SII-2

BIOACTIVE PEPTIDES DERIVED FROM FOOD PROTEINS AND PREVENTION OF LIFE-STYLE RELATED DISEASES

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Summary

Two opioid peptides, YPLDL and YPLDLF, were isolated from enzymatic digests of spinach ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) and named rubiscolin-5 and -6, respectively. These peptides were selective for delta-receptor and the latter was about 3 times more potent than the former. After oral administration in mice at the dose of 100 mg/kg, rubiscolin-6 showed analgesic activity in tail pinch test. It also stimutated learning performance at the same dose in passive avoidance experiment using step-through apparatus.

An immunostimulating peptide, MITLAIPVNKPGR, was isolated from a trypsin digest of soybean protein and named soymetide. Immunostimulating activy of soymetide was mediated by fMLP receptor. Interestingly, after oral administration in rats at a dose of 300 mg/kg (po.), soymetide-4 (MITL) protected alopecia (hair-loss) induced by etoposide, a cancer chemotherapy agent. Stimulation of IL-1 release by the peptide was involved in the mechanism.

Ovokinin(2-7), RADHPF, is a vasorelaxing peptide released from ovalbumin by the action of chymotrypsin. It lowered blood pressure of spontaneously hypersensive rats (SHR) after oral administration at a dose of 10 mg/kg. RPLKPW, which was designed by replacing 4 amino acid residues in ovokinin(2-7), exhibited hypotensive activity at a dose of 0.1 mg/kg (po.). This peptides was introduced into 3 homologous sites in soybean beta-conglycinin alpha' subunit by site-directed mutagenesis of the cDNA and expressed in E. coli. The minimum effective dose for hypotensive activity of the genetically modified beta-conglycinin alpha' subunit was 10 mg/kg (po.), which is about 1/200 that of ovalbumin.

INTRODUCTION

Since the isolation of an opioid peptide beta-casomorphin from casein peptone by Brantl et al., many bioactive peptides have been found in enzymatic digests of food proteins (1-8). Among them, angiotensin-I converting enzyme inhibitors preventing hypertension, casein phosphophopeptide stimulating calcium absorption, soybean protein lowering serum cholesterol, globin digest lowering serum triglycerides, and wheat globulin delaying starch digestion are admitted as constituents of

"Food for Specified Health Use" (FOSHU) in Japan. Here, we isolated and disigned new bioactive peptides which are expected to be effective in preventing life-style related diseases.

MATERIALS AND METHODS

Opioid activity and learnung performance was measured as described in previous papers (4, 9).

Stimulatory activity for phagocytosis by human polymorphonuclear leukocyte was measured by flowcytometry. To test protective effect on alopecia induced by cancer chemotherapeutic agent etoposide, which was given for 3 days at a dose of 1.0 mg/kg *ip*., peptide was given orally for 8 days at a dose of 300 mg/mg starting 5 days before the agent (10). Photograph was taken 7 days after the final dosage.

Antihypertensive activity in spontaneously hypertensive rats (SHR) was measured as described previously (11).

RESULTS AND DISCUSSION

Opioid peptide derived from plant Rubisco stimulating learning performance in mice

Some opioid peptides isolated from protein digests have YPX sequence; X are aromatic amino acid residues in beta-casomorphin (YPFPGPI) and hemorphin (YPWTQ) (1, 3), while X are non-aromatic residues in gluten exorphin A (GYYPT) , gluten exorphin C (YPISL) and neocasomorphin (YPVEPF) (4-6). We found that YPLDL and YPLDLF sequecnce existing in large subunit of ribulose-bisphosphate carboxylase (Rubisco) from many plant leaves have opioid activity and named them rubiscolin-5 and -6, respectively (12). Both are selective for delta-receptor as gluten exorphins A and C are. Rubiscolin-6 was about 3 times more potent than rubiscolin-5 (Table 1). Rubiscolin-6 showed

Table 1. Opioid activities and receptor affinities of rubiscolin-5 and -6.

Peptides	Opioid activities IC_{50} (μM)			Receptor affinities IC_{50} (μM)		
	GPI	MVD	GPI/MVD	[^{3}H]-DAMGO	[^{3}H]-Delt II	[^{3}H]-DPDPE
YPLDL rubiscolin-5	1110±71	51.0±6.6	21.8	1085±165	2.09±0.06	1.97±0.30
YPLDLF rubiscolin-6	748±207	24.4±3.6	30.7	>2000	0.93±0.04	0.90±0.29

analgesic activity by tail pinch test in mice after oral administration at a dose of 100 mg/kg). Previously, we found that gluten exorphins A stimulated learning performance in mice after oral administration at a dose of 300 mg/kg (po.). Rubiscolin-6 also stimulated learning performance in mice at a dose of 100 mg/kg (po.) in passive avoidance experiment using step-through apparatus (Fig. 1).

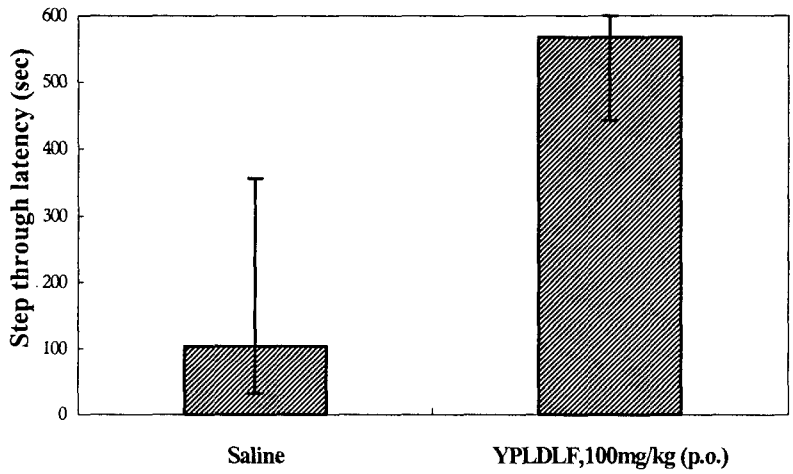

Fig. 1. Stimulation of learning performance by rubiscolin-6 in passive avoidance experiment

Immunostimulating peptide derived from soybean protein preventing alopecia induced by cencer-chemotherapy

We isolated a peptide stimulating phagocytosis by human polymorphonuclear leukocytes from a trypsin digest of soybean protein. The structure of the peptide was MITLAIPVNKPGR, which corresponds to the residues No.173-185 of beta-conglycinin alpha' subunit. Methionyl residue at the amino terminus was essential for the activity. Therefore, we named the peptide soymetide. In spite of the absence of formyl group at the amino terminus, soymetide showed weak affinity for fMLP receptor. Furthermore, the immunostimulating activity was blocked by Boc-MLF, an fMLP antagonist suggesting that it was mediated by fMLP receptor. Among truncated peptides, soymetide-4 (MITL) had marginal activity in stimulating phagocytosis in vitro However, the peptide was the most potent in stimulating TNF release after oral administration. Interestingly, soymetide-4 orally given for 8 days at a dose of 300 mg/kg completely prevented alopecia induced by etoposide, a cancer chemotherapy agent (Fig. 2). fMLP failed to show similar effect after oral administration. However, fMLP showed essentially the same effect after ip. administration suggesting that it is not absorbed from intestine. IL-1 induced by the peptide seems to be involved in anti-alopecic mechanism because it was blocked by Lys-D-Pro-Thr, an IL-1 inhibitor.

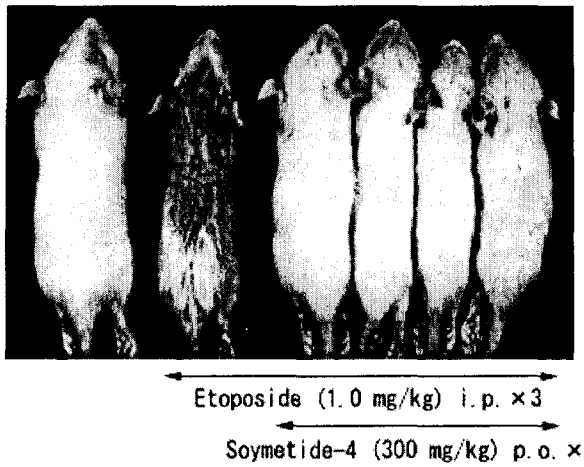

Fig. 2. Protective effect of soymetide-4 on cancer chemotherapy-induced alopecia.

Designing a genetically modified soybean protein preventing hypertension by introducing a potent deriveative of hypotensive peptide derived from ovalbumin

Previously, we isolated RADHPF as a nitric oxide-dependent vasorelaxing peptide from a chymotrypsin digest of ovalbumin and named it vokinin(2-7) (7). The peptide was derived from resisues No.359-364 of ovalbumin. The minimum effective dose of ovokinin(2-7) to lower blood pressure in SHR after oral administration was 10 mg/kg. If we assume that dose response of ovokinin(2-7) in human is equal to that in SHR, we have to take about 20 eggs a day to get the hypotensive effect. So, we tried to potentiate hypotensive activity of ovokinin(2-7) by replacing some amino acid residues, and to introduce the potent derivative obtained into food protein by site-directed mutagenesis of the gene. Among many of ovokinin(2-7) derivatives synthesized, RPLKPW was the most potent in hypotensive activity with a minimum effective dose of 0.1 mg/kg, which is 1/100 that of ovokinin(2-7).

Three sites having homology to RPLKPW were found in the primary structure of beta-conglycinin alpha'subunit. Then we introduced RPLKPW sequence by site-directed mutagenesis of expression vector for the alpha'subunit which was supplied by Prof. Utsumi. After expression in E. coli, the modified protein was recovered in the supernatant of the cell lysate, and purified by ammonium sulfate fractionation and ion exchange chromotography. The genetically modified beta-conglycinin alpha' subunit containing 3 RPLKPW sequence effectively loweded blood pressure of SHR after oral administration at a dose of 10 mg/kg. No hypotensive activity was observed at 5 mg/kg suggesting that minimum essential dose of the modified protein to be 10 mgkg. Native beta-conglycinin alpha' subunit produced in E. coli did not reduce blood pressure even at a dose of 20 mg/kg (Fig. 3).

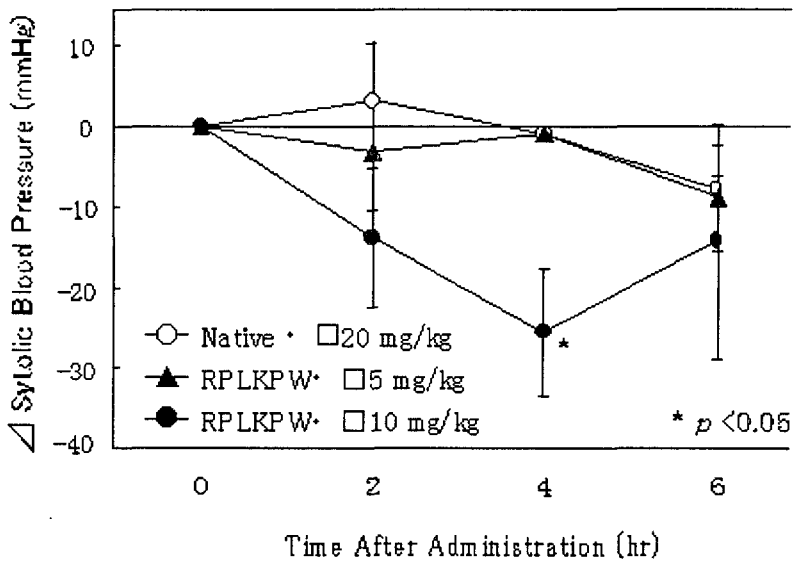

Fig. 3. Anti-hypertensive activity of RPLKPW-containing beta-conglycinin alpha' subunit after oral administration in SHR.

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