RESEARCH ARTICLE

Direct induction of CCK and GLP-1 release from murine endocrine cells by intact dietary proteins

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Scope: Consumption of high-protein diets cause elevated levels of CCK and GLP-1. Although unknown, this might be due to protein breakdown by various proteases that originate from the gastrointestinal tract. This study investigated which dietary proteins, hydrolysates, or synthetic-peptides are most potent to affect secretion of CCK and GLP-1 in STC-1 cells known for satiety hormone release.

Methods and results: Addition of intact proteins to STC-1 cells exerted strong effects on secretion of satiety hormones. Casein, whey, and pea showed strongest effects on CCK release, whereas casein, codfish, egg, and wheat showed most pronounced effects on GLP-1 release. Egg-hydrolysate stimulated release of CCK and GLP-1, whereas all other tested hydrolysates and synthetic-peptides showed no significant effects on hormone release. Addition of a combination of trypsin and casein-hydrolysate, codfish, egg, egg-hydrolysate, sodium-casein, wheat-hydrolysate, or wheat resulted in additional stimulation of CCK release, compared to only the protein. Addition of a combination of DPP-IV and egg-hydrolysate, ovomucoid, or sodium-casein decreased GLP-1 levels.

Conclusion: This study showed that specific intact, or partially digested proteins, in contrast to protein-hydrolysates and synthetic-peptides, stimulated hormone release. We conclude that intact proteins exert strong effects on satiety hormone release, and may therefore provide potent dietary supplements for prevention or treatment of obesity.

Keywords:
Cholecystokinin / Glucagon-like peptide 1 / Intact dietary protein / Protein hydrolysate / Protein sources

1 Introduction

Food ingestion exerts a transient suppressive effect on appetite and further food intake. Among all properties of food, total energy content and the macronutrient composition (*e.g.* fat, carbohydrate, or protein) appear to be the major determinants of such satiety-related subjective feelings [1]. A strong body of evidence indicates that the three macronutrients differ in extent to which they suppress hunger and energy intake. Proteins have been shown to be more satiating than carbohydrates, which in turn are more satiating than fats [1–3]. Findings that some specific peptide structures derived from parent food proteins are biologically active have stimulated the search for bioactive peptides that may lead to the development of functional foods, targeting to control food intake and thus, body weight [3–5].

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Abbreviations: CCK, cholecystokinin; **GLP-1,** glucagon-like peptide 1; **DPP-IV,** dipeptidyl peptidase-IV; **LDH,** lactate dehydrogenase

Proteins and peptides have the capacity to affect food intake and appetite in several species, including humans [2, 4, 6, 7]. Many factors are known to play a role in this satiating effect, *e.g.* luminal proteins or luminal hydrolysates have been found to increase the secretion of satiety inducing gut hormones such as glucagon-like peptide 1 (GLP-1) [8] and cholecystokinin (CCK) [9, 10]. In humans, it is believed that digestion of protein is required to stimulate CCK release in plasma [11, 12]. After ingestion, proteins are in part hydrolyzed by pepsin in the stomach, and luminal digestion is completed in the small intestine by a number of pancreatic proteases, of which trypsin is the most important. The resulting peptides are able to stimulate the release of CCK and GLP-1 by the intestinal enterocytes.

Secretion of CCK in the *in vivo* situation is under negative feedback regulation by proteases present in pancreatic secretion. Active proteases in the intestinal lumen inhibit CCK secretion. Release of CCK is stimulated by a luminal CCK releasing factor, which is secreted from enteroendocrine cells within the gastrointestinal-tract, and binds to receptors on the secreting cells of the gut [13]. In rats, it was shown that under basal conditions, the luminal CCK releasing factor is broken down by trypsin and no CCK is secreted. Inactivation of intestinal proteases or binding of proteases by substrates such as proteins, results in stimulation of CCK secretion [14, 15]. GLP-1 stimulates glucose-dependent insulin secretion and insulin biosynthesis, inhibits glucagon secretion and gastric emptying rates, and inhibits food intake [16]. GLP-1 is released by endocrine cells, which are distributed throughout the small and large intestines [17]. After an initial nutrient-stimulated rise in circulating levels of GLP-1, the levels fall rapidly, largely due to renal clearance and the *N*-terminal degradation of the peptide by dipeptidyl peptidase IV (DPP-IV). McIntosh *et al.* observed with oral treatment of a DPP-IV inhibitor over a 12-wk period in DM2 rats a body weight decrease of 12.5%. Inhibitor-treated diabetic animals showed a marked improvement in glucose tolerance and increased insulin secretion [18]. However, the effects of DPP-IV on protein digestion and the release of satiety hormones are still unknown.

The nature of the protein provided as a factor affecting short-term food intake response has received relatively little investigation and the results obtained are diverse. For example, in STC-1 cells it was shown that soy, potato, and casein protein hydrolysates directly stimulated CCK release, whereas whey and pea protein hydrolysates were inactive in stimulating CCK release [19]. However, in rats food intake suppression in the hour after feeding is larger with whey compared with egg-albumin or soy protein [20], and in a human study it was shown that pea protein hydrolysates had the strongest effects on satiety [6]. This effect on the food intake may be due to specific physiological activities in the gut based on specific peptides, which comes available after appropriate digestion [21].

Overall, the data suggest that dietary compounds, in particular proteins, stimulate hormone release from enteroendocrine cells that contribute to the termination of the meal. Therefore, identification of either specific proteins, protein hydrolysates or even specific peptides with optimized satiety hormone releasing properties is an interesting target for the development of functional food products for weight management purposes.

The aim of this study was to investigate the effects of various intact dietary proteins, protein hydrolysates, and specific synthetic peptides on the secretion of the satiety hormones CCK and GLP-1. To that end the STC-1 cell line was incubated with these components. Cell line STC-1, derived from an intestinal tumor arising in double transgenic mice [22], is known to secrete CCK and GLP-1, and was shown to be sensitive to protein exposure [19]. The proteins, protein hydrolysates, and synthetic peptides applied were selected based on their potential ability to stimulate the release of satiety hormones. In addition, experiments were executed where the intestinal serine proteases trypsin and DPP-IV were added in combination with proteins, to estimate the effect of these hydrolyzed proteins on the CCK and GLP-1 levels.

2 Materials and methods

2.1 Proteins, hydrolysates, and synthetic peptides

Ovomucoid and soybean were obtained from Worthington Biochemicals (Huissen, The Netherlands). Free tryptophan, egg-hydrolysate, egg protein, wheat-hydrolysate, wheat protein, whey protein, pea protein, casein-hydrolysate, sodium-casein, and codfish protein were obtained from Dutch Protein Services (DPS, Tiel, The Netherlands), pea protein from Cosucura (Pisane, Cosucra, Warcoing, Belgium), pea protein from Nutralys (SM, Nutralys, France), and pea protein hydrolysate from Triballat (HP90, Triballat, France). All other pea hydrolysates, a selected number of specific peptides were provided by DSM Food Specialties (Delft, the Netherlands). The pea hydrolysates pea B1, pea HB1, pea B2, pea HB2, pea B3, and pea HB3 were obtained by enzymatic hydrolysis using subtilisin (EC 3.4.21.62; Protex 6L from Danisco, Leiden, The Netherlands) at pH 8.0 in combination with a proline-specific endoprotease (EC 3.4.21.26; from DSM Food Specialties) at pH 6.0. With the pea HB1, the pea HB2, and the pea HB3 samples, a heat treatment was applied to inactivate the proteases used. The hydrolysates pea B1, pea B2, and pea B3 were not heat treated. The pea B1 and the pea HB1 samples represent the total hydrolysate, the pea B2 and pea HB2 samples represent supernatants obtained after centrifugation. The pea B3 and the pea HB3 samples represent 10 kDa permeates of the pea B1 and the pea HB1 sample, respectively.

The synthetic peptides used in this study (TAP, peptide VPI, opioid BCM-7, and peptide GRGRGRG) were selected for their possible potential to induce satiety. The peptide TAP (sequence: APFDDDK) (synthesized by Pepscan Presto, Lelystad, The Netherlands) is the octapeptide that is

cleaved from trypsinogen in the duodenum, resulting in active trypsin. To protect the pancreas from auto-digestion by its own digestive enzymes, most pancreatic enzymes are produced as inactive precursors (''zymogens''). Procolipase is such a pre-lipase that, upon entry into the duodenum, is activated by active trypsin. During this activation step, an N-terminal pentapeptide (Ala-Pro-Gly-Pro-Arg), also known as ''enterostatin'' is released from the procolipase. The enterostatin present within the intestinal lumen is known to act as a satiety signal for fat intake in rats. The TAP peptide is the ''pre-sequence'' of trypsinogen, which was introduced in this study to identify a possible effect on satiety. Tripeptide VPI, also called Diprotin C (synthesized by Pepscan Presto) is a known DPP-IV inhibitor [23]. Tryptophan could affect the release of satiety hormones [24]. Tryptophan residues are known as satiating agents in rats [25]. Opioid BCM-7 (sequence: YPFPGPI) (synthesized by Pepscan Presto) is a peptide present in casein. Although its function is unknown, the literature suggests that it delays gastro intestinal transit times, and stimulates mucus production [26]. Peptide GRGRGRG (synthesized by Pepscan Presto) has been shown to stimulate CCK and GLP-1 releases [27].

2.2 Cell culture conditions

STC-1 cells were a gift from by Dr. Douglas Hanahan (University of California, San Francisco), and were maintained in Dulbecco's Modified Eagles Medium (Invitrogen) with 10% fetal bovine serum (Invitrogen), 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin as additional supplements, at 37°C in 5% CO_2/air humidity. All studies were performed on cells with passage number 31.

2.3 Cell secretion studies

Two sets of secretion assays were performed. The first set was performed to study the effects of dietary proteins, hydrolysates, and peptides on the secretion of several satiety hormones. Briefly, three days before the experiment, STC-1 cells were seeded in 24-well plates (1.0×10^5 cells/well). On the day of the experiment, cells were washed twice with PBS, followed by addition of protein, hydrolysates, and peptides (1 mg/mL) to each well. After an incubation period of 2 h at 37°C ($n = 3$), the supernatant was collected for the measurement of CCK, total GLP-1, and lactate dehydrogenase (LDH).

In the second set, the effects of the dietary proteins, protein hydrolysates, and synthetic peptides combined with intestinal serine proteases were determined on the secretion of satiety hormones. Briefly, on the day of experiment, cells were incubated with a mixture of protein (1 mg/mL) with human trypsin (human pancreas, EC: 3.4.21.4; Athens Research, GA, USA) or human DPP-IV (Athens Research), for 30 min at 37°C ($n = 3$). CCK and GLP-1 levels were determined in the supernatant.

2.4 Determination of CCK and GLP-1 levels in supernatant

CCK levels were determined using the RIA from Euria-CCK (Euro-Diagnostica AB, Malmö, Sweden), with a detection limit of 0.3 pmol/L. The intra-assay variation ranges from 2.0 to 5.5% and the inter-assay variation from 4.1 to 13.7%. Cross-reaction with gastrin is $\leq 0.5\%$. GLP-1 levels were determined using the RIA from Linco Research, MO, USA. The detection limit of this kit was 3–333 pM. The intra-assay variation ranges from 10 to 23% and the inter-assay variation from 22 to 38%. There is no cross-reaction with GLP-2 and glucagon (0.01 and 0.2%, respectively).

2.5 Determination of lactate dehydrogenase levels

To check for lysis of the STC-1 cells due to the presence of active proteases in the tested protein hydrolysates, LDH levels were determined. To measure LDH, the LDH detection kit from Clontech (Clontech Laboratories, CA, USA) was used. Briefly, the cell-free culture supernatant was collected and incubated with the reaction mixture from the kit. LDH activity was determined by a colorimetric assay: in the first step, NAD^+ was reduced to $NADH/H^+$ by the LDH-catalyzed conversion of lactate to pyruvate. In the second step, a catalyst included in the reaction mixture (diaphorase) transfers H/H^+ from $NADH/H^+$ to the tetrazolium salt INT, which is reduced to a formazan dye. The amount of dye produced is proportional to the number of lyzed cells [28]. Free tryptophan residues are known as a satiating agent in rats.

2.6 Statistical analysis

The descriptive and statistical analyses were performed with SPSS, version 11.0. The means of the variables are presented with their standard deviation (mean $\pm$ SD). Comparison of means of the secretion studies was done using the one-way ANOVA, with the Bonferroni test as *post hoc* test. A p-value of less than 0.05 was considered statistically significant.

3 Results

3.1 Effects of intact proteins, protein hydrolysates, and specific synthetic peptides on CCK and GLP-1 release

A negative control (only Hanks Buffered Salt Solution buffer) was tested with every assay. Addition of this negative control to STC-1 cells resulted in CCK levels of 25.6 ± 1.2 pM and GLP-1 levels of 849.3 ± 64 pM. To correct for the small differences in the release of the satiety hormones, the negative control was subtracted from each assay. As shown in Fig. 1, the addition of various intact proteins to STC-1 cells resulted

in increased CCK levels in the supernatant. Intact casein, pea, and wheat protein were able to significantly elevate the release of CCK into the supernatant (45.9±4.8, 46.1±4.0, 53.8±7.3 pM, respectively) compared with the negative control (only Hanks Buffered Salt Solution buffer). Also the addition of protein hydrolysates to STC-1 cells (Fig. 1B) resulted in significantly elevated CCK levels. Addition of egg-hydrolysate and the pea hydrolysates pea B1, and pea B2 exerted the most pronounced effects (45.9±6.2, 107.5±7.5, 108.8±8.1, 130.1±3.2 pM, respectively). None of the selected synthetic peptide sequences or free tryptophan, all potentially of interest in relation to satiety, were able to elevate CCK levels compared to the negative control (Fig. 1C). Figure 2 shows the effects on GLP-1 release by STC-1 cells. Addition of intact casein, codfish, egg, pea, whey, and wheat protein significantly increased the release of GLP-1 into the supernatant (4255.9±3587, 4889.4±4403, 16662.3±12239, 7488.3±5023, 7847.3±4856, 7765.4±2371 pM, respectively; see Fig. 2A). Addition of protein hydrolysates to STC-1 cells (Fig. 2B) resulted in significantly elevated GLP-1 levels with egg-hydrolysate and pea B2 hydrolysates (15670.2±4965 and 13891.8±2106 pM, respectively). Neither the selected synthetic peptide sequences nor the free tryptophan was not able to elevate GLP-1 levels compared with the negative control (data not shown).

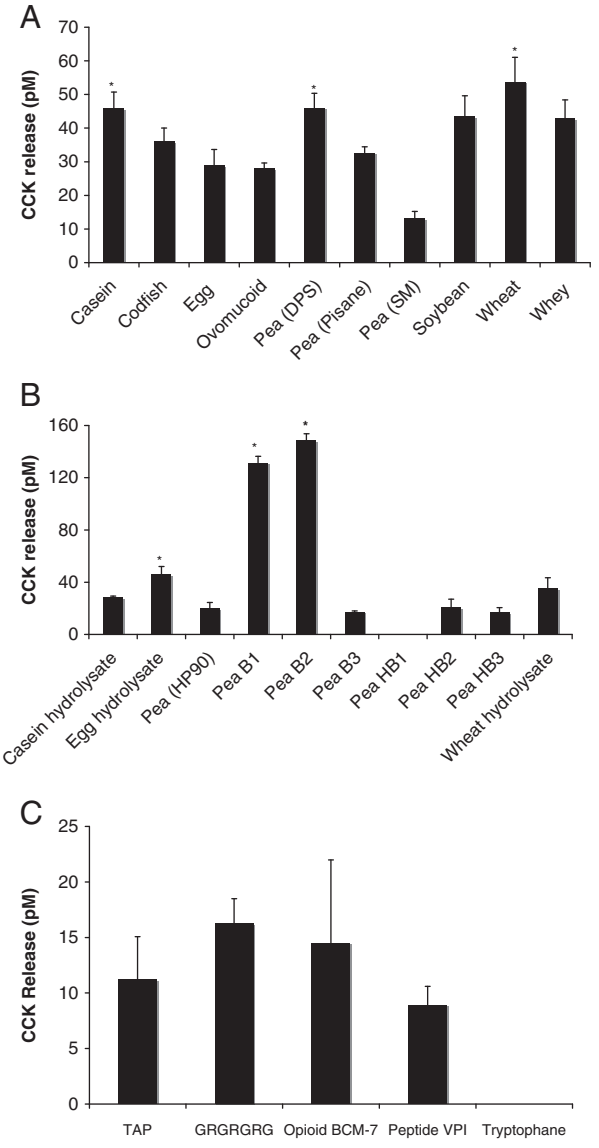

Figure 1. Effects of dietary proteins, hydrolysates, and peptides on CCK release. The levels of CCK were measured in the supernatant of STC-1 cells after being exposed to proteins for 2 h. Results are expressed as mean±SD of four individual experiments. (A) shows that addition of intact proteins to the cells. Casein, pea (DPS), and wheat were able to increase the release of CCK from the STC-1 cells when compared with the negative control. (B) shows that addition of egg-hydrolysate, pea B1, and pea B2 increase the release CCK, whereas all the tested peptides (C) are not able to elevate CCK release compared with the negative control. *$p<0.05$ when compared with the negative control.

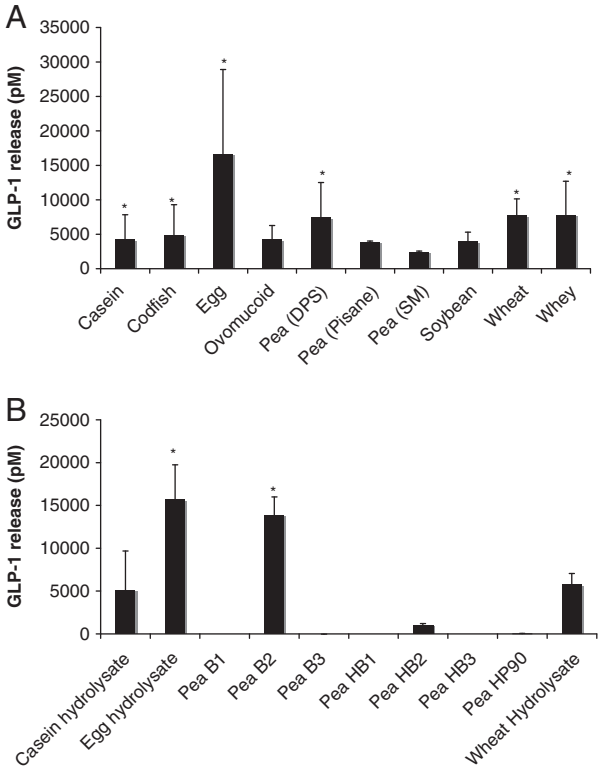

Figure 2. Effects of dietary proteins, hydrolysates, and peptides on GLP-1 release. The levels of GLP-1 were measured in the supernatant of STC-1 cells after being exposed to proteins for 2 h. Results are expressed as mean±SD of four individual experiments. (A) shows that addition of intact proteins to the cells. Casein, egg, pea, whey, and wheat were able to increase the release of GLP-1 from the STC-1 cells when compared with the negative control. (B) shows that addition of egg-hydrolysate and pea B2 increase the release GLP-1. *$p<0.05$ when compared with the negative control.

3.2 LDH release from STC-1 cells

To check for hormone release caused by lysis of the STC-1 cells, LDH release was measured. Pea hydrolysates provided by DSM Food Specialties were prepared using two different proteases (see Section 2). Among these, the pea "HB" samples were heated to inactivate these proteases but the pea "B" samples were not. By measuring LDH, cell lysis can be determined. As shown in Fig. 3, addition of intact pea protein and pea HB2 cause increased levels of LDH (19.0 ± 0.6 and 27.2 ± 3.2 IU/L, respectively) when compared with the negative control (8.3 ± 1.5 IU/L), but the cell layer was still intact after the incubation period. After addition of pea B2 or a relevant concentration of the subtilisin protease used during pea hydrolysis, the cell layer was visibly broken down after the incubation period, and LDH levels (93.3 ± 0.9 and 140.7 ± 2.2 IU/L, respectively) were significantly increased when compared with the negative control, but also when compared with intact pea and pea HB2.

3.3 Effects of trypsin and DPP-IV in combination with intact proteins, protein hydrolysates, and specific synthetic peptides on CCK secretion

The effects of the tested dietary proteins in combination with trypsin and DPP-IV on CCK release by STC-1 cells are presented in Table 1. Decreased levels of CCK were observed after addition of active trypsin combined with ovomucoid, all pea products (with the exception of pea HB1), the TAP peptide and peptide GRGRGRG, the opioid BCM-7 peptide or tripeptide VPI. Increased levels of CCK release were observed after addition of active trypsin in combination with either casein, casein-hydrolysate, codfish, egg, egg-hydrolysate, wheat-hydrolysate, or whey protein. All other proteins in combination with trypsin did not affect CCK release differently compared with exposure to only protein. Addition of DPP-IV resulted in a decreased CCK release after addition of DPP-IV and casein, ovomucoid, pea (DPS), egg-hydrolysate, and tripeptide VPI. Increased levels of CCK were observed after combining DPP-IV with codfish, egg, casein-hydrolysate, wheat-hydrolysate, TAP, and GRGRGRG. All other proteins in combination with DPP-IV did not affect CCK release.

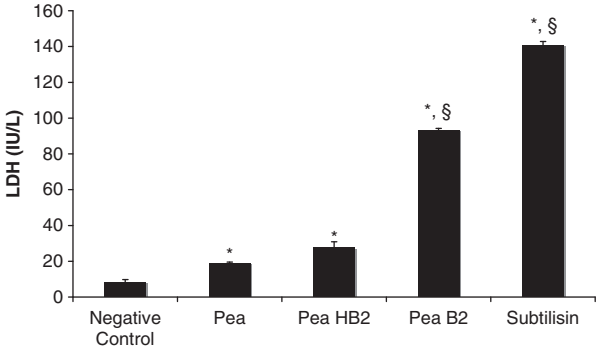

Figure 3. LDH levels after 30 min of exposure to pea, pea B2, pea HB2, and subtilisin. The levels of LDH were measured after an incubation period of 30 min with pea, pea B2, pea HB2 or subtilisin. The results are expressed as mean ± SEM of three individual experiments. Addition of these substances to the STC-1 cells resulted in a significant increase in LDH levels when compared with the negative control. Addition of pea B2 and subtilisin were also significantly increased when compared with pea protein. $^{*}p < 0.05$ when compared with the negative control. $\S p < 0.05$ when compared with pea protein.

3.4 Effects of trypsin and DPP-IV in combination with intact proteins, protein hydrolysates, and specific synthetic peptides on GLP-1 secretion

Both with the addition of trypsin and DPP-IV there was no visible indication of lysis in the cell layer, so all hormone levels secreted into the supernatant were due to addition of the proteins. The effects of the tested dietary proteins in combination with trypsin and DPP-IV on GLP-1 release by STC-1 cells are presented in Table 2. Decreased levels of GLP-1 were also observed after addition of trypsin with ovomucoid, intact pea (DPS), pea (Pisane), intact pea (SM), egg-hydrolysate, pea-hydrolysate HP-90), pea HB2, pea HB3, and wheat-hydrolysate. All other proteins in combination with trypsin did not affect GLP-1 release. Addition of DPP-IV resulted in a decreased release of GLP-1 after addition of DPP-IV with ovomucoid, pea (DPS), egg-hydrolysate, and peptide VPI. Increased levels of GLP-1 were observed after addition of DPP-IV with TAP to the STC-1 cells. All other proteins in combination with DPP-IV did not affect GLP-1 release.

4 Discussion

The present study was designed to identify potent appetite suppressing dietary proteins or protein derivates by determining the modulating effects on intestinal CCK and GLP-1 release in a murine intestinal cell line. We are the first to demonstrate that not only protein hydrolysates [19], but also intact proteins are able to directly modulate the release of the satiety hormones CCK and GLP-1. We also showed that in some cases the hydrolysates do not affect hormone release, whereas the intact protein does stimulate hormone release. Furthermore, we showed for the first time to our knowledge, that proteases such as trypsin, DPP-IV, and subtilisin modulate the effects of proteins on satiety hormone release.

Some previous studies in rats have shown that intact proteins, but not amino acids or carbohydrates, stimulate CCK release [29]. The present study also demonstrated that intact proteins were able to stimulate the release of CCK and GLP-1. Under physiological circumstances, little intact protein will enter the small intestine. To use intact proteins as a possible therapeutic option for weight management, gastric degradation has to be prevented. There are some

Table 1. CCK release after a 30-min incubation period with proteins, hydrolysates, and peptides in combination with trypsin or DPP-IV

	CCK release (pM)		
	Only protein	Protein with trypsin	Protein with DPP-IV
Casein	21.2±3.1	25.1±2.4[a)]	14.6±1.0[a)]
Codfish	24.9±1.2	45.3±3.1[a)]	32.0±1.8[a)]
Egg	16.3±3.2	30.7±3.1[a)]	22.5±7.2
Ovomucoid	34.2±2.1	23.5±1.0[a)]	16.7±1.3[a)]
Pea (DPS)	57.2±3.8	30.6±6.6[a)]	18.8±3.1[a)]
Pea (Pisane)	57.4±2.3	16.7±1.8[a)]	46.0±1.5
Pea (SM)	39.3±2.6	21.5±3.1[a)]	41.2±1.1
Soybean	13.1±4.9	18.4±5.0	16.7±3.1
Wheat	27.5±3.1	30.6±2.3	27.4±8.6
Whey	11.9±1.9	27.5±6.2[a)]	13.3±1.5
Casein-hydrolysate	15.7±2.7	32.1±5.4[a)]	24.7±3.9[a)]
Egg-hydrolysate	26.1±6.9	40.4±3.1[a)]	15.2±4.3[a)]
Pea (HP90)	42.3±3.4	25.9±1.6[a)]	47.8±5.2
Pea B1	131.4±4.1	122.5±3.8[a)]	127.8±4.8
Pea B2	148.7±7.2	135.4±6.8[a)]	132.1±8.0[a)]
Pea B3	30.8±3.6	20.5±1.9[a)]	26.9±5.7
Pea HB1	16.8±1.1	13.8±1.0	16.2±1.2
Pea HB2	32.6±2.0	24.5±1.6[a)]	30.9±2.1
Pea HB3	32.9±3.3	23.5±3.8[a)]	30.1±4.0
Wheat-hydrolysate	18.0±2.5	31.8±3.1[a)]	26.0±1.7[a)]
TAP	35.7±1.9	18.7±1.6[a)]	74.3±2.6[a)]
GRGRGRG	35.0±2.3	24.8±2.6[a)]	53.5±2.2[a)]
Opioid BCM-7	40.8±5.3	25.3±4.2[a)]	49.3±7.8
Peptide VPI	34.0±3.4	17.7±2.0[a)]	75.2±2.2[a)]
Tryptophan	24.9±4.1	18.9±1.0	25.1±4.3

The levels of CCK were measured in the supernatant of STC-1 cells after being exposed to protein with trypsin or protein with DPP IV for 30 min. Results are expressed as pmol/L and represent mean±SD of four individual experiments. Addition of trypsin to intact proteins, hydrolysates or peptides resulted in changed levels of CCK, with the exception of soybean, wheat, Pea Hb1, and tryptophan. Addition of only DPP-IV decreased CCK release. Combining DPP-IV with egg-hydrolysate, ovomucoid, pea protein, or sodium casein, resulted in a decrease of CCK. Addition of DPP-IV with casein-hydrolysate, codfish, wheat-hydrolysate, TAP, GRGRGRG, or peptide VPI resulted in an increase of CCK release. Addition of DPP-IV with other tested proteins showed no effect.
a) Significantly different compared with the addition of only protein ($p<0.05$).

studies describing coatings for enteric delivery, such as pH-triggered coatings, pressure-sensitive coatings, or time-release coatings [30–34]. For duodenal delivery of relatively large amount of protein, a pH-sensitive coating is suitable.

Human studies have shown that plasma levels of CCK and GLP-1 differ after intake of proteins from different origins [3, 6, 29, 35, 36]. These data suggest that amino acids and/or specific amino acid sequences are of importance in stimulating the release of CCK and GLP-1. It was previously shown that protein hydrolysates are able to stimulate CCK and GLP-1 release by the STC-1 cells [19, 37]. Cordier-Bussat *et al.* showed that addition of protein hydrolysates from meat, casein, and soybean to STC-1 cells increased CCK and GLP-1 release, whereas a mixture of free amino acids or undigested proteins was weak stimulants of CCK and GLP-1 release [4, 37, 38].

After ingestion, the proteins are hydrolyzed by pepsin in the stomach, and by, among others, trypsin in the small intestine. Some of the hydrolysates used in this study were prepared using the serine proteases subtilisin and a proline-specific endoprotease. However, after ingestion, these hydrolysates will still be cleaved by other serine proteases such as trypsin and DPP-IV. We found that the addition of intact casein, intact codfish, intact egg, intact pea (DPS), intact wheat, egg-hydrolysate, pea B1, and, pea B2 showed strongest effects on the release of CCK and GLP-1. However, after addition of trypsin or DPP-IV, most products are less potent to stimulate the release of CCK and GLP-1. The breakdown of the proteins by trypsin and DPP-IV seems to influence the ability of the proteins to stimulate hormone release. This might be due to the enzymatic effects of the proteases on the proteins and hydrolysates, which might cleave the protein in its ''inducing'' site, thereby loosing its effects to induce an increase in hormone release. Another possibility is that the cells used in this study were not able to detect the small peptides created upon the second enzymatic digestion. Addition of the synthetic peptides that were potentially of interest in relation to satiety also did not show any effects on the release of CCK and GLP-1 by the STC-1 cells.

The STC-1 cell line provides a suitable screening model to study the effects of food compounds on hormone release.

Table 2. GLP-1 release after a 30-min incubation period with proteins, hydrolysates, and peptides in combination with trypsin or DPP-IV

	GLP-1 release (pM)		
	Only protein	Protein with trypsin	Protein with DPP-IV
Casein	1664.7±745	576.1±249	1157.9±372
Codfish	1820.7±694	548.6±322	1924.6±347
Egg	1152.0±972	461.3±251	1201.2±487
Ovomucoid	929.5±112	381.1±39[a)]	567.0±56[a)]
Pea (DPS)	3149.2±572	607.9±261[a)]	1002.0±286[a)]
Pea (Pisane)	1955.8±0.9	33.5±1a)	1680.8±241
Pea (SM)	1572.8±0.7	77.5±14a)	1258.2±236
Soybean	839.7±343	1427.0±160	1253.2±171
Wheat	2250.5±742	1105.4±457	1869.3±371
Whey	1722.9±651	694.7±304	843.7±326
Casein-hydrolysate	957.5±487	610.7±333	695.4±243
Egg-hydrolysate	1837.7±414	642.2±251[a)]	583.8±207[a)]
Pea (HP90)	115.6±8	377.8±89[a)]	72.2±9
Pea B1	89.5±20	113.0±10	79.6±4
Pea B2	11 392.8±1971	14 631.8±1671	171 18.4±1019
Pea B3	72.2±6	143.4±61	116.1±31
Pea HB1	N.D.	N.D.	N.D.
Pea HB2	555.1±26	264.0±7[a)]	561.2±23
Pea HB3	157.1±61	72.2±6[a)]	221.0±7
Wheat-hydrolysate	1129.7±556	332.7±172[a)]	924.7±278
TAP	30.6±12	35.1±16	90.5±12[a)]
GRGRGRG	54.4±3	48.2±9	74.0±16
Opioid BCM-7	35.7±5	74.6±32	47.5±5
Peptide VPI	68.7±21	21.2±5	116.4±5[a)]
Tryptophan	58.2±4	39.5±11	70.4±24

N.D.: Not detectable. The levels of GLP-1 were measured in the supernatant of STC-1 cells after being exposed to protein with trypsin or protein with DPP-IV for 30 min. Results are expressed as pmol/L and represent mean±SD of four individual experiments. Addition of trypsin to ovomucoid, intact pea protein, pea-hydrolysates, egg-hydrolysate, or wheat-hydrolysates resulted in decreased levels of GLP-1. All other proteins, hydrolysates, and peptides were not affected Addition of only DPP-IV decreased GLP-1 release. Combining DPP IV with ovomucoid, pea protein from DPS, or egg-hydrolysate, resulted in a decrease of GLP-1. Addition of DPP-IV with pea 11B, TAP, or peptide VPI resulted in an increase of GLP-1 release Addition of DPP-IV with other tested proteins showed no effect.
a) Significantly different compared with the addition of only protein ($p<0.05$).

Many samples can be tested in a very short amount of time, and the responses of the cells seem to differentiate between specific compounds. However, there are also some drawbacks related to using this cell line. It originates from mice, so the effects on hormone release may not necessarily be translatable to the effects in human duodenal tissue. Also, the concentrations used for the products may be different when testing the same product in an *in vivo* situation. Human intervention studies must always be performed before final conclusions can be drawn whether the product affects food intake and weight regulation. We also demonstrated in this study that it is important to include a marker for cell lysis, in order to exclude a possible increase of CCK and GLP-1 in the supernatant due to cell damage.

In summary, we showed that most proteins were able to directly affect hormone release in the STC-1 cell line, and that in most cases, intact proteins and egg-hydrolysate were more potent than the other protein hydrolysates or synthetic peptides. We also demonstrated that most proteins lost the ability to increase the release of CCK and GLP-1 after the addition of trypsin or DPP-IV. Overall, we conclude that intact proteins or proteins that were partially hydrolyzed by trypsin or DPP-IV, such as codfish, egg, pea, and wheat exert stronger effects on satiety hormone release than the completely hydrolyzed proteins tested in this study. Hence, dietary protein supplements in which gastric degradation is prevented, *i.e.* by the application on an enteric coating of the proteins, may provide a novel strategy for the control of food intake and body weight.

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