RESEARCH ARTICLE

Calcium-sensing receptor mediates dietary peptide-induced CCK secretion in enteroendocrine STC-1 cells

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Scope: Dietary peptides are potent stimulators of cholecystokinin (CCK) secretion, but the sensing mechanism in CCK-producing cells is poorly understood. Recently, it has been demonstrated that the calcium-sensing receptor (CaSR) mediates CCK secretion induced by amino acids. We investigated the role of CaSR in CCK secretions induced by various protein hydrolysates (egg albumin, meat, casein, azuki bean, soybean ß-conglycinin, and potato) in the enteroendocrine cell line STC-1.

Methods and results: CCK secretions in response to these hydrolysates were measured in the STC-1 cells with or without CaSR antagonist (NPS 2143) treatment. Changes in intracellular calcium concentration ($[Ca^{2+}]_i$) in response to protein hydrolysates were measured in Human embryonic kidney (HEK) 293 cells transfected with CaSR-expression vector. Protein hydrolysates-induced CCK secretions were decreased by CaSR antagonist treatment, except meat hydrolysate-induced secretion. Protein hydrolysates increased $[Ca^{2+}]_i$ in CaSR-transfected HEK 293 cells. CaSR antagonist treatment suppressed low molecular weight fractions of azuki hydrolysate-induced CCK secretion, but the secretion induced by both low and high molecular weight fractions of ß-conglycinin hydrolysate. Further, CCK secretion induced by peptide fractions (>500 Da) derived from various protein hydrolysates were also reduced by CaSR antagonist.

Conclusion: These results demonstrate that CaSR plays a significant role in sensing various dietary peptides in triggering CCK secretion in enteroendocrine cells.

Keywords:
Calcium-sensing receptor / Cholecystokinin / HEK 293 cells / Protein hydrolysate / STC-1 cells

1 Introduction

Luminal chemosensing is involved in the digestion and absorption of nutrients through neurohormonal control. Gut hormones mediate nutrient signals through the vagus nerve from the gut to the brain [1]. The secretion of gut hormones is induced by dietary components through the activation of nutrient sensors, including specific G protein-coupled receptors (GPCRs) and transporters in enteroendocrine cells [2, 3].

The gut hormone cholecystokinin (CCK) is secreted from enteroendocrine "I cells" located in the proximal small intestine, which regulates gallbladder contraction, pancreatic enzyme secretion, gastric emptying, and appetite. Dietary proteins, peptides, and fatty acids stimulate CCK secretion in mice, rats, and humans [4]. Although a recent paper showed contradictory result that intestinal tissue from rats did not respond to proteins and peptides for CCK release [5], previous studies have demonstrated that protein hydrolysates and fatty acids directly stimulate I cells to trigger CCK secretion in rats [6], in isolated I cells [7, 8], and in the enteroendocrine cell line STC-1 [4, 9].

The extracellular calcium-sensing receptor (CaSR) is one member of the class C family of GPCRs. CaSR was cloned from bovine parathyroid gland and had been well known to be

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Abbreviations: **βcon**, β-conglycinin; **CaSR**, calcium-sensing receptor; **CCK**, cholecystokinin; **HEK**, human embryonic kidney; **L-Phe**, L-Phenylalanine

involved in serum calcium homeostasis [10]. CaSR is not only activated by extracellular calcium but is also activated by multiple ligands, such as aromatic amino acids [11], polyamines [12, 13], and basic polypeptides (protamine, poly-lysine, and poly-arginine) [14, 15]. Expression of CaSR has been demonstrated in various tissues, including the CCK-producing cells in the intestine [16].

CCK secretions induced by specific amino acids and peptides have been reported to be mediated by CaSR. L-Phenylalanine (L-Phe) but not D-Phenylalanine has been known to stimulate CCK secretion [8, 17]. The activation of CaSR is required for CCK secretion induced by L-Phe in STC-1 cells [18] and in primary CCK-producing cells [8, 16]. Previously, we demonstrated that an arginine-rich ß51–63 peptide (VRIRLLQRFNKRS) in soybean ß-conglycinin activates CaSR to stimulate the secretion of CCK in STC-1 cells [19]. Recently, several γ-glutamyl peptides, such as γ-Glu-Cys-Gly (glutathione) and γ-Glu-Val-Gly, were identified as CaSR agonists [20]. However, it is still unclear whether CaSR is commonly involved in the CCK secretion induced by protein hydrolysates.

In this study, we investigated whether the CCK secretion induced by various protein hydrolysates is affected by CaSR antagonist treatment in the murine CCK-producing enteroendocrine cell line STC-1. Activation of CaSR by various protein hydrolysates was examined in Human embryonic kidney (HEK) 293 cells transfected with a CaSR-expression vector. Furthermore, we investigated whether the effect of the protein hydrolysates are dependent on the molecular weight of the peptides or free amino acids within these hydrolysates.

2 Materials and methods

2.1 Materials

Cell culture consumables (Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin/streptomycin, and trypsin-EDTA solution) were purchased from Invitrogen (Carlsbad, CA). Fura-2-AM and Pluronic F-127 were obtained from Molecular Probes (Leiden, the Netherlands). NPS 2143 was chemically synthesized by method described in the literature [21], and its activity was determined using HEK 293 cells that were transiently transformed with CaSR [20]. Poly-L-lysine solution (0.1%), Hepes, albumin chicken egg hydrolysate (AEH), peptone enzymatic hydrolysate type I from meat (MHY), peptone from potatoes (potato), and tryptone from casein (CTP) were purchased from Sigma (St. Louis, MO). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise specified. Some protein hydrolysates (ß-conglycinin (ßcon), azuki, and casein) were prepared by previously described methods [22]. The molecular weight distributions of the protein hydrolysates (Table 1) were determined with size-exclusion chromatography in an FPLC system (AKTA explorer 10S, GE Health Sciences). The free amino acid composition of the protein hydrolysates (Table 2) was determined with a previously described method [23].

Table 1. Molecular weight distributions of protein hydrolysates

Protein hydrolysate	Average molecular weight	% of total area		
		MW >2000	MW 500–2000	MW <500
AEH	567.0	2.7	34.4	62.9
MHY	4279.1	62.6	25.1	12.3
Azuki	3496.3	53.0	35.2	11.8
Casein	5270.7	73.4	16.9	9.7
ßcon	6011.2	58.7	30.3	11.0
Potato	612.3	4.6	28.6	66.8
CTP	918.0	8.2	51.9	39.9

These profiles were obtained by FPLC using a Superdex Peptide 10/300 GL column.

Table 2. Free amino acid composition in protein hydrolysates

	Protein hydrolysate						
	AEH	MHY	Azuki	Casein	ßcon	Potato	CTP
	μg/5 mg protein hydrolysate						
Asp[a)]	3	15	8	–	–	29	–
Glu[b)]	22	39	11	–	–	30	6
Ser	43	34	–	–	–	113	13
Gly	24	31	–	–	–	26	15
His	7	15	–	–	–	41	11
Arg	50	109	4	–	–	100	75
Thr	20	26	–	–	–	87	24
Ala	24	39	–	–	–	44	13
Plo	4	7	–	–	–	8	5
Tyr	21	14	–	–	3	91	18
Val	22	38	–	–	–	139	44
Met	35	9	–	–	–	30	34
Cys	11	–	–	29	–	13	31
Ile	17	28	–	–	–	155	68
Leu	101	75	–	–	7	240	197
Phe	32	42	–	–	–	77	68
Trp	6	10	–	–	–	14	24
Lys	40	49	–	–	3	99	175

a) Includes Asn.
b) Includes Gln; –, not detected.

2.2 Cell culture

STC-1 cells (a gift from Dr. Hanahan, University of California, San Francisco, CA) and HEK 293 cells (JCRB9068, Human Science Research Resources Bank, Osaka, Japan) were grown in Dulbecco's modified Eagle's medium (Invitrogen, Cat. No. 12 100–038) supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, and 500 μg/mL streptomycin in a humidified 5% CO_2 atmosphere at 37°C. Cells

were routinely subcultured by trypsinization upon reaching 80–90% confluency.

2.3 CCK secretion study in STC-1 cells

STC-1 cells were seeded in 48-well culture plates at a density of 1.25×10^5 cells/well, and grown for 2–3 days until they reached 80–90% confluency. Cells were washed twice with Hepes buffer to remove the culture media and then exposed to the test agents (dissolved in Hepes buffer) for 60 min at 37°C. The Hepes buffer (pH 7.4) had the following composition: 140 mM NaCl, 4.5 mM KCl, 20 mM Hepes, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose, and 0.1% BSA. Following incubation, the supernatants were collected and centrifuged at 800 g for 5 min at 4°C to remove the remaining cells. Supernatants were then stored at −50°C until measurement of the CCK concentration using a commercial enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Belmont, CA). The primary antiserum provided in this kit cross-reacts 100% with sulfated and non-sulfated CCK (26–33), CCK-33 (porcine), caerulein, gastrin-1 (human), and big gastrin-1 (human). The antiserum also cross-reacts 12.6% with CCK (30–33) and 0% with pancreatic polypeptide (human) and Vasoactive intestinal peptide (human, porcine, and rat). Because STC-1 cells do not express detectable levels of gastrin [9], we selected an EIA kit in which the antibody cross-reacts with gastrin. The coefficients of intra-assay and inter-assay variation were $< 5\%$ and $< 14\%$, respectively.

2.4 Measurement of cytotoxicity in STC-1 cells

Cytotoxic effects on STC-1 cells were determined by release of lactate dehydrogenase (LDH) in the supernatant of STC-1 cells exposed to protein hydrolysates with or without CaSR antagonist as described above. The measurement of LDH was performed by using Cytotoxicity detection kit (Roche, Basel, Switzerland) according to the instrument manual. Cytotoxicity was calculated as relative release (%) of LDH after exposure to protein hydrolysates ± CaSR antagonist compared to total LDH (as 100%) released by treatment with lysis reagent.

2.5 Measurement of intracellular calcium concentration ($[Ca^{2+}]_i$) in HEK 293 cells expressing CaSR

HEK 293 cells were grown on 0.025% poly-L-lysine-coated coverslips (1.3 cm diameter) at a density of 1.0×10^5 cells/well in 24-well plate for 24 h, and then they were transiently transfected with an expression vector (pcDNA 3.1, Invitrogen) containing a mouse CaSR cDNA fragment (location between 521–3256 in NM_013 803) by using Lipofectamine 2000 for 48 h. As negative control, HEK 293 cells were treated only with Lipofectamine 2000 during transfection. We confirmed an increase in CaSR mRNA via RT-PCR in HEK 293 cells transfected with the CaSR vector but not in control cells. The $[Ca^{2+}]_i$ in cells grown on coverslips was determined using a dual-excitation spectrofluorophotometer (CAF-110; JASCO, Tokyo, Japan) with the Ca^{2+}-sensitive ratiometric dye fura-2-AM. Cells cultured on coverslips were loaded with 2 μM fura-2-AM dissolved in Hepes buffer (pH 7.4) containing 0.005% Pluronic F-127 at 37°C for 20 min. The concentration of calcium in the Hepes buffer was adjusted to 1.0 mM to observe CaSR activation clearly [19, 20]. After loading fura-2-AM, the coverslip was mounted into the holder and washed with Hepes buffer. The holder was then inserted into the cuvette of the spectrofluorophotometer, and the experimental liquid was continuously stirred in the cuvette at 1 000 rpm at 25°C. Fluorescence intensities were measured at an emission wavelength of 510 nm and excitation wavelengths of 340 and 380 nm. After stabilization of basal fluorescence, the calcium concentration was increased at 2 mM to confirm the function of transfected CaSR [11], and then cells were exposed to the test agents. The data are expressed as maximum change in the fluorescence ratio (Δ340 nm/380 nm) induced by exposure to the test agents.

2.6 Fractionation and separation of protein hydrolysates by size-exclusion chromatography

Ten (10) mg of protein hydrolysates were loaded onto a Superdex Peptide 10/300 GL column (GE Health Sciences,

Table 3. Molecular weight distribution of azuki and ß-conglycinin hydrolysate

Fraction	Azuki		ß-conglycinin	
	Molecular weight	% of total area	Molecular weight	% of total area
F1	>51 039	0.3	>24 933	0.3
F2	8466–51 039	10.4	8457–24 993	25.3
F3	2866–8466	30.8	4089–8457	17.6
F4	679–2866	38.0	1665–4089	27.0
F5	328–679	9.4	678–1665	18.2
F6	77–328	8.1	160–678	8.7
F7	<77	3.0	<160	2.9

These profiles were obtained by FPLC using Superdex Peptide 10/300 GL column.

Piscataway, NJ) connected to an FPLC system (AKTA explorer 10S, GE Health Sciences) with 0.1% trifluoroacetic acid as the running buffer. The flow rate was controlled at 0.5 mL/min, and the absorbance was monitored at 214 nm. The procedure was performed repeatedly in isocratic mode with fixed volume fractionations (1 mL each). Lyophilized fractions were dissolved in Hepes buffer for CCK secretion studies to identify the active fraction(s). Fractions of azuki hydrolysate and ßcon hydrolysate were reconstituted based on their chromatographic elution profile (Table 3). Fractions of other protein hydrolysates were reconstituted without the small molecule fractions (< 500 Da) in order to obtain peptide fractions with a molecular weight greater than 500 Da.

2.7 Statistical analysis

Results are expressed as means ± SEM. Statistical significance compared to control treatments was assessed by Student's *t*-test ($p < 0.05$).

3 Results

3.1 CaSR is involved in protein hydrolysate-induced CCK secretion in STC-1 cells

All of the protein hydrolysates increased CCK secretion from STC-1 cells with different potencies. CCK secretions induced by protein hydrolysates were significantly decreased by the presence of CaSR antagonist treatment compared to vehicle treatment (Fig. 1A and B), except the secretion induced by MHY. However, secretion was not completely abolished by the antagonist in several hydrolysates. Protein hydrolysates did not induce LDH release in the presence or absence of CaSR antagonist (Fig. 1C).

3.2 Effect of PepT1 inhibitor on protein hydrolysate-induced CCK secretion

The oligopeptide transporter PepT1 is a potential dietary peptide sensor [24, 25]. Although it was reported that PepT1 is only slightly expressed in STC-1 cells [7], we tested whether PepT1 is involved in protein hydrolysate-induced CCK secretion by using the PepT1 inhibitor 4-aminomethyl benzoic acid (4-AMBA, Sigma) [24]. CCK secretions induced by the various protein hydrolysates were not reduced by the treatment with 4-AMBA (Fig. 2).

3.3 Protein hydrolysates induce intracellular Ca^{2+} mobilization in CaSR-transfected HEK 293 cells

To test the specific and direct CaSR activation by protein hydrolysates, changes in $[Ca^{2+}]_i$ were measured in

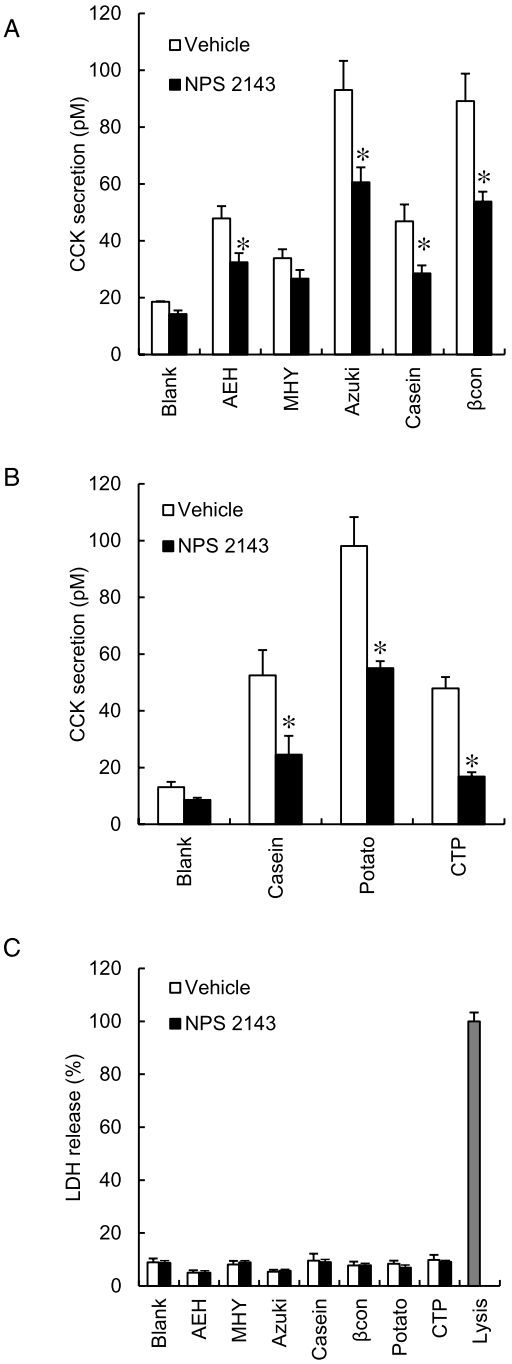

Figure 1. Effect of CaSR antagonist treatment on protein hydrolysate-induced CCK secretion in STC-1 cells. STC-1 cells cultured in 48-well plates were exposed to various protein hydrolysates at 2.5 (potato) or 5 mg/mL (other hydrolysates) for 60 min after a 10 min pretreatment with 25 μM NPS 2143 or its vehicle (0.1% DMSO). (A) and (B) were independently performed. CCK concentrations in the supernatant were measured by EIA. Values are means ± SEM of 3–4 wells. Significant differences ($p < 0.05$, Student's *t* test) between vehicle and NPS 2143 treatments are indicated with asterisk signs (*). (C) Cytotoxicity was measured by LDH in the supernatant. Lysis was used as high control. Values are means ± SEM of 3–4 wells.

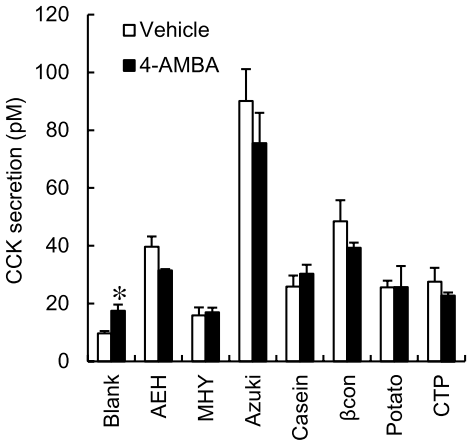

Figure 2. Effect of PepT1 inhibitor on protein hydrolysate-induced CCK secretion in STC-1 cells. STC-1 cells cultured in 48-well plates were exposed to protein hydrolysates at 2.5 mg/mL (potato) or 5 mg/mL (other hydrolysates) for 60 min after a 10 min pretreatment with 10 mM 4-AMBA. CCK concentrations in the supernatant were measured by EIA. Values are means ± SEM of 3–4 wells. Significant differences ($p < 0.05$, Student's *t* test) between vehicle and 4-AMBA treatments are indicated as asterisk sign (*).

CaSR-transfected HEK 293 cells. Many hydrolysates dose-dependently induced the increase in $[Ca^{2+}]_i$ in CaSR-transfected cells but not in control cells (Fig. 3A). In particular, azuki, ßcon, and egg hydrolysates induced a higher increase in $[Ca^{2+}]_i$ compared with other protein hydrolysates. These hydrolysates-induced increments in $[Ca^{2+}]_i$ were abolished by NPS 2143 treatment (data not shown).

3.4 Effect of CaSR antagonist on CCK secretion induced by molecular size fractions of protein hydrolysates

Potent hydrolysates (azuki and ßcon) were fractionated by size-exclusion chromatography to identify active peptide(s) that stimulate CCK secretion via or not via CaSR activation. Single CCK-releasing peptides were not isolated because active peptides were widely distributed throughout fractions in both hydrolysates (Fig. 4). Fractions (F4 in both hydrolysates) with the highest peptide content showed the highest activity. In the azuki hydrolysate, CCK secretion induced by relatively small peptides (F4-F6) was partially but significantly suppressed by the presence of CaSR antagonist (Fig. 4A and C). CCK secretion was suppressed by CaSR antagonist treatment in every fraction of ßcon hydrolysate (Fig. 4B and D).

3.5 Peptide fractions rather than free amino acids are responsible for CaSR-mediated CCK secretion

To investigate whether peptides or free amino acids in protein hydrolysates are involved in CaSR-mediated CCK secretion, low-molecular-weight fractions (MW < 500 Da) were

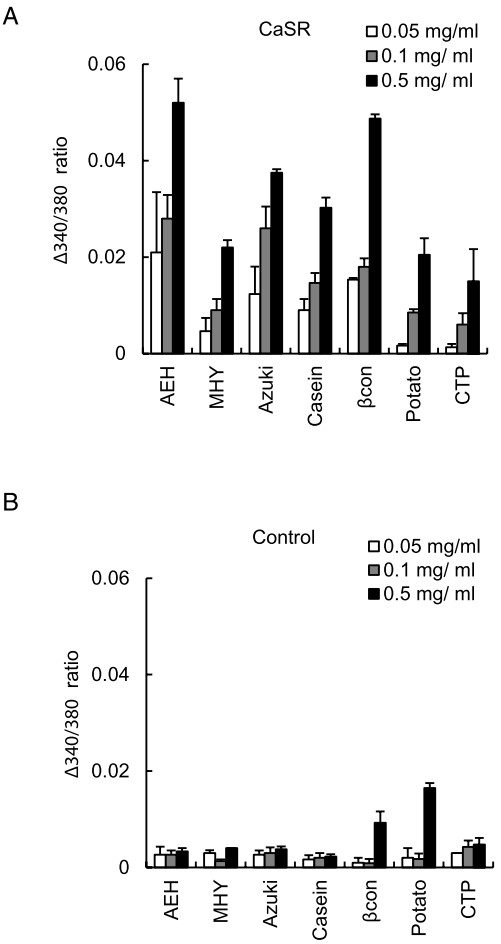

Figure 3. Effect of protein hydrolysates on the intracellular Ca^{2+} concentration in CaSR-expressing HEK 293 cells. HEK 293 cells were transfected with mouse CaSR expression vector (A) or treated only with Lipofectamine 2000 as a negative control (B). Cells cultured on coverslips in 24-well culture plates were loaded with fura-2-AM, and the emission fluorescence ratio (340 nm/380 nm, 510 nm excitation wavelength) was measured, showing changes in the intracellular Ca^{2+} concentrations. HEK 293 cells were exposed to different concentrations of protein hydrolysates in the presence of 2 mM Ca^{2+}. The data are expressed as the maximum change in the fluorescence ratio (Δ340 nm/380 nm).

removed from protein hydrolysates with size-exclusion chromatography. Protein hydrolysates without the < 500 Da fractions were able to induce CCK secretion (Fig. 5). CCK secretions induced by these hydrolysates were decreased by the presence of the CaSR antagonist.

4 Discussion

Luminal dietary proteins and peptides stimulate CCK secretion from "I cells" located in the proximal intestine. The existence of sensors for dietary proteins and peptides has been

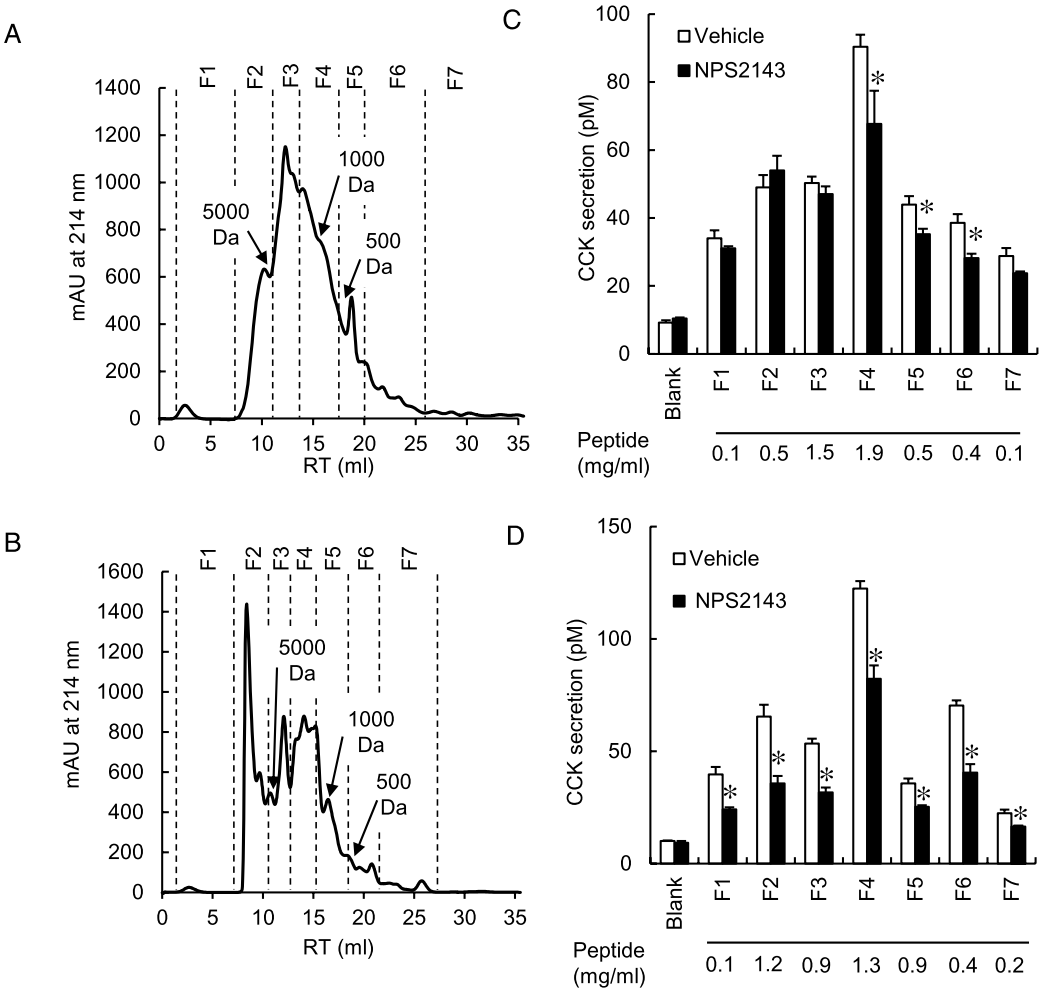

Figure 4. Separation of protein hydrolysates by size-exclusion chromatography and CCK-releasing activity of their fractions in the presence or absence of the CaSR antagonist. Fractions (F1-F7) from azuki hydrolysate (A) and ßcon hydrolysate (B) were collected based on the chromatogram obtained using the 10/300 GL Superdex Peptide column. CCK-releasing activity was measured in each fraction (F1-F7) separated from azuki hydrolysate (C) and from ßcon hydrolysate (D) in the presence of the CaSR antagonist (25 μM NPS 2143) or vehicle (0.1% DMSO). Concentrations of the hydrolysates were adjusted to represent the content of each fraction in 5 mg/mL of original hydrolysate, calculated based on the area of each fraction. Values are means ± SEM of 3–4 wells. Significant differences ($p < 0.05$, Student's t test) between vehicle and NPS 2143 treatments are indicated with asterisk signs (*).

speculated for many years, but reports on the identification of such receptors are still limited. To date, GPR93 [26] and Pept1 [24] have been reported as potential dietary peptide receptors. Recent papers have demonstrated that CaSR is involved in amino acid-induced CCK secretion by using enteroendocrine STC-1 cells [18] and primary CCK-producing cells [8, 16]. However, we have demonstrated that CaSR also functions as the sensor for a trideca peptide (ß51–63 peptide) derived from soybean ßcon to induce CCK secretion in STC-1 cells [19]. In addition, CCK secretion induced by a casein hydrolysate (tryptone) was partially impaired in isolated I cells prepared from CaSR-deficient mice [8]. Based on these studies, it was hypothesized that CaSR is also involved in CCK secretion induced by certain dietary peptides. In this study, we investigated whether CCK secretion induced by various protein hydrolysates was attenuated by a CaSR antagonist and whether CaSR is activated by protein hydrolysates.

CCK secretions induced by most protein hydrolysates were decreased by the presence of the CaSR antagonist in STC-1 cells (Fig. 1A and B) and protein hydrolysates did not show cytotoxic effect on STC-1 cells (Fig. 1C), suggesting the involvement of CaSR in CCK secretion induced by various protein hydrolysates. However, these CCK secretions were not completely abolished by CaSR antagonist treatment, and MHY-induced CCK secretion was not affected by the CaSR antagonist. NPS 2143 at 25 μM tested in the present study could be enough since it completely abolished CaSR-mediated CCK secretion in our previous study [18]. Our results showed that Pept1 is not responsible for CCK secretion in STC-1 cells (Fig. 2), consistent with the recent report that

used primary CCK-producing cells [7]. There may be other sensing mechanisms that mediate dietary peptide-induced CCK secretion. It is possible that various receptors, including CaSR and GPR93 [26], function as dietary peptide sensors in CCK-producing cells.

Protein hydrolysates induced an increase in intracellular calcium concentrations in HEK 293 cells expressing CaSR (Fig. 3) and the increments were abolished by CaSR antagonist treatment (data not shown), demonstrating specific activation of CaSR by protein hydrolysates. AEH induced the highest activation of CaSR (Fig. 3), but the AEH-induced CCK secretion and the involvement of CaSR in the secretion were relatively small compared with the other hydrolysates (Fig. 1A). Additionally, MHY had effect on CaSR activation to a similar degree compared to potato and casein hydrolysates (Fig. 3), though CaSR was not involved in MHY-induced CCK secretion (Fig. 1A). Such differences between CCK secretion in STC-1 cells and intracellular Ca^{2+} mobilization in CaSR-transfected HEK293 cells in response to AEH and MHY could be due to the involvement of other sensing mechanisms in CCK-producing cells. For example, prior to their detection by CaSR, AEH and MHY might be detected by other sensors that trigger CCK secretion.

CaSR mediated the CCK secretion induced by low-molecular-weight fractions of azuki hydrolysate (Fig. 4C), but it mediated the secretion induced by low- and high-molecular-weight fractions of ßcon hydrolysate (Fig. 4D). No correlation was seen between the molecular weight of the protein hydrolysates (Table 1) and CaSR activity or CCK releasing activity. These results suggest that CaSR senses various dietary peptides independently of their molecular weight. This is partially supported by previous studies demonstrating that CaSR is activated by peptides with various molecular weights, including tripeptides (γ-Glu-Cys-Gly [glutathione] and γ-Glu-Val-Gly)[20]), a tridecapeptide (soybean ß51–63 peptide) [19], and polypeptides (protamine and poly-L-lysine) [14]. It has been demonstrated that large peptide (poly-L-arginine) binds to transmembrane domain in CaSR [27], in contrast, small peptides (γ-glutamyl-peptides) are considered to bind Venus domain located on N-terminal for sensing amino acids [20].

Amino acids such as Phe, Trp, and His are well known CaSR agonists, and L-Phe (20 mM) stimulates CCK secretion through the activation of CaSR in CCK-producing cells [8, 16, 18]. Commercial protein hydrolysates contained relatively high amounts of free amino acids compared with the protein hydrolysates we prepared (Table 2), but all of the hydrolysates contained only small portion of free aromatic amino acids and His (maximum of ~0.5 mM in 5 mg/mL hydrolysate). Amino acid mixtures mimicking the free amino acid compositions of AEH and MHY did not stimulate CCK secretion (data not shown), similar to previous reports [28]. Furthermore, protein hydrolysates induced CaSR-mediated CCK secretion without low-molecular-weight fractions (Fig. 5). These results strongly suggest that relatively large peptides, rather than free amino acids, dipeptides, or tripeptides, are responsible for activating CaSR to trigger CCK secretion.

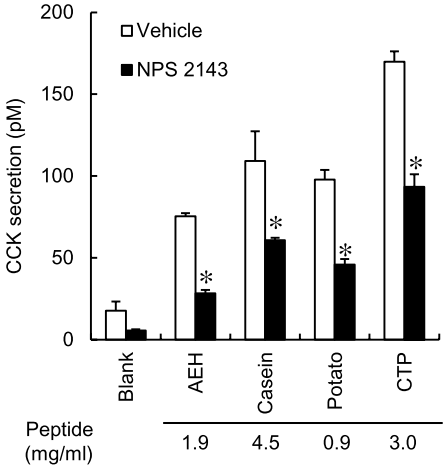

Figure 5. Effect of CaSR antagonist on CCK secretion induced by protein hydrolysates with no low molecular weight fractions. Low-molecular-weight fractions (<500 Da) were removed from protein hydrolysates using a size-exclusion column. STC-1 cells cultured in 48-well plates were exposed to these high-molecular-weight protein hydrolysates (MW > 500 Da) for 60 min after a 10 min pretreatment with 25 μM NPS 2143 or vehicle (0.1% DMSO). Concentrations of hydrolysates were adjusted as to represent the content in 2.5 mg/mL (potato) or 5 mg/mL of original hydrolysate (Table 1). CCK concentrations in the supernatant were measured by EIA. Values are means ± SEM of 3–4 wells. Significant differences ($p < 0.05$, Student's *t* test) between vehicle and NPS 2143 treatments are indicated with asterisk signs (*).

CaSR not only regulates serum calcium concentrations through the secretion of PTH but also modulates gastrointestinal functions such as gastric acid secretion, gut motility, and colonic fluid secretion [29]. Recent papers have demonstrated expression of CaSR in intestinal CCK-producing cells [16] and in gastric G-cells [30]. Taken together with our results, sensing of dietary peptides by CaSR would have physiological relevance for facilitating luminal digestion by enhancing pancreatic enzyme secretion through CCK release.

In summary, CCK secretion induced by various protein hydrolysates was partially lowered by CaSR antagonist treatment in STC-1 cells. Protein hydrolysates induced an increase in $[Ca^{2+}]_i$ in CaSR-expressing HEK 293 cells. CaSR-mediated CCK secretion was induced by peptides having various molecular weights. These results demonstrate the significant role of CaSR as a sensor for dietary peptides to stimulate CCK secretion in enteroendocrine cells.

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The authors have declared no conflict of interest.

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