prepared from swine blood, the origin of peptide 30–3 might be a protein other than globin that is not listed in the protein-sequence database.

These peptides inhibited the activity of angiotensin I-converting enzyme. The IC_{50} values were almost identical (5.8 μM for E-1, 7.4 μM for E-2, 2.1 μM for peptide 30–3 and 1.9 μM for H-1) although the C-terminal amino-acid residues of E-1, E-2 and peptide 30–3 were different. This result suggests that C-terminal amino acids are not important for angiotensin I-converting enzyme inhibitory activity. Ondetti et al. (1977) have reported that captopril inhibits enzyme activity with an IC_{50} value of 0.025 μM. We obtained a similar IC_{50} value (0.01 μM) for captopril (Table 1). Although the inhibitory potencies of the peptides isolated in the present experiments were lower than that of captopril, they were comparable to those of the peptide inhibitors of angiotensin I-converting enzyme reported so far, as summarized in Table 1.

It has been shown that captopril is a potent antihypertensive agent that decreases blood pressure in rats at a dose of 10 mg/kg given orally (Ondetti et al., 1977). We

Table 2
IC_{50} values for the peptides derived from hemoglobin on the angiotensin I-converting enzyme

Peptide	Content (μg)	Amino-acid sequence (location in swine hemoglobin β-chain)	IC_{50} (μM)
E-1	20.4	Phe-Gln-Lys-Val-Val-Ala (130–135)	5.8 (5.2–6.4)
E-2	54.3	Phe-Gln-Lys-Val-Val-Ala-Gly (130–136)	7.4 (7.0–7.8)
Peptide 30–3	5.9	Phe-Gln-Lys-Val-Val-Ala-Lys (n.f.)	2.1 (2.0–2.2)
H-1	187.2	Gly-Lys-Lys-Val-Leu-Gln (64–69)	1.9 (1.6–2.1)

Peptide content is the amount isolated from hemoglobin digest containing 310 mg peptides. IC_{50} values for angiotensin I-converting enzyme were determined using synthetic peptides and mean value with 95% confidence limit are shown. n.f., not found in the hemoglobin β-chain.

confirmed that the same dose of captopril decreased blood pressure in spontaneously hypertensive rats (Table 1). To know if the peptides isolated in the present experiments have antihypertensive effects, E-1 and H-1 were given to spontaneously hypertensive rats. Oral administration of E-1 and H-1 decreased the blood pressure of spontaneously hypertensive rats. The potency of these peptides was comparable to that of the angiotensin I-converting enzyme inhibitory peptides derived from milk casein and bonito bowels, although weaker than that of captopril (Table 1).

We also examined the effect of these peptides on the pressor effect of angiotensin I, to confirm that the antihypertensive effects of E-1 and H-1 are due to inhibition of angiotensin I-converting enzyme activity. In normotensive rats, oral administration of E-1 and H-1 inhibited the pressor effect of i.v. administered angiotensin I. This result suggests that both E-1 and H-1 are orally effective inhibitors of angiotensin I-converting enzyme and have a hypotensive effect.

Tables 1 and 2 also show that the inhibitory potencies of the peptides on angiotensin I-converting enzyme activity and the hypotensive effects are not necessarily correlated. Thus, peptide E-1, which had less inhibitory effect on the enzyme than H-1, showed stronger inhibition of the angiotensin I-induced pressor response than H-1 did. This difference may be due to a difference in the stability of the peptides in the gastrointestinal tract and a difference in their absorption ratio.

Both E-1 and H-1 were almost ineffective at 25 mg/kg whereas at 50 mg/kg they showed an almost maximum inhibitory effect on blood pressure, indicating that the effective range of these peptides is only 2-fold. In the in vitro assay, in contrast, 0.5-1.3 μM E-1 and H-1 showed approximately 20% inhibition whereas 8.4–21 μM E-1 and H-1 showed approximately 80% inhibition, indicating that the effective range is at least 16-fold. The reason for this difference remains to be clarified.

Lantz et al. (1991) reported that the hemoglobin β-chain contains an amino-acid sequence corresponding to hemorphin (34–39 amino-acid residues), which inhibits angiotensin I-converting enzyme activity. This finding, together with our results, indicates that the hemoglobin β-chain contains at least three-amino-acid sequences related to the inhibition of angiotensin-converting enzyme. Lantz et al. (1991) also suggested that hemorphin may be the endogenous modulator of angiotensin I-converting enzyme and hence blood pressure. In the present experiments, however, peptides corresponding to hemorphin was not identified. This difference may be due to the fact that our peptides were obtained under the conditions at high pH and high temperature. The possibility that the hemoglobin-derived peptides with angiotensin I-converting enzyme inhibitory activity are the endogenous modulators of blood pressure needs further examination.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

References

Alder-Nissen, J., 1986, Some fundamental aspects of food protein hydrolysis, in Enzymic Hydrolysis of Food Proteins, Elsevier, London, UK, pp. 9.

Braunitzer, G., B. Schrank, A. Stangl and U. Scheithauer, 1978, Die Sequenzanalyse des Haemoglobins des Schweines, Hoppe Seyler's Z. Physiol. Chem. 359, 137.

DeLoach, J.R., C.L. Sheffield and G.E. Spates, 1986, A continuous-flow high-yield process for preparation of lipid-free hemoglobin, Anal. Biochem. 157, 191.

Eriksson, C., 1982, Hemiron-enriched amino acid preparation and a process for the preparation of hemiron-enriched amino acid preparation from hemprotein, Eur. Pat. Appl. No. 61556.

Karaki, H., K. Doi, S. Sugano, H. Uchiwa, R. Sugai, U. Murakami and S. Takemoto, 1990, Antihypertensive effect of tryptic hydrolysate of milk casein in spontaneously hypertensive rats, Comp. Biochem. Physiol. 96C, 367.

Karaki, H., M. Kuwahara, S. Sugano, C. Doi, K. Doi, N. Matsumura, and T. Shimizu, 1993, Oral administration of peptides derived from bonito bowels decreases blood pressure in spontaneously hypertensive rats by inhibiting angiotensin converting enzyme, Comp. Biochem. Physiol. 104C, 351.

Kohama, Y., S. Matsumoto, H. Oka, T. Teramoto, M. Okabe and T. Mimura, 1988, Isolation of angiotensin-converting enzyme inhibitor from tuna muscle, Biochem. Biophys. Res. Commun. 155, 332.

Kohama, Y., Y. Nagase, H. Oka, T. Nakagawa, T. Teramoto, N. Maruyama, H. Tsujibo, Y. Inamori and T. Mimura, 1990, Production of angiotensin-converting enzyme inhibitors from baker's yeast glyceraldehyde-3-phosphate dehydrogenase, J. Pharmacobiol. Dyn. 13, 766.

Lantz, I., E. Glamsta, L. Talback and F. Nyberg, 1991, Hemorphines derived from hemoglobin have an inhibitory action on angiotensin converting enzyme activity, FEBS Lett. 287, 39.

Maruyama, S. and H. Suzuki, 1982, A peptide inhibitor of angiotensin I converting enzyme in the tryptic hydrolysate of casein, Agric. Biol. Chem. 46, 1393.

Maruyama, S., H. Mitachi, J. Awaya, M. Kurono, N. Tomizuka and H. Suzuki, 1987, Angiotensin I-converting enzyme inhibitory activity of the C-terminal hexapeptide of α_{S1}-casein, Agric. Biol. Chem. 51, 2557.

Maruyama, S., S. Miyoshi, T. Kaneko and H. Tanaka, 1989, Angiotensin I-converting enzyme inhibitory activities of synthetic peptides related to the tandem repeated sequence of a maize endosperm protein, Agric. Biol. Chem. 53, 1077.

Matsumura, N., M. Fujii, Y. Takeda and T. Shimizu, 1993a, Isolation and characterization of angiotensin I-converting enzyme inhibitory peptides derived from bonito bowels, Biosci. Biotech. Biochem. 57, 1743.

Matsumura, N., M. Fujii, Y. Takeda, K. Sugita and T. Shimizu, 1993b, Angiotensin I-converting enzyme inhibitory peptides derived from bonito bowels autolysate, Biosci. Biotech. Biochem. 57, 695.

Miyoshi, S., H. Ishikawa, F. Fukui, H. Tanaka and S. Maruyama, 1991a, Structure and activity of angiotensin-converting enzyme inhibitors in an α-zein hydrolysate, Agric. Biol. Chem. 55, 1313.

Miyoshi, S., T. Kaneko, Y. Yoshizawa, F. Fukui, H. Tanaka and S. Maruyama, 1991b, Hypotensive activity of enzymatic α-zein hydrolysate, Agric. Biol. Chem. 55, 1407.

Ohshima, G., H. Shimabukuro and K. Nagasawa, 1979, Peptide inhibitors of angiotensin I-converting enzyme in digests of gelatin by bacterial collagenase, Biochem. Biophys. Acta, 556, 128.

Ondetti, M.A., B. Rubin and D.W. Cushman, 1977, Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents, Science, 196, 441.

Skeggs, L.T., J.E. Kahn and N.P. Shumway, 1956, The preparation and function of the angiotensin-converting enzyme, J. Exp. Med. 103, 295.

Weeks, J.R. and J.A. Jones, 1960, Routine direct measurement of arterial pressure in unanesthetized rats, Proc. Soc. Exp. Biol. Med. 104, 646.

Yokoyama, K., H. Chiba and M. Yoshikawa, 1992, Peptide inhibitors of angiotensin I-converting enzyme from thermolysin digest of dried bonito, Biosci. Biotech. Biochem. 56, 1541.