Taste cell-expressed α-glucosidase enzymes contribute to gustatory responses to disaccharides

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The primary sweet sensor in mammalian taste cells for sugars and noncaloric sweeteners is the heteromeric combination of type 1 taste receptors 2 and 3 (T1R2+T1R3, encoded by *Tas1r2* and *Tas1r3* genes). However, in the absence of T1R2+T1R3 (e.g., in *Tas1r3* KO mice), animals still respond to sugars, arguing for the presence of T1R-independent detection mechanism(s). Our previous findings that several glucose transporters (GLUTs), sodium glucose cotransporter 1 (SGLT1), and the ATP-gated K^+ (K_{ATP}) metabolic sensor are preferentially expressed in the same taste cells with T1R3 provides a potential explanation for the T1R-independent detection of sugars: sweet-responsive taste cells that respond to sugars and sweeteners may contain a T1R-dependent (T1R2+T1R3) sweet-sensing pathway for detecting sugars and noncaloric sweeteners, as well as a T1R-independent (GLUTs, SGLT1, K_{ATP}) pathway for detecting monosaccharides. However, the T1R-independent pathway would not explain responses to disaccharide and oligomeric sugars, such as sucrose, maltose, and maltotriose, which are not substrates for GLUTs or SGLT1. Using RT-PCR, quantitative PCR, in situ hybridization, and immunohistochemistry, we found that taste cells express multiple α-glycosidases (e.g., amylase and neutral α glucosidase C) and so-called intestinal "brush border" disaccharide-hydrolyzing enzymes (e.g., maltase-glucoamylase and sucrase-isomaltase). Treating the tongue with inhibitors of disaccharidases specifically decreased gustatory nerve responses to disaccharides, but not to monosaccharides or noncaloric sweeteners, indicating that lingual disaccharidases are functional. These taste cell-expressed enzymes may locally break down dietary disaccharides and starch hydrolysis products into monosaccharides that could serve as substrates for the T1R-independent sugar sensing pathways.

gustation | sensory transduction | disaccharides | sucrase-isomaltase | maltase-glucoamylase

In humans, the heteromeric combination of type 1 taste receptors 2 and 3 (T1R2+T1R3, encoded by *TAS1R2* and *TAS1R3*) forms a sweet taste receptor responsive to sugars (e.g., glucose, fructose, sucrose), noncaloric sweeteners (e.g., aspartame, sucralose, saccharin, acesulfame K, rebaudioside A), and protein sweeteners (e.g., monellin, thaumatin, and brazzein), but not polysaccharides (1). The mouse sweet receptor (T1R2+T1R3) also responds to sugars, some of the same noncaloric sweeteners (e.g., sucralose, saccharin, acesulfame K, rebaudioside A), but not the protein sweeteners or polysaccharides. It is well established from multiple studies that T1R2+T1R3 is the major sweet taste receptor for sugars and likely the only sweet taste receptor for noncaloric sweeteners. For example, heterologous expression of human or mouse T1R2+T1R3 receptors in cultured cells recapitulates the host organism's response to sweeteners (2–4). KO mice lacking *Tas1r2* or *Tas1r3* have generally diminished responses to most sweet compounds as assessed by brief access lick assays, two bottle preference tests, and gustatory nerve recordings (5, 6).

However, in some studies, *Tas1r3* KO mice were found to still have significant behavioral and nerve responses to glucose and other sugars (5, 7). Many quantitative trait loci other than *Tas1r3* contribute to sweet taste perception in mice (8, 9). From this we inferred the presence of a sweet-sensing pathway that is independent of T1R3 (5, 7). We showed that multiple glucose transporters (GLUT2, GLUT4, GLUT8, and GLUT9), sodium glucose cotransporter 1 (SGLT1), and ATP-gated K^+ (K_{ATP}) channel subunits (KIR6.2 and SUR1) are present preferentially in the *Tas1r3*-expressing taste cells in mouse taste buds (10). Other groups (11–13) have confirmed some of these results. We proposed that the T1R-independent sweet pathway depends on uptake of glucose into *Tas1r3*-expressing taste cells, followed by its metabolism to ATP, which binds to K_{ATP}, closing the channel and depolarizing the sweet taste cell (10). The existence of two sweet pathways, both of which detect sugars, could explain why noncaloric sweeteners are fully cross-adapted by sugars, but sugars are only partially cross-adapted by noncaloric sweeteners (14, 15).

However, this proposed alternative pathway does not, on its own, explain the remaining taste responses of *Tas1r3* KO mice to the disaccharides maltose (5) and sucrose (5, 7). Dietary carbohydrates are hydrolyzed into constituent monosaccharides before uptake by enterocytes. Starch is partially hydrolyzed by extracellular enzymes, first in the oral cavity by salivary amylase (AMY1), and then in the small intestine by pancreatic amylase (AMY2). The end products of amylase-catalyzed starch hydrolysis are disaccharides like maltose and higher-molecular-weight oligomers of glucose; amylase cannot generate glucose from starch. Disaccharidases localized to the apical plasma membrane of enterocytes (brush border enzymes), such as

Significance

We previously showed that glucose transporters and the K_{ATP} metabolic sensor are coexpressed in sweet-responsive taste cells and could serve as sugar sensors in the absence of the sweet receptor (type 1 taste receptors 2 and 3). However, only monosaccharides are substrates for these transporters, whereas dietary carbohydrates are mostly polysaccharides and disaccharides. Here we show that the disaccharide-digesting enzymes maltase-glucoamylase and sucrase-isomaltase are expressed selectively in sweet taste cells. Pharmacological inhibition of these enzymes diminished taste nerve responses only to disaccharides. We hypothesize that these enzymes act in concert with salivary amylase to generate monosaccharide substrates for taste cell-expressed glucose transporters. The transported monosaccharides can then be metabolized to ATP to close K_{ATP} and activate the T1R-independent sweet taste pathway.

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maltase-glucoamylase (MGAM), sucrase-isomaltase (SIS), lactase (LCT), and trehalase (TREH) hydrolyze the disaccharides maltose, sucrose, lactose, and trehalose, respectively, to generate monosaccharides (16–19). Here, we used PCR, in situ hybridization, and immunohistochemistry to determine that multiple sugar- and starch-hydrolyzing enzymes are expressed in taste cells. We found that *Mgam, Sis, Lct, Treh, Amy1*, and neutral α-glucosidase C (*Ganc*) are all expressed in taste cells. The majority of *Tas1r3*-expressing taste cells express *Mgam* and *Sis*, as we previously showed for GLUTs and K_{ATP}. Furthermore, inhibition of MGAM and SIS specifically decreased gustatory nerve responses to the disaccharides sucrose and maltose. Our results indicate that the actions of these orally expressed digestive enzymes may contribute to the unique sweet taste of sucrose and other sugars by generating monosaccharide substrates for the T1R-independent sweet pathway.

Results

Carbohydrate-Digesting Enzymes Are Expressed in Taste Cells. To gain insight into the T1R-independent taste of disaccharides, we examined expression in mouse taste tissue of several carbohydrate-hydrolyzing enzymes. We first examined expression of the enzymes *Amy1* (salivary amylase), *Amy2* (pancreatic amylase), and *Ganc* in taste and nontaste tissues: mRNAs were from taste bud-containing [circumvallate (CV), foliate (FOL), and fungiform (FNG)] papillae and nontaste lingual epithelium (NT) tissues, along with Von Ebners gland (VEG), parotid (PAR) gland, and pancreas (PAN). PCR assays were then performed using primer pairs specific for cDNAs corresponding to *Amy1/2* (salivary and/or pancreatic forms), *Amy2*, and *Ganc*. By PCR the *Amy2* product was detected only from pancreatic cDNA, whereas an *Amy1/2* product was found in all tissues examined, indicating that all of the oral tissues tested (including the NT control) express only *Amy1* (Fig. 1*A*). PCR indicated that *Ganc* mRNA was present in all oral tissues, as well as in pancreas (positive control) (Fig. 1*A*). PCR assays with primer pairs against *Mgam* and *Sis* showed that their mRNAs were present in all taste tissues tested and jejunum (positive control), but were absent from NT and VEG (Fig. 1*B*). Gustducin served as a positive control for the taste tissues and for jejunum and was not expressed in VEG or NT (Fig. 1*B*). Quantitative evaluation by real-time PCR demonstrated highest expression of *Amy1* mRNA in VEG and PAR, followed by CV and FOL, with lowest expression in NT (Fig. 1*C*). Quantitation showed higher levels of *Mgam* and *Sis* mRNAs in CV and FOL papillae than in NT (Fig. 1 *D* and *E*).

The cDNA templates for the PCR experiments were derived from taste tissue containing a mixture of taste cells and surrounding epithelial and connective cells, from negative control NT tissue devoid of taste cells, or from positive control tissues (e.g., VEG, PAR, JEJ, PAN). To determine whether the mRNAs for these genes are indeed expressed in the taste cells themselves and/or elsewhere in the oral cavity, we carried out in situ hybridization with antisense and sense (control) probes for *Amy1/2, Mgam*, and *Sis*. In situ hybridization to taste bud-containing sections indicated that mRNAs for *Amy1/2, Mgam*, and *Sis* are selectively expressed in mouse taste cells in FNG, FOL, and CV papillae (Fig. 2). *Amy1/2* was also expressed in VEG (Fig. 2*D*), but *Mgam* and *Sis* were not (Fig. 2 *H* and *L*). Each antisense probe was validated in positive control tissues known to express these mRNAs: *Amy1/2* in parotid gland and *Mgam* and *Sis* in duodenum (Fig. S1 *A–C*). Unlike *Amy1/2*, mRNAs for *Mgam* and *Sis* were not expressed in the parotid, submandibular, and sublingual glands (Fig. S1 *D–I*), indicating that they are not secreted by the major salivary glands. To determine whether mRNAs for additional carbohydrate-digesting enzymes are expressed in taste cells, we carried out in situ hybridization with probes for lactase (*Lct*) and trehalase (*Treh*). Both *Lct* and *Treh* are selectively expressed in mouse taste cells in FNG, FOL, and CV papillae (Fig. S2 *A–D* and *F–I*). The *Lct* and *Treh* probes were validated in duodenum as the positive control tissue (Fig. S2 *E* and *J*).

Given that mRNA expression demonstrated above may not necessarily be correlated with protein expression, we also performed indirect immunohistochemistry to confirm the expression of MGAM, SIS, AMY1/2, GANC, and TREH proteins in taste cells. Immunoreactivity to MGAM, SIS, AMY1/2, GANC, and TREH was observed in mouse taste cells from all three types of papillae (Fig. 3). Primary antibodies against MGAM and SIS were previously validated with intestinal tissues (20). The anti–AMY1/2 antibody was validated against VEG (Fig. 3*C*, *Inset*). In addition, the primary antibodies against MGAM, SIS, and AMY1/2 were shown to be specific by preincubation with an excess of the specific immunogenic peptides used to generate each antibody (Fig. S3 *A–C*). Secondary antibodies were shown to be free of nonspecific immunoreactivity in tissue controls with primary antibodies omitted (Fig. S3*D*).

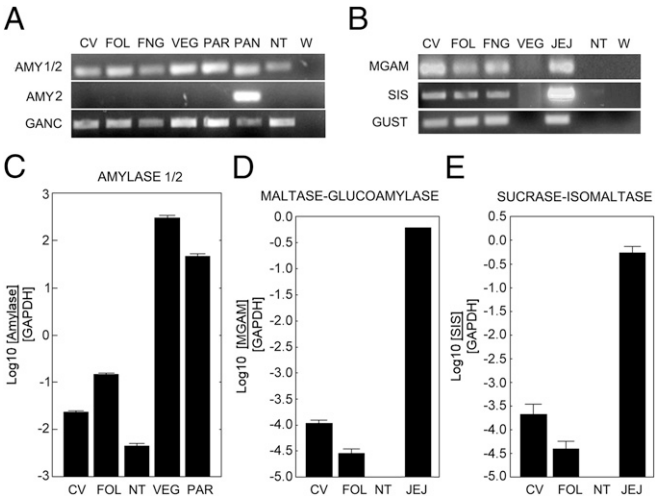

Fig. 1. Expression of mRNAs for α-glucosidases in gustatory and gastrointestinal tissues. (*A* and *B*) PCR amplification (35 cycles) of amylases (*Amy1/2*, salivary and pancreatic amylase; *Amy2*, pancreatic amylase), α-glucosidases (*Ganc*, neutral α-glucosidase C; *Mgam*, maltase-glucoamylase; *Sis*, sucrase-isomaltase), and gustducin (*Gust*) from mouse cDNAs from gustatory [CV, circumvallate papillae; FOL, foliate papillae; FNG, fungiform papillae; NT (non-taste lingual epithelium); PAR, parotid gland; VEG, Von Ebner's glands] and gastrointestinal tissues (PAN, pancreas; JEJ, jejunum). *Ganc* and *Amy1* are expressed in all gustatory tissues tested; *Amy2* is expressed only in pancreas. *Mgam* and *Sis* are expressed in all three types of taste papillae, as well as in jejunum (positive control), but not in nontaste tissue. (*C–E*) Taqman real-time PCR was used to quantitate expression in gustatory and gastrointestinal tissue cDNAs of *Amy1/2*, *Mgam*, and *Sis*. Elevated expression in CV and FOL cDNAs vs. NT cDNA are observed for all three enzymes. The expression of each gene is plotted as the logarithm of the ratio between its cycle threshold value and that of *Gapdh*.

Carbohydrate-Digesting Enzymes Are Expressed in Type II and III Taste Cells. The data above indicate by multiple independent means that several carbohydrate-hydrolyzing enzymes are present in taste cells. Were any of these enzymes to contribute to taste sensing of sucrose, maltose, or other disaccharides, they would most likely be found within or in proximity to those taste cells that detect sweet compounds by T1R-dependent and T1R-independent pathways (i.e., the T1R2+T1R3-positive subset of type II taste cells that also express glucose and other monosaccharide transporters and K_{ATP} channels). To examine this, we double-stained taste cells using an antibody against either the MGAM or SIS enzymes, along with second antibodies or transgenes that mark specific taste cell types. Double-staining with markers for type I taste cells (an antibody against NTPDase2; Fig. S4), for all type II taste cells (an antibody against TRPM5; Fig. 4), for the T1R3-positive subset of type II taste cells (T1R3-GFP; Fig. S5), or for type III taste cells [an antibody against serotonin (anti-5HT); Fig. S6], showed that both MGAM and SIS were most often found in type II taste cells (in both anterior and posterior fields), but also frequently in type III taste cells. In the small intestine, disaccharidases are localized to the apical plasma membrane of enterocytes with their catalytic domain exposed to the intestinal lumen. To determine whether these enzymes are also

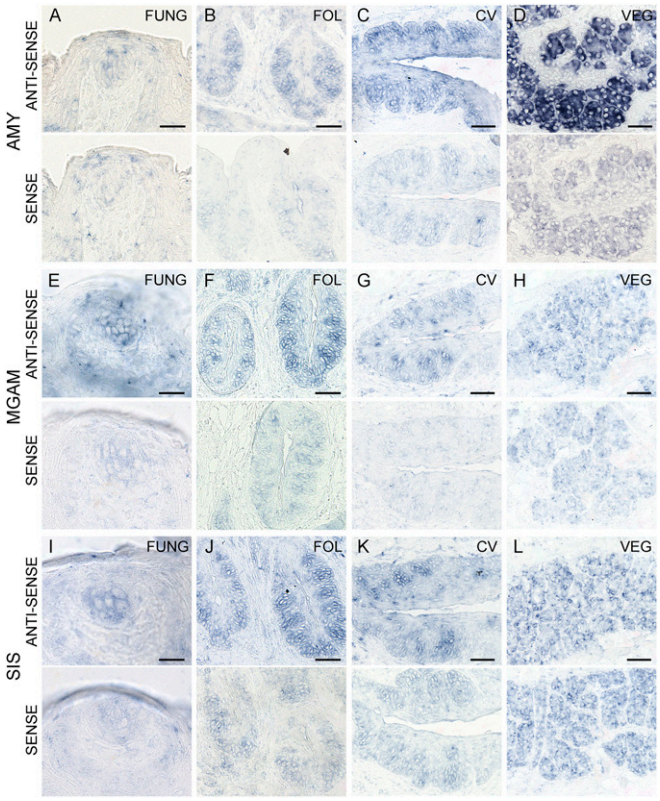

Fig. 2. Expression of α-glucosidase mRNAs in taste cells. In situ hybridization to taste bud-containing tissues from mouse FNG, FOL, and CV papillae and VEG was carried out with digoxigenin-labeled RNA probes for *Amy1/2* (*A–D*), *Mgam* (*E–H*), and *Sis* (*I–L*). Taste cell hybridization to antisense probes indicates expression of mRNAs for all three enzymes in FNG, FOL, and CV taste cells; *Amy1/2* mRNA also is observed in VEG. Hybridization of sense probe controls in and around taste cells indicative of nonspecific background was generally lower than with corresponding antisense probes. [Scale bars, 20 (*A*, *E*, and *I*) and 40 μm (*B–D*, *F–H*, and *J–L*).]

localized apically to areas of the taste bud exposed to the contents of the oral cavity, we double-stained taste cells with antibodies against either MGAM or SIS along with an antibody against VILLIN, a marker that labels taste receptor cell microvilli (21). We detected colocalization of both MGAM and SIS proteins with VILLIN in apical taste cell microvilli at the taste pore (Fig. S7).

Quantitation of taste cells in CV papillae that coexpress MGAM or SIS with TRPM5 or T1R3-GFP (Table S1) determined the following. (*i*) Among type II taste cells (assessed by their expression of TRPM5), 93% expressed MGAM, and 97% expressed SIS. Among the MGAM-expressing and SIS-expressing cells, 66% and 62%, respectively, were type II taste cells, based on expression of TRPM5. (*ii*) Among T1R3-GFP–expressing taste cells, 89% expressed MGAM, and 89% expressed SIS. Among the MGAM-expressing and SIS-expressing cells, 56% and 53% expressed T1R3-GFP, respectively. (*iii*) Forty-six percent of MGAM-expressing cells and 41% of SIS-expressing cells expressed 5HT, whereas 70% and 71% of 5HT-expressing type III cells expressed MGAM and SIS, respectively. In sum, most type II taste cells and the majority of T1R3-GFP expressing taste cells expressed the MGAM and SIS enzymes in CV papillae. Given the percentage of type II cells and T1R3 cells that express MGAM and SIS, many bitter responsive and potentially all umami responsive cells may also express both enzymes in addition to the sweet responsive cells. A similar pattern of expression was found in the FOL papillae. In addition, a majority of type III taste cells in CV and FOL papillae expressed both enzymes.

Oral Carbohydrate-Digesting Enzymes Contribute to Taste Nerve Responses to Disaccharides. The data above show that carbohydrate-digesting enzymes MGAM and SIS are present in type II and type III taste cells, including nearly all T1R3-expressing taste cells. Were these enzymes to function in the oral cavity, we would expect them to be inhibited by α-glucosidase inhibitors. To test this possibility and to determine whether the activity of these enzymes might contribute to taste responses to disaccharides, we recorded chorda tympani nerve responses of WT (C57BL/6) mice to a series of tastants before treatment, after incubation, and after washout of two different brush border enzyme inhibitors, miglitol and voglibose, applied to the dorsal surface of the tongue (22). Pretreatment and posttreatment washout of the inhibitors had no effect on nerve responses of WT mice to any of the taste stimuli (Fig. 5 *A* and *B*). However, incubation of the tongue with either inhibitor led specifically to decreased chorda tympani nerve responses to the disaccharides sucrose and maltose, but had no effect on nerve responses to the monosaccharides glucose (GLU) and fructose (FRU), the noncaloric sweeteners SC45647 (SC) and sucralose (SCR), or control stimuli representative of nonsweet taste qualities (i.e., salty (NaCl), sour [citric acid (CA)], bitter [quinine hydrochloride (QHCl)], and umami [monopotassium glutamate (MPG); Fig. 5 *A* and *B*]. Miglitol (500 μM) reduced chorda tympani nerve responses ($n = 7, 8$) to sucrose by 40% ($P < 0.01$) and to maltose by 25% ($P < 0.05$). Voglibose (10 μM) similarly reduced chorda tympani nerve responses ($n = 8, 9$) to sucrose by 40% ($P < 0.001$) and to maltose by 25% ($P < 0.05$).

To determine whether this effect was via a T1R-independent mechanism, we measured sensitivity of chorda tympani nerve responses of *Tas1r3* KO mice to the α-glucosidase inhibitor voglibose. Pretreatment and washout of voglibose had no effect on nerve responses of *Tas1r3* KO mice to any of the taste stimuli (Fig. 5*C*). Incubation with voglibose decreased nerve responses of *Tas1r3* KO mice ($n = 6$) to sucrose ($P < 0.01$) and maltose ($P < 0.01$) to background levels (i.e., comparable to their responses to the artificial sweeteners SC45647 and sucralose), but had no effect on nerve responses of these mice to the other sweet compounds (i.e., glucose, fructose, SC45647, and sucralose) or to the control nonsweet stimuli (i.e., NaCl, CA, QHCl, and MPG) (Fig. 5*C*). Thus, in the genetic absence of *Tas1r3*, pharmacological inhibition of disaccharidases eliminated all responses to the disaccharide sugars sucrose and maltose.

Discussion

Starch, a dietary staple for humans and rodents alike, is initially digested into oligo- and disaccharides by salivary and pancreatic amylases (23–25). The intestinal brush border enzymes MGAM, SIS, TREH, and LCT then convert disaccharides maltose, sucrose, trehalose, and lactose into readily absorbable monosaccharides (16, 17). In the small intestine, absorptive enterocytes take up free glucose and galactose via cotransport with sodium by SGLT1 and free fructose by GLUT5; these sugars are transported by GLUT2 across the basolateral aspect of the enterocytes into the bloodstream (26). We and others have shown that the sweet taste receptor T1R2+T1R3 and downstream signaling components, including gustducin and TRPM5, are present in enteroendocrine cells in the small intestine where they up-regulate enterocyte expression of SGLT1 and GLUT2 in response to dietary levels of sugars and sweeteners (27, 28).

The finding that taste cells express multiple carbohydrate-hydrolyzing enzymes previously thought to be present only in gut is striking, yet it seems unlikely that these taste cell-expressed enzymes play a significant role in nutrient absorption per se. It had previously been shown in rats that *Amy1/2* was expressed in both the VEG and the taste buds of the CV papillae, although the relative or absolute level of expression had not been quantified nor was the identity of the amylase isoform determined (29). Our RT-PCR results show that *Amy*1 is most highly expressed in the parotid gland and VEG, with lower but clearly detectable expression in the CV and FOL taste bud-containing tissues. By in situ hybridization, *Amy1/2* was found to be expressed in taste buds of the FNG, FOL, and CV papillae, but

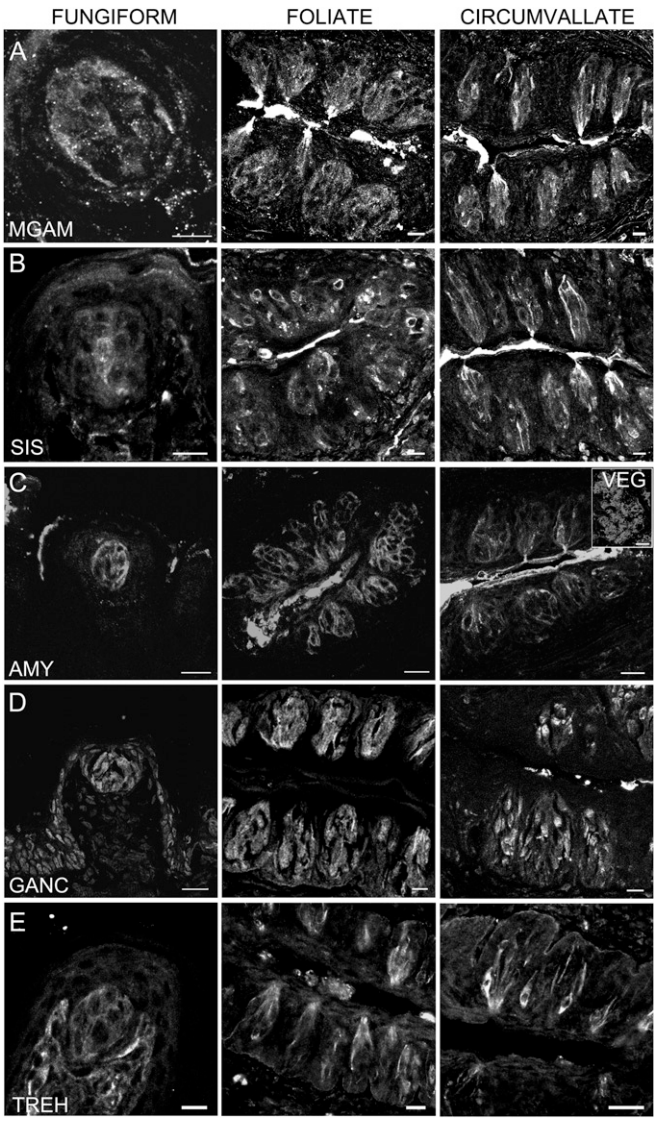

Fig. 3. Expression of α-glucosidase proteins in taste cells. Indirect immunofluorescence confocal microscopy of taste bud containing sections from mouse FNG, FOL, and CV taste papillae was carried out with specific polyclonal antibodies directed against AMY1/2, MGAM, SIS, GANC, and TREH. Immunofluorescence indicates expression in taste cells of all five enzymes. [Scale bars, *A* = 10 μm (FNG), 40 μm (FOL and CV); *B* = 40 μm (all); *C* = 80 μm (FNG), 20 μm (FOL and CV), and 40 μm (VEG); *D* = 80 μm (FNG), 40 μm (FOL and CV); *E* = 10 μm (FNG), 20 μm (FOL and CV).]

again at much lower levels than in VEG. The contents of the VEG are secreted directly into the trenches underlying the FOL and CV papillae (30), suggesting that AMY1 from the VEG and taste cells may act on dietary starch to generate locally elevated amounts of oligo- and disaccharides in close proximity to the taste buds. Our immunohistochemistry results show that the majority of taste cells that express MGAM and SIS are type II taste cells (TRPM5 positive), including nearly all T1R3-expressing taste cells. In addition, a sizable minority of the MGAM- and SIS-expressing cells is made up of 5HT-positive type III taste cells. That MGAM and SIS are localized at the taste pore and therefore exposed to the oral cavity may be crucial for their role in the T1R-independent pathway, as the monosaccharides released at the taste pore will be accessible to even those nearby cells that don't express these enzymes.

GANC is expressed in liver where it hydrolyzes terminal nonreducing (1–>4)-linked α-D-glucose residues from maltose and glycogen (31). Although glycogen phosphorylase catalyzes degradation of glycogen in the liver (32), GANC may also be involved in glycogen metabolism (31). GANC in taste cells is found predominantly within the nucleus, and we speculate that this may reflect a mechanism for regulating its activity, as in the case of the liver specific isoform of glucokinase (32).

Why are AMY1, GANC, and multiple brush border enzymes expressed in taste cells, particularly the T1R3-expressing type II taste cells? Likely they are contributing to the T1R-independent sweet sensing pathway in T1R3-positive taste cells and broadening the responsiveness of this pathway to carbohydrates and sugars beyond just glucose and any other monosaccharides that could be transported into these taste cells. In the absence of T1R3 (i.e., in *Tas1r3* KO mice) animals lose responses to noncaloric sweeteners, but retain much of their responses to sugars (5). We proposed that there are two sweet-sensing pathways (10). The heterodimeric sweet receptor T1R2+T1R3 mediates the T1R-dependent pathway by which T1R3-positive cells respond to both caloric and noncaloric sweeteners. In contrast, the T1R-independent sweet pathway, also found in the T1R3-positive cells, depends on uptake of glucose and other monosaccharides into these taste cells, followed by metabolism to ATP, which binds to K_{ATP}, closing the channel and depolarizing the sweet taste cell, triggering release of neurotransmitters and neuro-peptides (10). Although the T1R-dependent pathway would respond to all sweeteners and all sugars that bind to T1R2+T1R3, the T1R-independent pathway would only respond to those sweet compounds that can be transported into the T1R3-positive taste cells and then metabolized. To a first approximation then, only glucose, fructose, and galactose would be likely substrates for the T1R3-independent pathway. However, the disaccharides sucrose and maltose elicit robust nerve responses and preference responses in *Tas1r3* KO mice (5). We propose that the activity of MGAM and SIS can convert dietary oligosaccharides (including AMY1-generated starch hydrolysates) and disaccharide sugars in the oral cavity into monosaccharide substrates for taste cell-expressed monsaccharide transporters (e.g., GLUTs and SGLT1). Once transported into the taste cell, glucose and other monosaccharides would be metabolized to ATP, eliciting the closure of taste cell-expressed K_{ATP} and taste cell depolarization. Together, the T1R-dependent pathway and the T1R-independent pathway described here likely account for the entirety of taste responses to caloric sugars: simultaneously blocking both pathways reduces the response to sucrose and maltose to background levels.

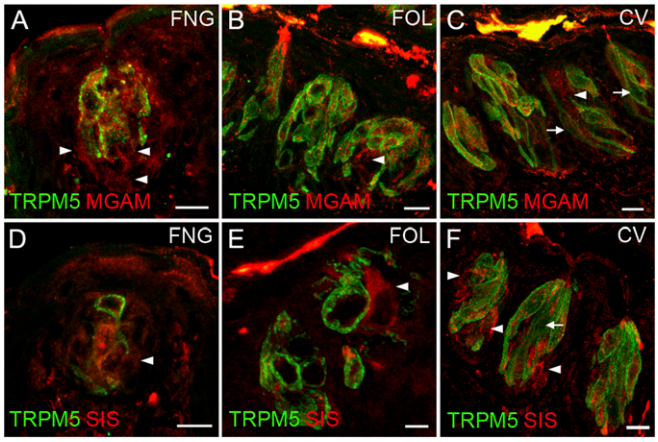

Fig. 4. Coexpression in taste cells of brush border enzymes with TRPM5. Indirect immunofluorescence confocal microscopy of taste bud-containing sections from mouse FNG, FOL, and CV papillae was carried out with antibodies against the brush border enzymes (MGAM or SIS) along with TRPM5 (a marker for type 2 taste cells). Overlaid images indicate frequent coexpression of TRPM5 with MGAM (*A*–*C*) and SIS (*D*–*F*). Arrowheads, single immunolabeling of brush border enzymes; arrows, single immunolabeling of TRPM5. (Scale bars, 40 μm.)

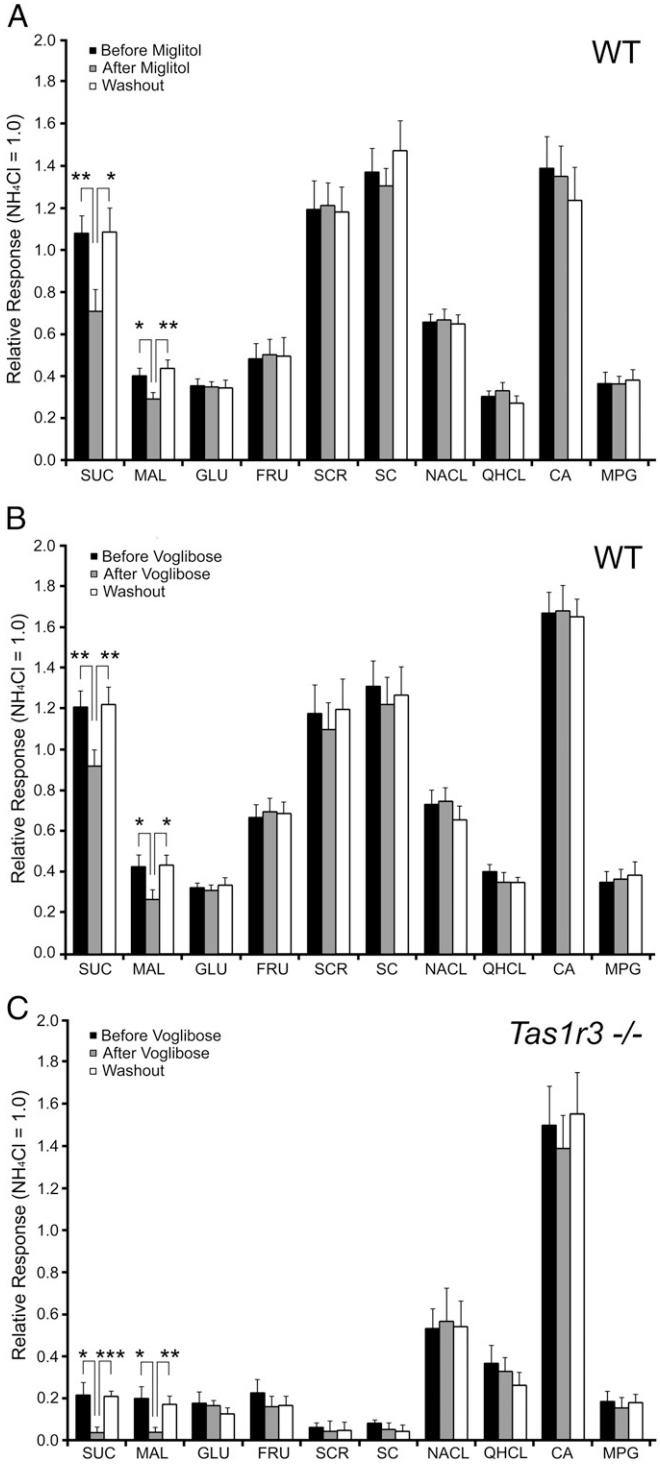

Fig. 5. Integrated whole-nerve recording from chorda tympani taste nerves of mice stimulated by lingual application of taste stimuli in the presence or absence of α-glucosidase inhibitors. Relative responses were normalized to the response to 100 mM NH_4Cl. Recordings from WT mice were taken before application (filled bars), after application (gray bars), and after 10-min wash out (open bars) of the α-glucosidase inhibitors (*A*) miglitol (500 μM) and (*B*) voglibose (10 μM). Both miglitol and voglibose significantly reduce the magnitude of nerve responses to sucrose (SUC) and maltose (MAL), but have no effect on the responses to other sugars, noncaloric sweeteners, or nonsweet tastants in WT mice. Recordings from *Tas1r3* KO mice (*Tas1r3*$^{-/-}$) were taken before application (filled bars), after application (gray bars), and after 10-min wash out (open bars) of voglibose (10 μM) (*C*). Voglibose significantly reduces the magnitude of nerve responses to SUC and MAL, but has no effect on the responses to other tastants in *Tas1r3* KO mice. Taste stimuli: 100 mM NH_4Cl, 500 mM SUC, 500 mM MAL, 500 mM glucose (GLU), 500 mM fructose (FRU), 1 mM SC45674 (SC), 100 mM NaCl, 10 mM quinine-HCl (QHCL), 10 mM citric acid (CA), 100 mM monopotassium glutamate (MPG). (n = 6–9; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

Our discovery of MGAM and SIS in taste cells is unprecedented. Although the location of GLUTs and SGLT1 in human oral mucosa was reported as early as 1999 (13), and their location in T1R3-positive taste cells of rodents was confirmed in 2011 (10–12), those studies were limited to glucose uptake and did not test transport of disaccharides. A sucrose stimulated sodium-preferring ion-transport system in canine lingual epithelium was reported in 1988, but SGLT1 was not identified in taste tissue at that time (33). Similarly, sucrose was shown to have the same detection threshold in WT and *Tas1r3* KO mice (7), and maltose and sucrose were shown to elicit nerve and preference responses in *Tas1r3* KO mice (5), but the presence of GLUTs and SGLT1 was not recognized in those studies. Our discovery of MGAM and SIS expression in mammalian taste cells explains the connected observations between those previous studies.

What is the purpose of having a T1R-independent sweet-sensing pathway in the same cells that express the T1R-dependent pathway? Much as K_{ATP} serves in the pancreas as a metabolic sensor of blood glucose levels, so too would the T1R-independent pathway serve as a sensor of metabolizable sugars. Coexpression of the brush border disaccharidases in the T1R3-positive taste cells, along with GLUTs and SGLT1, provides these cells with the ability to detect the caloric value of oligosaccharides and disaccharides, as well as of the starch hydrolysis products. Together these two pathways may serve as "coincidence detectors" for substances that are both sweet and have caloric value to provide a mechanism to evaluate the caloric value of a sweet substance. Presumably, sufficiently inhibiting K_{ATP} channels in T1R3 taste cells by elevated ATP would depolarize these cells and elicit a perception of sweetness. However, at low sugar levels that would only submaximally inhibit K_{ATP}, the addition of a noncaloric sweetener acting via T1R2+T1R3 would likely provide enhanced perception of sweet taste over that achieved by either sweetener alone. Under low metabolic conditions, the tonic activity of K_{ATP} channels would hyperpolarize the T1R3-positive cells, making it less likely that sweetener activation of T1R2+T1R3 depolarizes the taste cell. Together these two pathways underlie the unique sensory response to sucrose and other sugars. Responses to many noncaloric sweeteners, in contrast to responses to sugars, display delayed onsets and offsets (34) and lower maximal sweetness intensity (35). The higher peak-magnitude sweetness responses displayed by sugars in vivo may be explained if sugars act via nonsaturable transporters and saturable T1R2+T1R3, whereas noncaloric sweeteners act only on T1R2+T1R3. Sucrose may be the most preferred sugar because it initially stimulates the T1R2+T1R3 pathway but then yields glucose and fructose that could be transported into sweet taste cells via the T1R-independent pathway. In addition to a purely sensory role, the T1R-independent pathway may also have a role in regulating metabolism. Indeed, a robust cephalic phase insulin release (CPIR) can be induced by oral administration of glucose or sucrose, but not fructose in WT and *Tas1r3* KO mice (36). The CPIR improved glucose tolerance in both strains, buttressing the physiological importance of this pathway. Given our identification here of SIS in taste cells, particularly the T1R3-positive cells that also express GLUTs and K_{ATP}, orally administered sucrose would generate sufficient glucose to stimulate the T1R-independent pathway. All or at least a portion of the glucose-elicited CPIR may be in response to GLP-1 released directly from taste cells (37). Furthermore, leptin and other circulating hormones may affect sweet taste

sensitivity by acting directly on K_{ATP} channels in the T1R3-positive cells (38).

Materials and Methods

All experiments were performed under National Institutes of Health guidelines for the care and use of animals in research and approved by the Institutional Animal Care and Use Committee of Monell Chemical Senses Center or Kyushu University. All mice used for this study were in the C57BL/6J background. Transgenic mice expressing GFP under the promoter for T1R3 (T1R3-GFP) were as previously described (39). RNAs were isolated using the Pure-Link RNA mini kit from Life technologies. RT-PCR was done using Phire hot start II DNA polymerase from Life Technologies using intron spanning primer pairs (Table S2). qPCR was done using Taqman Gene Expression assays (Applied Biosystems). RNA probes for in situ hybridization were transcribed as previously described (10). Tissues for in situ hybridization and immunohistochemistry were prepared as previously described (10). Further detailed methods are provided in *SI Materials and Methods*.

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