Taste signaling elements expressed in gut enteroendocrine cells regulate nutrient-responsive secretion of gut hormones

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ABSTRACT
Many of the receptors and downstream signaling elements involved in taste detection and transduction are also expressed in enteroendocrine cells where they underlie the chemosensory functions of the gut. In one well-known example of gastrointestinal chemosensation (the “incretin effect”), it is known that glucose that is given orally, but not systemically, induces secretion of glucagon-like peptide 1 and glucose-dependent insulinotropic peptide (the incretin hormones), which in turn regulate appetite, insulin secretion, and gut motility. Duodenal L cells express sweet taste receptors, the taste G protein gustducin, and several other taste transduction elements. Knockout mice that lack gustducin or the sweet taste receptor subunit T1r3 have deficiencies in secretion of glucagon-like peptide 1 and glucose-dependent insulinotropic peptide and in the regulation of plasma concentrations of insulin and glucose in response to orally ingested carbohydrate—ie, their incretin effect is dysfunctional. Isolated small intestine and intestinal villi from gustducin null mice displayed markedly defective glucagon-like peptide 1 secretion in response to glucose, indicating that this is a local circuit of sugar detection by intestinal cells followed by hormone secretion from these same cells. Modulating hormone secretion from gut “taste cells” may provide novel treatments for obesity, diabetes, and malabsorption syndromes.

AM J CLIN NUTR 2009;90(suppl):822S–5S.

MOLECULAR BASIS OF TASTE SIGNALING
The vertebrate sense of taste depends on specialized epithelial receptor cells contained in taste buds located in the surface papillae of the tongue. Taste is initiated by the interaction of tastants with receptors and ion channels in the apical microvilli of taste receptor cells. Some taste transduction pathways convert chemical information into a cellular second messenger code (eg, cyclic nucleotides and inositol trisphosphate) (reviewed in reference 1). These messengers are components of signaling cascades that typically lead to taste receptor cell depolarization and Ca2+ release. In other cases, the transient itself may constitute all or part of the initial cellular signal (eg, Na+, K+, H+).

The sense of sweet taste is initiated by the binding of sugars or sweeteners to the sweet taste receptor: a heterodimer of 2 type 1 taste G protein–coupled receptors (T1R2 + T1R3) (2–6). The sweet taste receptor couples to heterotrimeric gustducin (α-gustducin, Gβ3, Gγ13) (7, 8). Gustducin’s α-subunit (α-gustducin) activates taste cell phosphodiesterase to decrease cyclic nucleotide concentrations (1), whereas gustducin’s βγ-unit (Gβ3-Gγ13) activates phospholipase Cβ2 to generate inositol trisphosphate and diacylglycerol (9). Ca2+ influx and release from internal stores activates taste-cell-expressed transient receptor potential channel type M5 (TRPM5) (10), a Ca2+-activated cation channel (11–13), which leads to taste cell depolarization and signaling to other taste cells in the bud and to gustatory afferent nerves.

TASTE MOLECULES ARE EXPRESSED IN THE GUT
The taste G protein gustducin, originally identified in taste receptor cells (7), is expressed also in the cells of the stomach and small intestine (14, 15). In recent work from our group (16, 17) gustducin’s 3 subunits (α-gustducin, Gβ3, Gγ13), along with many other taste-signaling elements (eg, T1r1, T1r2, T1r3, TRPM5, PLCβ2, and others) were also found to be expressed in L-type enteroendocrine cells of the small intestine. Indeed, entire taste-signaling pathways are present in human duodenal L cells (16). The roles of gustducin, taste receptors, TRPM5, and other taste-signaling elements expressed in gut endocrine cells are now being made clear from physiologic studies in knockout mice. The expression of taste-signaling elements in gut endocrine cells and the characterization of their function as the gut’s luminal glucose sensor that initiates the incretin response to elicit the release of glucagon-like peptide 1 (GLP-1) from L cells are described here and elsewhere (16, 17).

TASTE MOLECULES UNDERLY THE INCRETIN EFFECT
GLP-1, secreted from enteroendocrine L cells of the gut, is an incretin hormone that augments the release of insulin from the pancreas. The incretin effect—the observation that orally ingested glucose is a much more effective stimulator of insulin secretion from the pancreas than is intravenously injected glucose (18)—is primarily mediated by GLP-1. It was recently determined that sugars in the gut lumen act on the taste-signaling proteins T1r3 and gustducin that are expressed in L cells to elicit

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2 Supported in part by NIH grant DC03055 to RFM.
3 Presented at the “100th Anniversary Symposium of Umami Discovery: The Roles of Glutamate in Taste, Gastrointestinal Function, Metabolism, and Physiology,” held in Tokyo, Japan, 10–13 September 2008.
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the release of GLP-1 from enteroendocrine L cells (16). Analogously with their function in oral taste cells, the gut-expressed taste-signaling elements respond to sugars and artificial sweeteners in the gut’s lumen (16, 17). However, instead of transmitting their signals via gustatory afferents, these gut “taste cells” act via humoral mediators such as GLP-1 and glucose-dependent insulinotropic peptide (GIP).

**TASTE MOLECULES ARE EXPRESSED IN INTESTINAL ENTEROENDOCRINE CELLS**

Intestinal mucosal cells in humans and mice have been examined for the presence of α-gustducin, T1R taste receptors, and other known taste-signaling elements (16, 17). In human duodenal biopsy sections, α-gustducin was shown by indirect immunofluorescence to be present in 4 populations of intestinal mucosal cells: 1) GLP-1-expressing enteroendocrine L cells, 2) GIP-expressing enteroendocrine K cells, 3) GIP and GLP-1 co-expressing enteroendocrine K/L cells, and 4) mucosal cells (presumed to be brush cells) that expressed neither GLP-1 nor GIP (16). Most human duodenal L cells and a sizable minority of K cells expressed α-gustducin (16). Independent confirmation of the expression of α-gustducin in human duodenal enteroendocrine K and L cells came from laser capture followed by reverse transcriptase–polymerase chain reaction (16). This same technique was used to show that α-gustducin was absent from human duodenal enterocytes (16). In addition, multiple taste-signaling elements (T1R2, T1R3, G\textsubscript{\textalpha}3, G\textsubscript{\textbeta}13, PLC\textbeta}2, and TRPM5) were found to be co-expressed with α-gustducin and GLP-1 in human duodenal L cells (16).

**TASTE MOLECULES IN ENTEROENDOCRINE L CELLS UNDERLIE THE INCRETN EFFECT**

The function of taste-signaling elements in intestinal endocrine cells (mouse duodenal cells) has been examined by looking for the co-expression of taste-signaling elements. In mouse duodenum, GLP-1-expressing L cells frequently expressed α-gustducin and an α-gustducin marker (green fluorescent protein driven from the α-gustducin promoter) (Figure 1A). Mouse L cells in duodenum (Figure 1A), jejunum (Figure 1B), and ileum (16) frequently expressed α-gustducin along with GLP-2 and peptide YY (well known to be present in L cells).

Knockout mice lacking either α-gustducin (α-gust\textsuperscript{-/-}) or T1r3 (T1r3\textsuperscript{-/-}) were examined for their enteroendocrine cell responses to glucose in the gut lumen. To determine the effects of directly stimulating the gut’s enteroendocrine cells and to eliminate the potential effects of the oral taste receptor cells, glucose was gavage administered by inserting feeding needles directly into the stomachs of α-gustducin knockout mice (α-gust\textsuperscript{-/-}) and their wild-type (α-gust\textsuperscript{+/+}) littermates (Figure 2). Gavage-administered glucose led to a significant rise (P < 0.01) in plasma concentrations of GLP-1 in wild-type mice with a peak \(\approx\) 10 min after gavage (Figure 2A). In contrast, there was no significant rise in plasma concentrations of GLP-1 in the α-gust\textsuperscript{-/-} mice after glucose gavage (Figure 2A). Gavage-administered glucose led to a significant rise (P < 0.001) in plasma concentrations of insulin in wild-type mice with a peak \(\approx\) 45 min after gavage (Figure 2B). In contrast, there was a markedly delayed rise in plasma concentrations of insulin in α-gust\textsuperscript{-/-} mice after glucose gavage (Figure 2B). In the α-gustducin-null mice, insulin reached a peak \(\approx\) 60 min after gavage and remained elevated for 2 h (Figure 2B), which is a marked difference from wild-type mice whose insulin concentrations gradually returned to baseline between 60 and 120 min after gavage.

The α-gustducin-null mice also showed disrupted glucose homeostasis: plasma glucose concentrations were higher in α-gust\textsuperscript{-/-} mice compared with wild type after overnight fasting followed by feeding (Figure 2C). Furthermore, plasma glucose concentrations in α-gust\textsuperscript{-/-} mice remained high for \(>\) 2 h after feeding (Figure 2C).

α-Gustducin is expressed in brush cells of the stomach (16) as well as in intestinal enteroendocrine cells (Figure 1). Thus, it is possible that glucose administered by gavage into the stomach might be acting directly on the brush cells and indirectly on the intestinal L cells via brush-cell-released factors. To rule out such

**FIGURE 1.** A: Indirect immunofluorescence staining of mouse duodenum showing co-expression of α-gustducin (α-gust) with glucagon-like peptide 1 (GLP-1), and GLP-2. Intrinsic fluorescence of green fluorescent protein (GFP) shows the expression of GFP and GLP-2 in the α-gustducin-expressing cells of α-gustducin–GFP transgenic mice. B: Indirect immunofluorescence of mouse jejunum showing the co-expression of α-gustin with GLP-1, GLP-2, and peptide YY (PYY). Bars, 15 μm. Data are modified with permission from reference 16.
indirect effects, glucose was injected directly into the duodena of wild-type, \( \alpha \text{-gust}^{+/+} \) mice and wild-type \( \alpha \text{-gust}^{-/-} \) mice. In each animal, the duodenum was surgically isolated from the stomach and the distal portion of the small intestine, but remained in circulatory contact so that plasma concentrations of hormones could be monitored. After duodenal injection with glucose, plasma GLP-1 in wild-type mice peaked at 10 min after injection and then returned to baseline at 20 min (Figure 2D). In marked contrast, plasma concentrations of GLP-1 did not increase at all after duodenal injection with glucose in either \( \alpha \text{-gust}^{+/+} \) or \( \alpha \text{-gust}^{-/-} \) mice. The duodenum was ligated away from the stomach and the rest of the intestines, and circulatory contact was maintained. (E) Secretion of GLP-1 ex vivo from minced proximal duodenum from \( \alpha \text{-gust}^{-/-} \) and \( \alpha \text{-gust}^{+/+} \) mice in response to the addition of 10% glucose to the culture medium. (F) Secretion of GLP-1 ex vivo from isolated duodenal villi from \( \alpha \text{-gust}^{-/-} \) and \( \alpha \text{-gust}^{+/+} \) mice in response to the addition of 10% glucose to the culture medium. For in vivo experiments, \( n = 5–12 \) animals/genotype; in vitro experiments were carried out in triplicate and replicated at least twice. All values are means \( \pm \) SEMs. **** Statistical significance was determined by ANOVA: \(* P < 0.05, ** P < 0.01, *** P < 0.001. Data are modified with permission from Reference 16.

CONCLUSIONS

In 2 recent studies (16, 17), gustducin-coupled sweet taste receptors were found to be present in enteroendocrine cells in the proximal intestines of mice and humans. In the human duodenum, \( \alpha \text{-gust} \) was found in 3 types of enteroendocrine cells: K (GIP), L (GLP-1), and K/L (GIP and GLP-1) cells (16). In addition to expressing \( \alpha \text{-gust} \) and the sweet receptor subunits T1R2 and T1R3, human duodenal L cells also expressed several other taste-signaling molecules, including G\( \beta \)3, G\( \gamma \)13, PLC\( \beta \)2, and TRPM5 (16).

In mice, \( \alpha \text{-gust} \) was frequently found in L-type enteroendocrine cells but rarely in K cells. \( \alpha \text{-Gustducin} \) knockout mice were disrupted in their glucose homeostasis and hormonal responses to glucose within the lumen of the small intestine. The primary deficit was the failure of enteroendocrine cells to release GLP-1 in response to glucose within the gut lumen (16). The
absent GLP-1 response to glucose led to an abnormal insulin response and prolonged elevation of postprandial blood glucose. T1r3 knockout mice also failed to release GLP-1 from their duodenal cells after glucose injection into the intestine. Such data indicate that sweet receptors in intestinal L cells couple to heterotrimeric gustducin to detect extracellular glucose and then respond with secretion of GLP-1. In 2 L-cell lines, both α-gustducin and T1r3 were required for stimulating GLP-1 release in response to either sugars or noncaloric sweeteners (16, 17). The results indicate that the sensing of sugars and sweeteners by taste-signaling elements expressed in intestinal L cells in vivo leads to GLP-1 release from these same cells. (Other articles in this supplement to the Journal include references 19–47.)

The authors’ responsibilities were as follows—ZK, BM, and RFM: contributed to the design and analysis of the experiments and to writing the manuscript; RFM: wrote the final version of the manuscript. The travel expenses of the presenting author (RFM) associated with participation in the symposium and an honoree were paid by the conference sponsor, the International Glutamate Technical Committee, a nongovernmental organization funded by industrial producers and users of glutamate in food. ZK and BM had no conflicts of interest. RFM has a personal financial interest in Redpoint Bio. Redpoint Bio is a biotechnology company that identifies and develops compounds to improve the taste of pharmaceutical, food, and beverage products.

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