Expression patterns of T1R3 in duodenal epithelial cells with some gastrointestinal hormone*

Masafumi Oda1,2, Yuji Seta2, Shinji Kataoka3, Takashi Toyono2, Kuniaki Toyoshima2, and Yasuhiro Morimoto1

1Department of Oral Diagnostic Science, 2Division of Oral Histology and Neurobiology, 3Division of Anatomy, Department of Bioscience, Kyushu Dental College, Kitakyushu, Japan

Summary. Recent studies have demonstrated that taste receptors, T1Rs and T2Rs, are expressed not only in taste buds but also in the gut. In the duodenum, it is thought that the secretion of secretin (S cells) and somatostatin (D cells) is stimulated by acid, that gastrin (G cells) and cholecystokinin (CCK) (I cells) are stimulated by proteins and amino acids, and that serotonin (EC cells) secretion is triggered by mechanical stimulation sensing gut endocrine cells.

We examined the expression of T1R3 in the duodenum by reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization. Double-label studies showed that approximately 50% of secretin positive cells (S cells) were coexpressed with T1R3, and approximately 33% of AADC positive cells (EC cells) were coexpressed with T1R3. On the other hand, T1R3 never colocalized with somatostatin (D cells), gastrin (G cells), or CCK (I cells). These results suggest that S cells may regulate the secretion of secretin, stimulated not only by acid but also by sweet substances and/or amino acids. Similarly, EC cells may regulate the secretion of serotonin by stimuli other than a mechanical one.

Introduction

The taste system, which is made up of taste receptor cells clustered in taste buds at the surface of the tongue and the soft palate, plays a key role in the decision to ingest or reject food. Its function is therefore, essential to protect organisms against harmful toxins and select the most appropriate nutrients. Ingested nutrients stimulate the secretion of gastrointestinal hormones necessary for the coordinated processes of food digestion.

The T1Rs, G-protein-coupled receptors (GPCRs) that form two novel seven-transmembrane domains, are expressed in topographically distinct subpopulations of taste receptor cells and taste buds (Hoon et al., 1999). The functional expression of T1Rs is influenced by sweet and umami taste stimuli. A T1R2/T1R3 heterodimer serves as a chemical receptor for sweet tastes (diverse natural and synthetic sweeteners). T1R1/T1R3 heterodimers are receptors for the umami compound l-glutamate, a response enhanced by 5'-ribonucleotides (Nelson et al., 2001; Li et al., 2002).

Recent studies have demonstrated that solitary cells expressing taste cell signal transduction proteins exist in hollow organs. It has been previously shown that T1Rs are expressed in the gastrointestinal tract (GI) (Hofer et al., 1996; Wu et al., 2002; Dyer et al., 2005; Grundy, 2005). They are additionally expressed in the stomach, small intestine—including the duodenum, and colon in mice and humans, with the exception of T1R2, which has not been detected in the mouse or human stomach or in the mouse colon (Bezençon et al., 2007). There have been
numerous studies on the downstream cascade reactions of gastrointestinal chemosensing by taste receptor components (Margolskee et al., 2007; Rozengurt and Sternini, 2007; Jang et al., 2007; Mace et al., 2007; Shin et al., 2008; Sternini et al., 2008). T1R3 and gustducin in the gut regulate the secretion of glucagon-like peptide-1 (GLP-1)—an incretin hormone that promotes the augmented insulin release from the pancreas in response to glucose in the gut lumen more than to intravenously injected glucose (Kokrashvili et al., 2009)—as well as the expression of the Na+-glucose cotransporter 1 that transports dietary sugars from the intestinal lumen into absorptive enterocytes (Margolskee et al., 2007). There is a study showing that T1R3 is expressed in the brush cells and ghrelin-producing cells of the murine stomach (Hass et al., 2010). However, no reports have described the relationship between the initial reaction and hormone secretion. We therefore, aimed to determine whether the T1R3 that is a common component of sweet and umami receptors is the initial chemoreceptor for these secreting reactions.

Materials and Methods

Adult ICR mice of both sexes were used in this study. The use of these animals was approved by the Kyushu Dental College Animal Care and Use Committee and conformed to the National Institute of Health Guidelines.

RT-PCR

For RT-PCR, dissected duodenum tissue containing epithelium was incubated for 60 min at room temperature in RNH safer Stabilizer Reagent (Omega Bio-Tek, Norcross, GA, USA). For the extraction of RNA from tissues, we used the RNA Purification System (Roche Applied Science, Mannheim, Germany) and degraded the DNA by incubation at 37°C for 1 h with DNase. The resultant total RNAs were reverse transcribed using random 9-mers and Avian Myeloblastosis Virus (AMV) reverse transcriptase at 42°C for 15 h to yield single-stranded cDNAs. Following denaturation at 99°C for 5 min, PCR amplification was performed under the following conditions: 94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min, for a total of 35 cycles. The reaction was terminated after a 15-min elongation step at 72°C. The reverse transcriptase step was omitted in negative control samples to confirm the removal of all genomic DNA. Amplification products were analyzed in 2% agarose gels and visualized with ethidium bromide. Amplification products were subcloned and sequenced to confirm their identities. The primer sequences for mouse T1R3 were as follows:

Forward: 5'- TTGTGCTGTCACCTCTCCTG -3'
Reverse: 5'- TAAGCTAGCATGGCAAGGT -3'.

Tissue preparation

All mice were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg) and perfusion through the left ventricle with 4% paraformaldehyde (PFA) in a phosphate buffer, pH 7.4. Duodenums from perfused mice were fixed overnight in the same fixative. They were then rinsed overnight in a phosphate buffer containing 30% sucrose, embedded in OCT compound (Sakura, Torrance, CA, USA) and snap-frozen in a dry ice-isopentane mixture. Cryostat sections (5 μm) were mounted on Superfrost slides (Matsunami, Japan) and stored in airtight boxes at −80°C.

In situ hybridization

After a brief washing in PBS, treatment for 5 min each with 0.2 N HCl and PBS containing 1 μg/ml proteinase K (Wako, Osaka) at 37°C, the sections were refixed for 20 min in 4% PFA and then treated twice for 15 min with 2 mg/ml glycine/PBS. Sections were prehybridized for 30 min at room temperature in a hybridization buffer (50% deionized formamide/300 mM NaCl/5 mM EDTA/0.1% Tween 20/0.5% CHAPS/1% Blocking Buffer). Digoxigenin-labeled antisense and sense riboprobes were produced from plasmids containing T1R3. Hybridization was performed overnight at 67°C in a hybridization buffer containing 100 μg/ml tRNA, 0.05 mg/ml, and 0.5 μg/ml riboprobe. After hybridization, the slides were washed for 30 min in 2×SSC at room temperature, followed by 1 h in 2×SSC at 67°C, 1 h in 0.1×SSC at 67°C, and 30 min in maleic acid buffer (MABT) (0.1 M maleic acid/0.15 M NaCl/0.1% Tween 20) at room temperature. Next, the sections were incubated for 2 h with anti-digoxigenin-AP diluted 1:250 in 2% (wt/vol) blocking reagent (Roche). After a 15-min washing in MABT, a 5-min equilibration in a NBT/BCIP buffer [100 mM Tris-HCl (pH 9.5)/100 mM NaCl/50 mM MgCl2, and a 3-h incubation in the dark in NBT/BCIP buffer containing 10% polyvinyl alcohol and a NBT/BCIP mixture (Roche) diluted 1:50, the slides were washed for 5 min in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA and then rinsed in PBS.

Immunofluorescence

Following in situ hybridization, the sections were analyzed for the presence of gastrointestinal hormone-
secreting cells, secretin, somatostatin, gastrin, CCK, and Aromatic L-amino acid decarboxylase (AADC). The sections were rinsed in PBS and then incubated with primary rabbit anti-secretin (1:200; Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA), anti-somatostatin (1:1000; Invitrogen, Carlsbad, CA), anti-gastrin (1:1; Cell Marque, Rocklin, CA), anti-CCK (1:1000; Affinity BioReagents, Rockford, IL, USA), or AADC antibody (1:500; Gene Tex, Inc., Irvine, CA) overnight at room temperature in a humidified chamber. After rinsing with PBS, the sections were incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (Molecular Probes, Eugene, OR, USA) overnight at room temperature. The slides were rinsed with PBS and coverslipped with Vectashield (Vector Laboratories, Inc., Burlingame, CA).

Immunofluorescent and in situ hybridization images were obtained with an Olympus DP72 CCD camera mounted on an Olympus BX50 microscope. Digital images were acquired with DP2-BSW software, converted to TIFF format, and then contrast- and color-adjusted using Adobe Photoshop CS3 for Macintosh. Overlays of fluorescent and in situ hybridization images were also generated in Photoshop as follows: the in situ hybridization image was first inverted, and the blue and green channel portion deleted to black, leaving an inverted, pseudocolored red image. Then the green fluorescence image of the same field of view was pasted into the green channel, to produce the overlay.

**Data analysis**

For quantification, every second section was examined (to avoid counting the same cells). A positive cell was
Fig. 2. Expression of T1R3 in the mouse duodenum (A, B). T1R3 was expressed by a subset of cells in the duodenum epithelium (arrow), whereas T1R3-expressing cells were not observed in the other part of duodenum. Scale bars: 40 μm (A), 10 μm (B).

Fig. 3. Double labeling with in situ hybridization for T1R3 and immunohistochemistry for AADC and secretin. In situ hybridization images of T1R3 expression are shown in the left column (red; A and D), immunofluorescence values of AADC and secretin are shown in the right column (green; C; AADC, D; secretin), and the computer-generated overlays of in situ hybridization and immunohistochemistry images are shown in the middle column (B: AADC, E: secretin). Approximately 50% of T1R3-positive cells were also immunoreactive for AADC (arrows). However, there were approximately 67% of the AADC-positive cells that lacked T1R3 expression (arrowheads). Scale bars: 10 μm.
marked as immunopositive if the staining revealed that the nuclear profile of the cell was present in the section. T1R3-positive cells were considered to be labeled with antisense RNA probes if the staining was circumnuclear within a section. The double-labeled cells were then assessed and tallied via a digital overlay as described above.

Results

RT-PCR analysis

To determine T1R3 gene expression in the mouse duodenum, we performed RT-PCR experiments (Fig. 1). When the RT-PCR was performed using RNA prepared from the epithelium of circumvallate papilla and the

Fig. 4. Double labeling with *in situ* hybridization for T1R3 and immunohistochemistry for each hormone. *In situ* hybridization images of T1R3 expression are shown in the left column (red; A, D, G), immunofluorescence of hormones is shown in the left column (green; C: CCK, F: gastrin, I: somatostatin), and the computer-generated overlays of in situ hybridization and immunohistochemistry images are shown in the middle column (B: CCK, E: gastrin, H: somatostatin). Enteroendocrine cells positive for other gastrointestinal hormones (arrowheads: CCK, gastrin, and somatostatin) did not express T1R3 (arrows). Scale bars: 10 μm
duodenum, an amplification product of the expected size (610bp) was obtained with primer sets specific for mouse T1R3 and sequenced to confirm identities. However, no amplification product was obtained using RNA prepared from the duodenum without reverse transcription.

Expression of T1R3 gene in the mouse duodenum

To determine the expression patterns of T1R3 in the mouse duodenum, in situ hybridization was performed. T1R3 mRNA was found in the epithelium of the duodenum, where T1R3 was expressed by a subset of cells T1R3-expressing cells were not observed in other parts of the duodenum (Fig. 2).

Double staining for T1R3 and each hormone

To assess further which cells express T1R3 in the duodenum, we performed double labeling using in situ hybridization for T1R3 and immunohistochemistry for some gastrointestinal hormones (AADC, CCK, gastrin, secretin, and somatostatin). A previous study demonstrated that AADC is present in EC cells in the small intestine (Facer et al., 1979). Approximately 50% of T1R3-positive cells were also immunoreactive for AADC (51.1%; 186/364, Fig.3A–C, Table 1). However, there were approximately 67% of AADC-positive cells that lacked T1R3 expression (66.7%; 186/559, Fig.3A–C, Table 1). Secretin is a marker of S cells in the small intestine (Bayliss and Starlig, 1902). Approximately 40% of T1R3-positive cells were also immunoreactive for secretin (39.8%; 65/163, Fig.3D–F, Table 1). Approximately 50% of the secretin-positive cells that lacked T1R3 expression (46.7%; 65/122, Fig.3D–F, Table 1).

On the other hand, enteroendocrine cells positive for other gastrointestinal hormones (CCK, gastrin, and somatostatin) did not express T1R3 (Fig.4, Table 1).

Discussion

The present study has shown for the first time that T1R3 is expressed with secretin (S cell) and AADC (EC cell) of the mouse duodenal epithelium. However, T1R3 did not coexpress with gastrin (G cell), somatostatin (D cell), or CCK (I cell) in the mouse duodenum.

The T1R family (T1R1, T1R2, and T1R3) belong to subclass 3 of the GPCR superfamily. T1R1 and T1R3 combine to function as an umami receptor, while T1R2 and T1R3 form a sweet receptor in the taste buds. Recent studies have shown that the T1R family is also expressed in the small intestine and colon. Furthermore, Taniguchi (2004) showed that T1R3 is expressed in the intrahepatic bile duct and pancreas. Thus, the T1R family is expressed in various parts of the digestive system, and it is possible that these receptors may play a role in chemical sensations in these tissues.

Enteroendocrine cells are specialized endocrine cells of the GI tract. They produce hormones such as

| Table 1. Extent of overlap of epithelial cells of duodenum expressing TIR3 mRNA with cells immunopositive for hormones. |
|---|---|---|---|---|---|
| In situ hybridization hormone | TIR3 n=364 AADC n=559 Coexpression n=186 | TIR3 n=163 Secretin n=122 Coexpression n=65 | TIR3 n=88 CCK n=160 Coexpression n=0 | TIR3 n=180 Gastrin n=102 Coexpression n=0 | TIR3 n=146 Somatostatin n=140 Coexpression n=0 |
| TIR3 n=178 (51.1%) | TIR3 n=98 | TIR3 n=88 | TIR3 n=180 | TIR3 n=146 |
| AADC n=373 | secretin n=65 (39.8%) | CCK n=160 | gastrin n=102 | somatostatin n=140 |
enteroglucagon (A cell), cholecystokinin (CCK) (I cell), glucose-dependent insulinotropic polypeptide (K cell), gastrin (G cell), neurotensin (N cell), secretin (S cell), serotonin (EC cell), and somatostatin (D cell). Enteroeocrine cells sense nutritive and non-nutritive properties of luminal food and, in response, release hormones from their basolateral aspect. Secretin, discovered by Bayliss and Starling (1902), is secreted by S cells, which are evenly distributed over the duodenal epithelium. S cells are activated mainly by gastric acid but also by digested products of fat (longer than 14 carbons), protein, and bile acids (Li et al., 1990; Chey and Chang, 2003). This causes secretion of a large volume of fluid containing a high concentration of bicarbonate (Chey and Chang, 2001; Chang and Chey, 2001). Gastrin is secreted by G cells distributed mainly over the pyloric antrum as well as the duodenum. These G cells are activated by stimuli such as amino acids or peptides. Secretion of gastrin by these cells is suppressed by acid or somatostatin. This hormone prompts the parietal cells of the stomach to secrete HCl and stimulates mucosal growth in the acid-secreting part of the stomach (Walsh, 1990).

Somatostatin is a hormone found in various tissues of the body, where it plays various roles. This hormone, secreted by D cells in the duodenum, is activated by acid. Evidence for the inhibition of gastrin secretion by antral somatostatin was first reported in 1979 (Saffouri et al., 1979). In addition, a reciprocal paracrine pathway exists in which gastrin secretion acts to restore somatostatin secretion (Schubert et al., 1991).

Cholecystokinin (CCK) is secreted by I cells distributed throughout the intestinal mucosal epithelium. The physiological actions of CCK include the stimulation of pancreatic secretion and gallbladder contraction, regulation of gastric emptying, and induction of satiety. The I cells are activated by lipids (longer than 12 carbons) or amino acids and induce the secretion of enzyme-rich pancreatic juice (McLaughlin et al., 1999; Lal et al., 2001; Dockray, 2003).

Aromatic L-amino acid decarboxylase (AADC) belongs to the a-family (subgroup II) of pyridoxal 5-phosphate (PLP) dependent enzymes (Bertoldi and Borri Voltattoni, 2003). AADC catalyzes the second enzymatic step in the synthesis of the neurotransmitters dopamine and serotonin through the decarboxylation of the substrates L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) in neuronal and also non-neuronal cells (Nagatsu, 1991). The activity of this enzyme has been localized to various areas of the mammalian organs, including in the EC cells of the mammalian gastrointestinal tract (Facer et al., 1979). Mechanical stimulation releases 5-hydroxytryptamine (5-HT) from EC cells with the subsequent activation of intrinsic primary afferents that carry electrical signals to the submucosal ganglia (Cooke, 2000), and 5-HT initiates peristaltic and secretory reflexes to transmit information to the central nervous system (Gershon and Tack, 2007; Sikander et al., 2009).

Recent studies have suggested that epithelial cells expressing T1Rs in taste buds and the GI system share common downstream regulation. S cells, which contain secretin, are activated by acid (Chey and Chang, 2003). In the present study, 50% of the S cells expressed T1R3 mRNA. T1R3 is a component of the amino acid receptor, but not the sour receptor. This suggests that S cells may be classified into two groups: one is T1R3 expressing cells, which may be activated by specific substances; the other is non-T1R3 expressing cells that may be activated by acidity. Similarly, 33% of EC cells that contain AADC expressed T1R3 mRNA. However, T1R3 and AADC never coexpress in the taste buds; T1R3 is expressed in type II cells, whereas AADC is expressed in type III cells in the taste buds. It is interesting that both GI tract cells and taste bud cells share common taste receptor signaling systems, but they show different expression patterns.

Although G and I cells are activated by amino acids or peptides, we did not observe the expression of any T1R3 in these cells. Effector neurons, cholinergic neurons, bombesin/GRP, and VIP neurons regulate the release of gastrin and somatostatin. In addition, distention of the antrum also regulates the release of gastrin and somatostatin (Schubert and Makhlouf, 1992). GRP is found in porcine gastrointestinal tissues and pancreases (Yanaihara et al., 1981) as well as in neurons of the colon and small intestine (Gonzalez et al., 2008), indicating that it is possible that the release of hormones by G and D cells also may be regulated by neural regulation. Regulation of gastrin secretion occurs almost entirely through neural regulation and not via G cells alone. This relationship can be postulated to apply to D cells because there is a reciprocal interplay between gastrin and somatostatin: somatostatin inhibits gastrin secretion in the pyloric antrum.

Luminal perfusion with 0.5% peptone increased gastrin secretion and decreased somatostatin secretion (Schubert et al., 1992). The T1R3-expressing cells may transmit signals of the luminal peptone to neurons, but this phenomenon has yet to be confirmed. The monitor peptide (trypsin-sensitive CCK-releasing peptide) was found in the pancreatic juice, and its amino acid sequence was determined (Iwai et al., 1987). When it was absorbed by a specific antibody, no further pancreatic enzyme secretion was observed in response to the intraduodenal infusion of a trypsin inhibitor (Fushiki et al., 1989). The
intraluminal monitor peptide releases cholecystokinin from the small intestine (McVey et al., 1999), and it is well established that CCK secretion is stimulated by amino acids (Liddle, 1995). It has been reported that the metabotropic glutamate receptors (mGluRs) are sensitive to monosodium L-glutamate in vivo (Chaudhari et al., 2001) and lead to increased intracellular IP3 concentrations via PLC β. In addition, these receptors are expressed in taste cells (Ninomiya et al., 2000; Toyono et al., 2003, 2007). In the present study, T1R3 mRNA was not expressed in G, D, or I cells. These results suggest that G, D, and I cells may express another receptor for amino acids, like mGluRs.

In conclusion, the present study demonstrates that subsets of S cells and EC cells in the mouse duodenum express T1R3. The function of taste receptors in the gastrointestinal epithelium remains to be revealed. Further studies that target taste receptors will provide a better understanding of their function within the gastrointestinal epithelium.

References

Bayliss WM, Starling EH: The mechanism of pancreatic secretion. *J Physiol* 28: 325-353 (1902).

Bertoldi M, Borri Voltattorni C: Reaction and substrate specificity of recombinant pig kidney Dopa decarboxylase under aerobic and anaerobic conditions. *Biochim Biophys Acta* 1647: 42-7 (2003).

Bezençon C, le Coutre J, Damak S: Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells. *Chem Senses* 32: 41-49 (2007).

Chang TM, Chey WY: Neurohormonal control of exocrine pancreas. *Curr Opin Gastroenterol* 17:416-25 (2001)

Chaudhari N, Landin AM, Roper SD: A metabotropic glutamate receptor variant functions as a taste receptor. *Nat Neurosci* 3: 113-119 (2000).

Chey WY, Chang TM: Neural hormonal regulation of exocrine pancreatic secretion. *Pancreatology* 1: 320-35(2001).

Chey WY, Chang TM: Neural control of the release and action of secretin. *J Physiol Pharmacol* 54 (Suppl 4): 105-112 (2003).

Cooke HJ: Neurotransmitters in neuronal reflexes regulating intestinal secretion. *Ann N Y Acad Sci* 915: 77-80 (2000).

Dockray GJ: Luminal sensing in the gut: an overview. *J Physiol Pharmacol* 54 (Suppl 4): 9-17 (2003).

Dyer J, Salmon KS, Zibrik L, Shirazi-Beechey SP: Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. *Biochem Soc Trans* 33: 302-305 (2005).

Facer P, Polak JM, Jaffe BM, Pearse AG: Immunocytochemical demonstration of 5-hydroxytryptamine in gastrointestinal endocrine cells. *Histochem J* 11: 17-21 (1979).

Fushiki T, Kajiura H, Fukuoka S, Kido K, Semb T, Iwai K: Evidence for an intraluminal mediator in rat pancreatic enzyme secretion: reconstitution of the pancreatic response with dietary protein, trypsin and the monitor peptide. *J Nutr* 19: 622-627 (1989).

Gershon MD, Tack J: The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 132: 397-414 (2007).

Gonzalez N, Moody TW, Igarashi H, Ito T, Jensen RT: Bombesin-related peptides and their receptors: recent advances in their role in physiology and disease states. *Curr Opin Endocrinol Diabetes Obes* 15: 58-64 (2008).

Grundy D: Sensory signals from the gastrointestinal tract. *J Pediatr Gastroenterol Nutr* 41 (Suppl 1): S7-9 (2005).

Hass N, Schwarzenbacher K, Breer H: T1R3 is expressed in brush cells and ghrelin-producing cells of murine stomach. *Cell Physiol Biochem* 33: 493-504 (2010).

Hofer D, Puschel B, Drencuhahn D: Taste receptor-like cells in the rat gut identified by expression of alpha-gustducin. *Proc Natl Acad Sci USA* 93:6631-6634 (1996).

Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, Zuker CS: Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* 96:541-551 (1999).

Iwai K, Fukuoka S, Fushiki T, Tsujikawa M, Hirose M, Tsunasawa S, Sakiyama F: Purification and sequencing of a trypsin-sensitive cholecystokinin-releasing peptide from rat pancreatic juice. Its homology with pancreatic secretory trypsin inhibitor. *J Biol Chem* 262: 8956-8959 (1987).

Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou H, Kim HH, Xu X, Chan SL, Juhaszova M, Bernier M, Mosinger B, Margolskee RF, Egan JM: Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci USA* 104: 15069-15074 (2007).

Kokrashvili Z, Mosinger B, Margolskee RF: T1r3 and alpha-gustducin in gut regulate secretion of glucagon-like peptide-1. *Ann N Y Acad Sci* 1170:91-4 (2009).

Lal S, Kirkup AJ, Brunsden AM, Thompson DG, Grundy D: Vagal afferent responses to fatty acids of different chain length in the rat. *Am J Physiol Gastrointest Liver Physiol* 281: G907-915 (2001).
Li P, Lee KY, Chang TM, Chey WY. Mechanism of acid-induced release of secretin in rats: Presence of a secretin releasing factor. *J Clin Invest* 262: 8956-8959 (1990).

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E: Human receptors for sweet and umami taste. *Proc Natl Acad Sci USA* 99: 4692-4696 (2002).

Liddle RA: Regulation of cholecystokinin secretion by intraluminal releasing factors. *Am J Physiol* 269: G319-327 (1995).

Mace OJ, Affleck J, Patel N, Kellett GL: Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol* 582: 379-392 (2007).

Margolskee RF, Dyer J, Kokashvili Z, Salmon KS, Ilegems E, Daly K, Maillet EL, Ninomiya Y, Mosinger B, Shirazi-Beechey SP: T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proc Natl Acad Sci USA* 104: 15075-15080 (2007).

McLaughlin J, Grazia Lucà M, Jones MN, D’Amato M, Dockray GJ, Thompson DG: Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. *Gastroenterology* 116: 46-53 (1999).

McVey DC, Romac JM, Clay WC, Kost TA, Liddle RA, Vigna SR: Monitor peptide binding sites are expressed in the rat liver and small intestine. *Peptides* 20: 457-464 (1999).

Nagatsu T: Genes for human catecholamine-synthesizing enzymes. *Neurosci Res* 12: 315-45 (1991).

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS: Mammalian sweet taste receptors. *Cell* 106: 381-390 (2001).

Ninomiya Y, Nakashima K, Fukuda A, Nishino H, Sugimura T, Hino A, Danilova V, Hellekant G: Responses to umami substances in taste bud cells innervated by the chorda tympani and glossopharyngeal nerves. *J Nutr* 130: 950S-953S (2000).

Rozenburg E, Sternini C: Taste receptor signaling in the mammalian gut. *Curr Opin Pharmacol* 7: 557-562 (2007).

Saffouri B, Weir G, Bitar K, Makhlfou G: Stimulation of gastrin secretion from the perfused rat stomach by somatostatin antiserum. *Life Sci* 25: 1749-1753 (1979).

Schubert ML., Makhlfou GM: Neural, hormonal, and paracrine regulation of gastrin and acid secretion. *Yale J Biol Med* 65: 553-560 (1992).

Schubert ML, Jong MJ, Makhlfou GM: Bombesin/GRP-stimulated somatostatin secretion is mediated by gastrin in the antrum and intrinsic neurons in the fundus. *Am J Physiol* 261: G885-889 (1991).

Schubert ML, Coy DH, Makhlfou GM: Peptone stimulates gastrin secretion from the stomach by activating bombesin/GRP and cholinergic neurons. *Am J Physiol* 262: G685-689 (1992).

Shin YK, Martin B, Golden E, Dotson CD, Maudsley S, Kim W, Jang HJ, Mattson MP, Drucker DJ, Egan JM, Munger SD: Modulation of taste sensitivity by GLP-1 signaling. *J Neurochem* 106: 455-463 (2008).

Sikander A, Rana SV, Prasad KK. Role of serotonin in gastrointestinal motility and irritable bowel syndrome. *Clin Chim Acta* 403: 47-55 (2009).

Sternini C, Anselmi L, Rozengurt E: Enteroendocrine cells: a site of 'taste' in gastrointestinal chemosensing. *Curr Opin Endocrinol Diabetes Obes* 15: 73-78 (2008).

Taniguchi T: Expression of the sweet receptor protein, T1R3, in the human liver and pancreas. *J Vet Med Sci* 66: 1311-1314 (2004).

Toyono T, Seta Y, Kataoka S, Kawano S, Shigemoto R, Toyoshima K: Expression of metabotropic glutamate receptor group I in rat gustatory papillae. *Cell Tissue Res* 313: 29-35 (2003).

Toyono T, Kataoka S, Seta Y, Shigemoto R, Toyoshima K: Expression of group II metabotropic glutamate receptors in rat gustatory papillae. *Cell Tissue Res* 328: 57-63 (2007).

Walsh JH: Role of gastrin as a trophic hormone. *Digestion* 47 (Suppl 1): 11-16 (1990).

Wu SV, Rozengurt N, Yang M, Young SH, Sittren-Smith J, Rozengurt E: Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. *Proc Natl Acad Sci USA* 99: 2392-2397 (2002).

Yanaihara N, Yanaihara C, Mochizuki T, Iwahara K, Fujita T, Iwanaga T: Immunoreactive GRP. *Peptides* 2: 185-91 (1981).