Intestinal Signaling of Proteins and Digestion-Derived Products Relevant to Satiety

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ABSTRACT: Luminal nutrients stimulate enteroendocrine cells through the activation of specific receptors to release hormones that inhibit appetite and promote glucose homeostasis. While food protein is the macronutrient with the highest effect on satiety, the signaling on the protein digestion products at the gut is poorly understood. This perspective aims to highlight the existing gaps in the study of protein digestion products as signaling molecules in gastrointestinal enteroendocrine cells. Because dietary protein digestion can be modulated by the technological processes applied to food, it is possible to target gut receptors to control food intake by formulating specific food ingredients or protein preloads.

KEYWORDS: *food proteins, gastrointestinal digestion, satiety, enteroendocrine cells, gastrointestinal hormones*

INTRODUCTION

Energy homeostasis and satiety are centrally regulated by a complex coordinated system, but this regulation also includes peripheral organs, such as the pancreas, adipose tissue, and gastrointestinal tract. In the gut, ingested food induces satiety by two different ways: (i) mechanical stimulation, which is mediated by gastrointestinal nerve endings and release of peptide hormones, and (ii) sensing of the luminal contents by enteroendocrine cells, which also involves the release of gastrointestinal hormones. These hormones play an important role in the digestive process, regulating not only appetite and satiety but also gastric emptying and ileal brake.

Understanding the mechanisms that connect food gastrointestinal digestion, nutrient sensing at the gut, and satiety signaling are crucial to designing foods with an adequate physiological response. Dietary protein is the macronutrient with the highest satiating effect compared to an isoenergetic intake of fat and carbohydrates. Products derived from protein digestion have a more potent effect on gastrointestinal-released hormones than other nutrients, but while mechanisms of carbohydrate sensing are better understood, those for protein and lipid sensing are less well-defined. It is known that bypass surgery reduces food intake by pushing nutrients to the distal gut, causing an increased postprandial release of satiety hormones via chemosensory mechanisms and a reduced food intake.[1] Because protein digestion is considerably affected by the technological treatment applied, it is possible to design food ingredients or protein preloads targeting the initiation of satiety signals from the gastrointestinal tract, simulating the effects of gastric bypass. The formulation of ingredients by the application of food technologies to target the distal gastrointestinal tract is an attractive option and would open new possibilities to control food intake and diabetes.

The aim of this perspective is to combine the available data about gastrointestinal digestion of dietary proteins with the updated information on protein-derived products as signaling molecules at the level of gastrointestinal enteroendocrine cells to induce the release of hormones relevant to satiety. A critical view is given regarding the poor characterization of protein hydrolysates in studies performed at the cellular level and the importance of food digestion and technological treatments of the ingredients evaluated in animal or human studies. Insights on the possibilities of food technology to modify protein digestion, leading to different hormone release and satiety signaling at the gut, are given.

FOOD PROTEIN DIGESTION

Upon digestion, food undergoes physical and chemical changes mediated by mastication, peristaltic movements, pH, and the action of different enzymes that disintegrate food to release nutrients, which will be used by the organism. In the case of proteins, they have different susceptibility to pH and proteases determining their digestion rate in each gastrointestinal compartment. In addition, food protein digestion is affected by the protein conformation, the presence of cross-linkages between protein chains, bound metals, or polyphenols, the particle size, and the presence of antinutritional factors, such as trypsin and chymotrypsin inhibitors. In addition, interindividual variability is also significant and can be influenced by age, health status, or the use of common drugs, such as antacid medication.

Each part of the gastrointestinal tract has its own function in the digestion of food proteins. After the formation of the food bolus in the mouth, it is swallowed and enters the stomach, where proteins find an acidic environment (in the range of pH 1.5−3). Dietary proteins are denatured at this low pH and are hydrolyzed by the action of pepsin.[2] Biosurfactants, such as phosphatidylcholine, also play a role in protein unfolding to make them more accessible to the enzyme. Pepsin is secreted

in the stomach in the form of inactive pepsinogen and is converted to the active enzyme by autocatalysis at pH below 5. The specificity of pepsin is less than that of trypsin and is known to cleave the peptide chain after hydrophobic amino acids, preferably after phenylalanine and leucine. The behavior and susceptibility of food protein in the stomach vary greatly with the protein source and the structural characteristics of the protein. One of the best described examples is the protein fraction of milk, where the casein fraction coagulates in the stomach as a result of the acidic environment, delaying gastric emptying. On the contrary, whey proteins remain soluble at the gastric pH and are rapidly emptied from the stomach, leading to faster amino acid absorption in comparison to casein. On the basis of these different gastric-emptying rates and postprandial metabolic responses, casein and whey proteins are termed as slow and fast proteins, respectively, in parallel with carbohydrates.[3] In a human study with protein snacks containing casein and whey protein, flow rates of nitrogen in jejunum and the rate of appearance of amino acids in plasma were significantly faster for whey protein than for casein but the level of gastrointestinal and pancreatic hormones was not affected. Only a lower secretion trend was obtained for glucagon-like peptide-1 (GLP-1) in the casein group compared to the whey protein group.[4]

In general, globular and compact dietary proteins, such as β-lactoglobulin, bean phaseolin, wheat gluten, or soy glycinin, or proteins rich in disulfide bonds, such as lysozyme, are resistant to pepsin digestion. Other proteins with random structures, such as β-casein, or globular proteins with looser structures, such as hemoglobin, are susceptible to the action of pepsin.[5] Digested dietary proteins in the stomach are generally degraded into peptides, and only traces of free amino acids can be detected, as shown in a porcine model and by *in vitro* simulated gastrointestinal digestion.[6] Gastric digestion can be altered (delayed or enhanced) by applying different technological processes usually employed in the food industry. Heat-induced unfolding or, on the contrary, heat-induced aggregation can affect protein susceptibility to pepsin. For instance, heat treatment produces an increased resistance of casein to be hydrolyzed by pepsin, while β-lactoglobulin is found to be more susceptible.[7] Stomach emptying rates, which control further signaling and absorption in the intestine, are also affected by gastric disintegration and, thus, food structure. A reduced gastric-emptying rate, i.e., increased gastric residence time, has been related to augmented subjective satiety signals in humans. It has been shown that solid and liquid foods with the same macronutrient composition have different effects on gastric emptying and intestinal satiety hormone release. For instance, in a study with liquid and gelled lipid−protein emulsions, a faster release of nutrients into the lumen by the liquid diet was found, inducing a more rapid nutrient sensing at the proximal part of the small intestine, because higher gastric inhibitory polypeptide (GIP) levels were found in the plasma of liquid-diet-fed rats.[8] However, it is accepted that an increasing caloric content is more effective than increasing viscosity in slowing gastric emptying, although viscosity is important in the subjectively perceived fullness. Current knowledge suggests that satiety signals derived from food texture are integrated with those directly derived by food composition, triggering gut hormone release at the gut and an overall experience of satiety.

Once the chyme enters the small intestine, pancreatic proteases are secreted together with bicarbonate, which neutralizes the chyme to a more favorable pH for the activity of intestinal enzymes. All pancreatic proteases are secreted to the intestine in the form of inactive zymogens. Trypsinogen is cleaved by brush border enteropeptidase to trypsin, while trypsin catalyzes the hydrolysis of the other zymogens to their active forms. Trypsin is the most selective protease and cleaves the C terminus to lysine and arginine; chymotrypsin hydrolyses peptide bonds adjacent to hydrophobic amino acids; while elastase cleaves the C terminus to alanine, glycine, and serine. In addition, carboxypeptidases A and B remove amino acids from the carboxyl end, preferably hydrophobic and positively charged amino acids, respectively. Peptides at this level are also hydrolyzed by aminopeptidases located at the brush border membrane. After the action of the pancreatic enzymes, around 30% of ingested nitrogen is in the form of free amino acids but a large fraction corresponds to peptides (ca. 70%).[2] Several peptidomic studies in human or porcine jejunal samples have shown that most peptides at this level are between 2 and 14 amino acids, although some longer fragments have been found. Dependent upon the protein source, the characteristics of peptides resistant to digestion are different. Casein-derived resistant peptides found at human jejunum are rich in proline and phosphorylated serine, while whey proteins are a source of peptides rich in negatively charged residues.[6] Plant protein digestibility at this level is affected by the presence of protease inhibitors, polyphenols, saponins, and phytic acid, or the presence of complex carbohydrates that hinder the access of enzymes to the protein. All of these factors explain the lower protein digestibility rates accepted for plant proteins in comparison to those of animal proteins. Digestibility in the small intestine can also be influenced by the processing techniques applied to food, and thereby, this affects protein arrival at the colon. In general, processing techniques causing protein denaturation, protein degradation, or deactivation of enzyme inhibitors induce an increase in protein digestibility, while a decrease in digestibility is observed with technologies causing protein aggregation or the formation of cross-linkages or inducing the Maillard reaction.[5] In a human study with fat emulsions stabilized with sodium caseinate or transglutaminase-cross-linked sodium caseinate, the postprandial profiles of glucose, insulin, cholecystokinin (CCK), appetite, and satiety were affected through the decrease of the protein digestion in the cross-linked emulsion, although there were no significant effects on the gastric-emptying rates or the overall lipid digestion.[9] Understanding how these technologies affect protein digestibility, especially the release of protein digestion products found in the intestine, which act as signaling messengers in enteroendocrine cells, will help to design new strategies to modulate food intake and weight management.

In addition, intestinal microbiota can ferment the protein that reaches the colon, approximately 12−18 g of protein/day depending upon the amount and quality of the ingested protein. The metabolic activities of luminal bacteria and the production of certain bacterial proteins able to activate the host satiety pathways have been proposed as the putative mechanisms of the microbiota to influence the food intake and energy balance of the host. Moreover, it has been suggested that the amino acid composition and digestibility of dietary proteins can determine composition and metabolic activity of the gut microbiota. Dependent upon the dietary protein, significant changes in short-chain fatty acids, ammonia, amines, and gases have been reported. The arrival of a high amount of

Table 1. Main Types of Enteroendocrine Cells Expressed in the Gastrointestinal Tract, Localization, Hormone Secretion, Functions on Gastrointestinal Tract and Food Intake, and Nutrients Acting as Stimuli[a]

cell	localization	cell type	hormone secretion	function	nutrient as stimuli	references
A	stomach	closed	ghrelin	stimulation of food intake	fasting	11 and 12
				stimulation of growth hormone secretion	inhibited by nutrients	
D	stomach	closed	somatostatin	inhibition of gastrin release	acid	11
	duodenum			reduction of acid secretion		
G	stomach (pyloric antrum)	open	gastrin	stimulation of gastric acid secretion	digested protein	12
	duodenum			induction of pepsinogen secretion	gastric distension	
					inhibition by acid	
EC	stomach, small and large intestine	closed	serotonin	food intake modulation	glucose	11
				induction of gastric and pancreatic secretion	lipids (SCFA)	
				increase small bowel motility	stimulation by distension, pH	
I	proximal small intestine (duodenum and jejunum)	open	CCK	inhibition food intake	digested protein	11 and 12
				inhibition of gastric emptying	lipids (LCFA)	
				stimulation of gallbladder contraction and pancreatic enzyme secretion		
S	duodenum	open	secretin	inhibition gastric acid secretion	acid	11
				inhibition of gastric emptying	digested protein	
				stimulation of bicarbonate fluid production from the pancreas		
K	proximal small intestine (duodenum and jejunum)	open	GIP, xenin	inhibition of food intake	lipids	11 and 13
				inhibition of gastric emptying and acid secretion	carbohydrates	
				glucose-induced insulin secretion		
				regulation of lipid metabolism in adipose tissue		
L	distal ileum and colon	open	GLP-1, GLP-2, PYY, OXM, glicentin	inhibition food intake	carbohydrates (monosaccharides)	11 and 13
				inhibition of gastric emptying and acid secretion	lipids (MCFA, LCFA)	
				stimulation of glucose-dependent insulin secretion	digested protein	
				inhibition of glucagon release		
M	duodenum	open	motilin	stimulates gastrointestinal motility	lipids	12
					bile acids	
					acid	
N	jejunum and ileum	open	neurotensin	stimulation of gastric acid secretion	lipids	12
				stimulation of pancreatic and biliary secretion		
				inhibition of gastric and small intestinal motility		

[a]LCFA, long-chain fatty acids; MCFA, medium-chain fatty acids; SCFA, short-chain fatty acids; EC, enterochromaffin cells; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; OXM, oxyntomodulin; GIP, gastric inhibitory peptide; and PYY, peptide YY.

undigested protein at the colon has been related to changes in the microbiota, gut barrier, and immune system, which have been associated with the development of colon cancer and inflammatory bowel diseases.[10] Therefore, the studies of protein digestibility from different dietary sources by proteomic and metabolomic techniques are gaining increased relevance as well as the suitable protein/carbohydrate ratio in the diet to avoid adverse health effects.

■ ENTEROENDOCRINE CELLS AND SECRETED GASTROINTESTINAL HORMONES

The gut is considered the largest endocrine organ as a result of not only the high number of endocrine cells present but also the high variety of hormones produced. The enteroendocrine cells represent around 1% of the total epithelial intestinal cells and are found throughout the gastrointestinal epithelium among absorptive enterocytes, mucus-secreting goblet cells, opioid-releasing cells, and Paneth cells.[11] Most enteroendo-

crine cells have the apical side covered with microvilli, which are in contact with luminal contents, and these are called "open-type". In contrast, "closed-type" enteroendocrine cells do not make contact with the lumen. It is now accepted that open-type enteroendocrine cells act as sensors of the luminal content or physical or chemical stimuli and, as a response, produce a variety of hormonal regulators for the gastrointestinal tract, metabolism, and food intake. For recent reviews on enteroendocrine cells as sensors of luminal contents, see refs 12 and 13.

Traditionally, enteroendocrine cells have been classified into at least 15 cell subtypes, according to their morphology and the main secreted hormones. They are distributed along the gastrointestinal tract and are able to react to different stimuli (Table 1). Those related with protein sensing are A-like cells on the gastric mucosa, which release ghrelin, G cells, located in the pyloric antrum and duodenum, releasing gastrin, S and I cells, located in the duodenum and proximal small intestine,
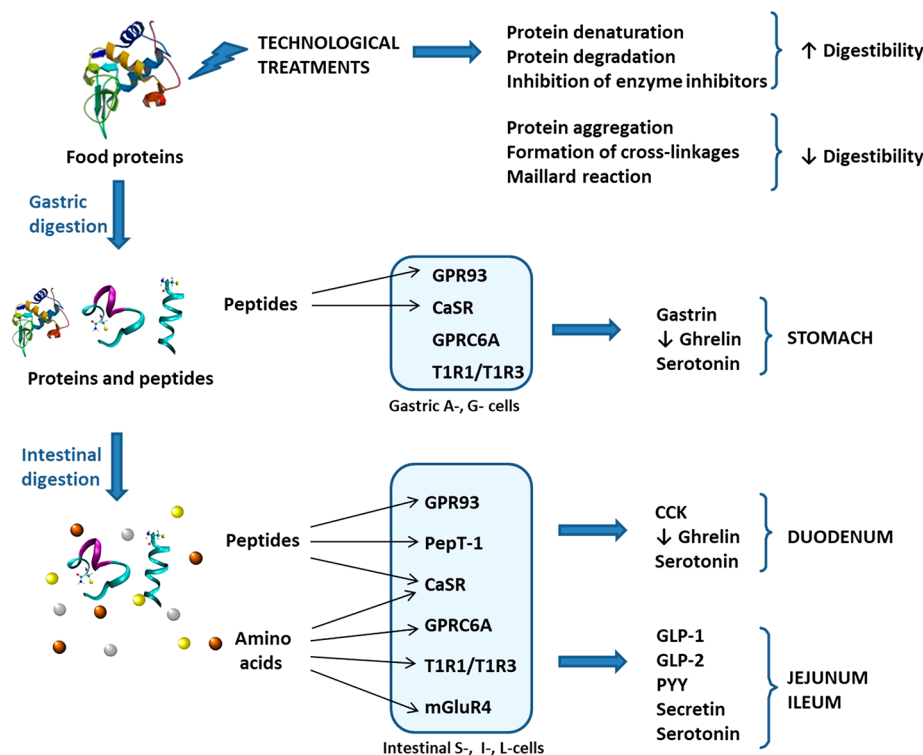
Figure 1. Schematic overview of protein digestion through the gastrointestinal tract and protein digestion products acting as signaling molecules in enteroendocrine cells. Food processing alters protein digestibility, enhancing or delaying it. Peptides released at the stomach activate GPCRs, mainly GPR93 and CaSR, which are able to sense peptides and stimulate the release of hormones related to food intake. At the intestine, a large amount of free amino acids is released together with peptides. At this level, different GPCRs can be activated by peptides and free amino acids, inducing hormone secretion.

which produce secretin and CCK, respectively, and L cells located in the distal small intestine and large intestine, which secrete GLP-1, GLP-2, PYY, glicentin, and oxyntomodulin. Enterochromaffin cells distributed along the gastrointestinal tract (stomach and small and large intestine) secrete serotonin, and D cells, which release somatostatin, are also involved in protein sensing in the gastrointestinal tract.[11]

Ghrelin-secreting A-like cells are closed-type enteroendocrine cells located in the stomach and buried within the epithelial mucosa, making only contact with the bloodstream. G cells, at the gastric antrum, detect the luminal contents and, in particular, protein breakdown products and secrete gastrin. The secretion of gastrin has been related to the amount and quality of the ingested protein. CCK is secreted predominantly from I cells in the duodenal and jejunal mucosa of the small intestine. This hormone reduces food intake by increasing post-meal satiation in humans, but as a result of its short half-life, it does not affect the time between meals (satiety). Protein and fat produce a greater CCK release than carbohydrate or fiber.[14] GLP-1 is produced as proglucagon in the gut, brain, and pancreas and is cleaved in different active forms depending upon the tissue. In the intestine, proglucagon is processed to GLP-1, GPL-2, and glicentin, which is further hydrolyzed to oxyntomodulin.[14] As occurs with the CCK, as a result of its rapid degradation, GLP-1 exerts a short-term effect on food intake. GLP-1 is also involved in the ileal brake, the mechanism that regulates gastric emptying. In addition, this hormone as well as the gastric inhibitory peptide (GIP) leads to glucose-dependent insulin secretion by acting on pancreatic β cells, although through different receptors. For this reason, these two hormones, GLP-1 and GIP, are known as insulinotropic

incretin peptides. However, dietary fat and carbohydrates are the major stimuli for the secretion of GIP, while ingested protein seems to have no effect on the secretion of this insulinotropic peptide. Carbohydrates are also a strong stimulus for GLP-1 secretion, although there is ongoing controversy regarding whether proteins or carbohydrates induce higher plasma levels of this hormone. Some studies have reported a higher postprandial GLP-1 response after high-fat or high-protein meals than after high-carbohydrate meals.[15] L cells also secrete PYY, and this hormone mediates ileal and colonic brakes, slows gastric emptying, and promotes digestion to increase nutrient absorption. It is mainly released in the distal parts of the gastrointestinal tract, ileum, colon, and rectum, although smaller amounts of PYY can also be found in the upper small intestine. Fat and protein are potent stimulators of PYY release to a greater extent than carbohydrates, and studies in PYY null mice have shown the important role of PYY in protein-mediated satiation.

Recent literature has evidenced a high degree of transcriptional overlap between different enteroendocrine cell populations, and a revision of the classical classification has been proposed. This has brought the idea that enteroendocrine cells might be plastic and modifiable, for instance, by dietary changes or gastric bypass surgery. Gastric bypass surgery results in an increased postprandial concentration of GLP-1 and PYY in humans and animal models.[1] Similarly, intra-duodenal administration of intact pea protein produced an increase in CCK levels compared to oral protein administration in lean and obese subjects, although GLP-1 levels were only augmented in obese subjects.[16] The current consensus on gastrointestinal chemosensory mechanisms is

Table 2. Main Receptors and Transporters Expressed in Gastrointestinal Enteroendocrine Cells Related to Sensing of Protein Digestion Products[a]

receptor	expression in human gastrointestinal tract	enteroendocrine cell type	nutrient ligand	antagonist	physiological effect	references
GPR93/GPR92/LPAR5	along gastrointestinal tract; higher expression at mid-intestine (distal jejunum)	D, G, I, and L cells	peptides	YM-254890	protein sensing in G cells; increase CCK expression and secretion	20 and 23
CaSR	along gastrointestinal tract, pancreatic and ductal cells	5-HT, L, G, D, and I cells	free amino acids: L-Phe, L-Trp, L-His > L-Ala > L-Ser, L-Pro, L-Glu > L-Asp; cationic peptides: Arg and Lys-rich peptides, soybean β-conglycinin f(S1−63) γ-glutamyl peptides (γ-Glu-Cys-Gly and γ-Glu-Val-Gly); di- and trivalent cations	NPS2143; Calhex231; thapsigargin	regulation of secretion of digestive enzymes; CCK secretion; GLP-1 secretion	20, 27, and 33
GPRC6A	gastric and intestinal mucosa	gastric D and G cells, intestinal L cells	calcium, other cations and basic amino acids: L-Lys > L-Arg, L-ornithine > other amino acids	calindol; NPS2 143; 2-phenyl-indole-derived compounds 1−3	glucose metabolism; GLP-1 secretion in intestinal L cells	20
T1R1−T1R3	small intestine pancreatic islets	I, K, and L cells	monosodium glutamate; L-amino acids; nucleotides: inosine monophosphate and guanosine monophosphate	lactisole (T1R3); fibrates; phenoxy herbicides	glucose metabolism; GLP-1 secretion; PYY and CCK secretion	20
mGluR4 and mGluR1	along gastrointestinal tract; higher expression distally (proximal colon)		L-Glu			20
GPR142	stomach and small intestine	G, K, and L cells	L-Trp essential amino acids; peptides	CLP-3094	GLP-1 secretion; glucose metabolism	17
PEPT1	from small intestine to colon	I and L cells	di- and tripeptides; peptidomimetics	4-(aminomethyl)benzoic acid (4-AMBA)	CCK secretion (indirectly); GLP-1 secretion	33 and 37

[a]References are included in the text. Three-letter code is used for amino acids.

that luminal contents are able to interact and activate G-protein-coupled receptors (GPCRs) and amino acid and peptide transporters located in the membranes of enteroendocrine cells, which produce peptide hormone release (Figure 1). GPCRs have been classified into six families (from A to F), but only types A and C have shown to play a role in nutrient sensing, while class B GPCRs include those which respond to hormones, such as GIP and GLP-1. Concretely, those receptors related to the sensing of protein digestion products are the G-protein-coupled receptor family C group 6 member A (GPRC6A), the umami taste receptor T1R1/T1R3, the calcium-sensing receptor (CaSR), the metabotropic glutamate receptors, and the peptide receptor GPR93,[17] as will be detailed in the next section.

TARGETING PROTEIN SENSING RECEPTORS IN ENTEROENDOCRINE CELLS BY PROTEIN DIGESTION PRODUCTS

Nutrients released after gastrointestinal digestion are traditionally described as "fuel" or energy sources, but now they are also considered as signaling molecules able to regulate the activity of enzymes and control different physiological processes, such as food intake. This is an especially well-known event in the gastrointestinal tract. There, food digestion products act as strong stimuli for the secretion of gut and pancreatic hormones through interaction with GPCRs. While the mechanisms of carbohydrate sensing are relatively well-understood, sensing protein digestion products is less described. Often they are referred to as peptones or hydrolysates without considering their composition with regard to the presence of free amino acids or the characteristics of peptides. This lack of characterization of the substrate has led to controversial results, although several reports have pointed to a clear specificity of certain peptide sequences to induce hormone release in cellular models.[18,19] This section focuses on the GPRCs related with the sensing of protein digestion products and the molecules responsible for their activation (Table 2) as well as the current evidence on cell cultures, animal models, or humans.

GPR93 (also known as GPR92 or LPAR5) is expressed more than other amino acid or fatty acid nutrient sensors in the small intestine in mice and humans.[20] It is expressed in G cells, located at the stomach (pyloric antral), and L cells, located at the distal small intestine and colon. In fact, colonic L cells immunoreactive to GLP-1 and PYY have been found to be frequently associated with GPR93. Although gastric G cells are able to sense protein breakdown products by two other protein-related receptors (GPRC6A and CaSR), the relative amount of receptor GPR93 is much higher than that of the other two receptor types. It has also been suggested that peptides are more effective than amino acids in stimulating the secretion of gastrin in the stomach.[21] In line with the proposed plasticity of the enteroendocrine cells, a protein-induced increased GPR93 expression has been observed after high protein feeding for 35 days, although it returned to the baseline level after 84 days.[21] This receptor was activated by a protein hydrolysate in STC-1 cells, leading to an induction of CCK expression and secretion.[22] In agreement with the involvement of a Gα_q-coupled receptor, a hydrolysate from soybean β-conglycinin showed CCK secretion in the same cell line, which was blocked after treatment with a Gα_q protein inhibitor (YM-254890).[23] Interestingly, several reports have demonstrated that CCK secretion is induced by peptides to a higher extent than by free amino acids. Commercial hydrolysates from egg albumen, meat, soybean, and casein have demonstrated stimulation of CCK release and gene transcription to a higher extent than a mixture of free amino acids or intact protein (bovine serum albumin).[24] Similarly, a hydrolysate of blue whiting muscle was found to induce CCK and GLP-1 secretion in STC-1 cells, while its oral administration in rats reduced short-term food intake, which was correlated to CCK and GLP-1 plasma levels.[25] Other studies have shown certain selectivity of peptides inducing CCK secretion. For instance, no effect on CCK secretion in STC-1 cells was found with whey protein-derived di- or tripeptides containing aliphatic side chains, and only peptides with five or more amino acids were effective at stimulating CCK release.[26] Caron and co-workers suggested that the CCK secretagogue activity of hemoglobin-derived peptides was linked to the high content of aromatic residues in their sequences.[19] Therefore, further research is needed to identify the peptide molecules able to activate this receptor and the real physiological implications by carrying out *in vivo* studies.

The receptor linked to the secretion of a range of gastrointestinal hormones by activation with L-amino acids and oligopeptides is CaSR. It is widely expressed not only along the gastrointestinal mucosa but also in pancreatic and ductal cells, where it is responsible for regulating the secretion of digestive enzymes. In enteroendocrine cells, CaSR requires calcium to elicit the opening of a voltage-gated calcium channel. In the absence of calcium, amino acids or other agonists for this receptor are ineffective. This receptor has been found to be present in 5-hydroxytryptamine-secreting cells rather than L cells.[20] CaSR can adopt multiple conformational states; it is stabilized by different ligands that induce one or more signaling pathways.[12] It is activated by calcium and other di- and trivalent cations and L-amino acids. The rank order of amino acid affinity for the receptor has been proposed as L-phenylalanine, L-tryptophan, L-histidine > L-alanine > L-serine, L-proline, L-glutamic acid > L-aspartic acid.[27] The amino acid phenylalanine induces CCK release in STC-1 cells by activation of CaSR and intracellular calcium mobilization, and the use of a specific CaSR antagonist blocks this response.[28] In mice native I cells, the role of this receptor on CCK secretion in response to L-phenylalanine was demonstrated.[29] Basic polypeptides, i.e., lysine- and arginine-rich peptides, such as, the peptide soybean β-conglycinin 51−63, have also been found to stimulate CaSR in STC-1 cells.[30] In addition, several γ-glutamyl peptides, such as γ-Glu-Cys-Gly (glutathione) and γ-Glu-Val-Gly, were identified as CaSR agonists. CCK secretion was induced by different dietary protein hydrolysates, and the effect was suppressed by treatment with the CaSR antagonist NPS2143, demonstrating the role of this receptor in sensing dietary peptides.[31] Although protein hydrolysates contained free amino acids, low-molecular-weight peptides (<1000 Da) have been suggested as the best stimuli of CCK secretion via CaSR activation,[32] although more studies are needed to confirm this point. This receptor has also been suggested to be involved in the GLP-1 secretion triggered by an enzymatic meat hydrolysate, but the exact peptide composition inducing this signaling has not been characterized.[33]

GPRC6A is closely related to CaSR and is expressed in gastrin G cells, I cells, and somatostatin-secreting D cells. Immunohistochemical studies have shown that this receptor co-localizes with gastrin and somatostatin in the gastric

mucosa, implying that it could be involved in the secretion of these peptides. It is activated by calcium, cations, and L-amino acids, especially positively charged amino acids, such as arginine, lysine, and ornithine, although it also senses other amino acids (alanine, serine, etc.). However, some studies with GPRC6A null mice have suggested that this receptor is not necessary for the effects of high-protein diets in mice,[34] and the release of GLP-1 induced by basic amino acids can also occur independently of this receptor.[35] In addition, because this receptor is poorly expressed at the cell surface and tends to be retained intracellularly in humans, its physiological relevance upon sensing of protein-derived products is questionable.

Taste receptors (TRs) are GPCRs comprising two major families: the T1R family composed of three receptors (T1R1, T1R2, and T1R3) that function as dimers to detect umami (T1R1 + T1R3) and sweet (T1R2 + T1R3) and a large family of T2Rs that detect an array of diverse bitter compounds. The umami receptor T1R1/T1R3 has been detected in I, K, and L cells, being highly expressed distally, in the ileum.[20] In humans, this receptor is particularly sensitive to L-glutamate, while in rodents, a wider range of amino acids can stimulate CCK release mediated by this receptor. In pigs, G-protein-related signaling messengers of this receptor, α-gustducin and α-transducin, are upregulated in response to high-protein diets.[36] However, it is not clear whether this receptor is involved in the control of hormone secretion in humans. In addition, certain redundancy has been proposed to occur because L-amino acids can also be detected by other gastrointestinal receptors, such as the umami taste sensing and the glutamate-selective GPCRs, mGluR4 and mGluR1.

Sensing of dietary protein in the gastrointestinal tract is more complex because it is also dependent upon peptide transporters, such as PEPT1, and amino acid transporters. PEPT1 is a proton-coupled di- and tripeptide transporter, which has been detected in L cells from the small intestine to the colon. The murine tumoral STC-1 cell line and isolated native I cells express PEPT1 transcripts, but present data suggest that this transporter does not directly mediate CCK secretion in response to a protein hydrolysate.[37] However, this transporter was related to the GLP-1 secretion in murine primary colonic cultures in response to peptones and dipeptides.[33]

■ CRITICAL VIEW AND FUTURE PERSPECTIVES

Protein is a key dietary macronutrient and plays an important role on the satiety effect of foods. The importance of nutrient sensing in the gut has been recognized for glucose homeostasis and food intake. While the mechanisms by which carbohydrates and lipids are detected in the gastrointestinal tract are becoming better understood, the detection and pathways followed by protein digestion products are not well-known. The main reasons for this gap are (i) the multifaceted and redundant signaling in the gut for the different protein digestion products and (ii) the complexity of protein digests, which, in turn, can be modified with the technological treatment applied, as demonstrated in different foods: native casein versus sodium caseinate, heated versus unheated whey protein, and well-done versus raw or medium-done meat. Protein digestion products have a huge effect on the secretion of gastrointestinal hormones, and the sensing of these products at enteroendocrine cells is mediated through GPCRs. Several receptors and transporters have been related to the detection of free amino acids (CaSR, GPRC6A, T1R1/T1R3, and

mGluRs). However, proteins are digested into peptides in the stomach, and at more distal locations (jejunum), around 70% of protein nitrogen is found in the form of peptides. The receptor GPR93 is able to detect peptides and has been found to be the most highly expressed receptor in the small intestinal mucosa, peaking in the distal jejunum. Recently, receptor CaSR, traditionally linked to amino acid sensing, has also been related to the detection of small size peptides. Because the peptide composition varies with the protein source and the food structure, treatment, and composition, it would be important to identify the peptide sequences able to activate these receptors. One of the limitations in the study of the signaling at the enteroendocrine level is the poor characterization of the protein hydrolysates studied, often termed as peptones, which has led to controversial conclusions. The field of nutrient sensing receptors is evolving rapidly but also the analytical techniques to sequence a high number of peptides included in complex mixtures using peptidomic approaches. More studies are thus needed to identify those protein digestion products able to induce a metabolic and behavioral response through the exposure of gut enteroendocrine to the gastrointestinal digests of food proteins and the activated pathways.

It is known that nutrients administered in the distal gut, by gastric bypass surgery or infusion, produce a huge postprandial release of satiety and incretin hormones, reducing food intake. It has also been pointed out that protein digestion products have potent effects on hormone release and glycaemic control, which can be used with therapeutic benefits. Because protein digestion is severely influenced by technological treatments, it is possible to modify the gastrointestinal degradation of food to achieve a different postprandial response in the gut and, thus, satiety feelings. For instance, delaying gastric emptying by the formation of a dense protein network with a fine pore structure to restrict pepsin accessibility during the gastric phase can be accomplished by heat-induced gelation of proteins. Interestingly, food structure and food processing are now being considered in many of the nutritional animal and human studies focused on food intake and satiety. The tools to predict the gastrointestinal behavior of food are also being developed, with static and dynamic devices able to simulate gastrointestinal digestion of food, even at the molecular level. The validation and comparability of these digestion protocols with human and animal digestion are being demonstrated, showing excellent performance. The knowledge of the sites, mechanisms, and receptors involved in protein sensing as well as the way proteins are digested and how the technological treatment affects the kinetic release of protein digestion products in the gut would allow for the design of ingredients for the control of food intake, weight, and glycaemia. It is thus an exciting time to perform multidisciplinary studies by connecting the fields of food technology, food digestion, proteomic characterization, and enteroendocrine signaling to develop successful ingredients to control food intake and glucose homeostasis.

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