Transformation of postigestive glucose responses after deletion of sweet taste receptor subunits or gastric bypass surgery

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Geraedts MC, Takahashi T, Vigues S, Markwardt ML, Nkobena A, Cockerham RE, Hajnal A, Dotson CD, Rizzo MA, Munger SD. Transformation of postigestive glucose responses after deletion of sweet taste receptor subunits or gastric bypass surgery. Am J Physiol Endocrinol Metab 303: E464–E474, 2012. First published June 5, 2012; doi:10.1152/ajpendo.00163.2012.—The glucose-dependent secretion of the insulinotropic hormone glucagon-like peptide-1 (GLP-1) is a critical step in the regulation of glucose homeostasis. Two molecular mechanisms have separately been suggested as the primary mediator of intestinal glucose-stimulated GLP-1 secretion (GSGS): one is a metabotropic mechanism requiring the sweet taste receptor type 2 (T1R2) + type 3 (T1R3), while the second is a metabolic mechanism requiring ATP-sensitive K+ (KATP) channels. By quantifying sugar-stimulated hormone secretion in receptor knock-out mice and in rats receiving Roux-en-Y gastric bypass (RYGB), we found that both of these mechanisms contribute to GSGS; however, the mechanisms exhibit different selectivity, regulation, and localization. T1R3−/− mice showed impaired glucose and insulin homeostasis during an oral glucose challenge as well as slowed insulin granule exocytosis from isolated pancreas islets. Glucose, fructose, and sucrose evoked GLP-1 secretion from T1R3−/−, but not T1R3+/−, ileal explants; this secretion was not mimicked by the KATP channel opener glibenclamide. T1R2−/− mice showed normal glycemic control and partial small intestine GSGS, suggesting that T1R3 can mediate GSGS without T1R2. Robust GSGS that was KATP channel-dependent and glucose-specific emerged in large intestines of T1R3−/− mice and RYGB rats in association with elevated fecal carbohydrate throughout the distal gut. Our results demonstrate that the small and large intestines utilize distinct mechanisms for GSGS and suggest novel large intestine targets that could mimic the improved glycemic control seen after RYGB.

glucagon-like peptide-1; insulin; T1R3; glucose-stimulated potassium ion channel; enteroendocrine cells.

THE BODY TIGHTLY REGULATES blood glucose levels, and disruption of the homeostatic mechanisms that underlie normal glycemic control can have significant deleterious effects. For example, the prolonged hyperglycemia associated with type 2 diabetes mellitus (T2DM) increases the risk of cardiovascular disease, neuropathy, retinopathy, kidney disease, and death (66). Hormonal signals arising in the gastrointestinal tract are key components of the homeostatic mechanisms controlling blood glucose levels after a meal. Ingestion of carbohydrate and other nutrients promotes the secretion of insulinotropic hormones such as glucagon-like peptide-1 (GLP-1) from the gut, resulting in a surge of insulin production before blood glucose levels rise (11, 32). This early response contributes to increased glucose disposal during absorption and helps to prevent hyperglycemia. GLP-1 mimetics and inhibitors of GLP-1 degradation help increase insulin biosynthesis and secretion from pancreatic β-cells and are valuable additions to previous treatment regimens for T2DM patients (11, 32).

Despite the importance of intestinal glucose sensing and glucose-stimulated gut hormone secretion, the mechanisms underlying these processes have remained elusive. The distinct glucose-sensing mechanisms found in the pancreas and in the gustatory epithelium have each been suggested as models for stimulus-secretion coupling in the intestine. Pancreatic β-cells couple glucose detection to insulin secretion through a metabolic mechanism that includes the facilitative glucose transporter GLUT2, the glycolytic enzyme glucokinase, and the G protein-coupled sweet taste receptor composed of the subunits Tas1r1 and Tas1r3 (38, 50, 73). These receptor proteins, as well as the taste receptor-associated G protein α-gustducin, are also expressed outside the gustatory system, including in the respiratory system (70), pancreatic islets (34, 49), and intestinal epithelial cells (12, 27, 31, 43, 56, 67, 78). In the pancreas, sweet taste receptor activation has been reported to potentiate glucose-stimulated insulin secretion (34, 49). In the intestine, this receptor has been implicated in
glucose absorption, whereas α-gustducin has been shown to be critical for glucose-stimulated incretin secretion (31, 41–43) from enterotoendocrine cells. Mice lacking α-gustducin exhibit decreased expression of the Na⁺-glucose cotransporter SGLT1 in the intestine, elevated blood glucose levels, a delayed rise in plasma insulin levels, and a diminished incretin response after a glucose challenge compared with controls (31, 43). However, the role of the sweet taste receptor in gut hormone release remains controversial. For example, while the sweet taste receptor agonist sucralose can promote GLP-1 secretion from human and mouse enterotoendocrine cell lines (31, 43), this artificial sweetener failed to elicit GLP-1 secretion from primary cultures of mouse enterotoendocrine L cells (55). Furthermore, oral ingestion or intragastric infusion of artificial sweeteners does not increase circulating GLP-1 levels in humans or rodents (17, 18, 39) although the sweet taste receptor inhibitor lactisole reduces plasma glucose-dependent blood GLP-1 levels in humans receiving an intragastric glucose load (65). Therefore, there remains a need to elucidate the physiological mechanisms underlying glucose detection and glucose-stimulated hormone secretion.

Changes in gastrointestinal anatomy, such as those resulting from gastric bypass surgeries, may also impact GSGS (6, 32–36). Bariatric surgery procedures such as Roux-en-Y gastric bypass (RYGB) are becoming common treatments for extreme obesity. These procedures reduce caloric intake by restricting the capacity of the gastrointestinal tract to hold food (e.g., by reducing the size of the stomach or its ability to expand) and/or by reducing the amount of nutrients absorbed (e.g., by shortening the length of small intestine that is exposed to ingested food) (58, 69). RYGB has both a restrictive and malabsorptive component, since this procedure reduces the stomach to a small pouch and bypasses much of the small intestine (69). A large majority of T2DM patients receiving RYGB (or other bariatric procedures with a malabsorptive component) exhibit resolution of their dysglycemia within days to weeks of the surgery, well before significant weight loss occurs (7). This effect is so dramatic that some have asked whether bariatric procedures should be considered as treatments for T2DM (46, 59). Of course, there is understandable caution about using a major surgical procedure for T2DM treatment. Could the anti-T2DM effects of bariatric surgery be mimicked? Unfortunately, the mechanisms underlying this effect are not known (68–69). While several hypotheses have been proposed, two are particularly prominent (58, 69). The “forget hypothesis” predicts that RYGB removes tissues that produce diabetogenic factor and thus removes an obstacle to normal glucose homeostasis. The “hindgut hypothesis,” by contrast, predicts that an enhanced release of one or more insulinotropic factors, such as GLP-1, emerges from the distal gut. These two possibilities are not mutually exclusive, and there is evidence supporting each (e.g., see Refs. 8, 57, 69) as well as for additional mechanisms.

In this study, we investigated the role of the sweet taste receptor in postprandial glucose sensing. Our results reveal a mechanistic complexity that resolves the previous controversy over whether receptor-dependent or metabolic pathways mediate GSGS, indicate a role for the sweet taste receptor in the regulation of insulin secretion from pancreatic islets, and provide an explanation for changes in hindgut GLP-1 secretion after bariatric surgery.

**MATERIALS AND METHODS**

**Animals.** T1R2 and T1R3 gene-targeted mice (80) were maintained by heterozygote inbreeding. Brief access taste tests (15, 20) were used to confirm sweet taste aguesia in these lines. Sprague-Dawley rats were ∼500 g and aged 18 wk at the time of surgeries (see below). All animal procedures were approved by the University of Maryland, Baltimore (mouse studies), or Pennsylvania State University-Hershey (rat studies) Institutional Animal Care and Use Committees.

**Glucose tolerance tests.** Mice were fasted for 18 h before testing. In the oral glucose tolerance test (OGTT), mice received a glucose gavage (5 g glucose/kg body wt) delivered in the esophagus by syringe, as in Ref. 31. For the intraperitoneal glucose tolerance test (IPGTT), mice received an intraperitoneal injection of glucose (2.5 g glucose/kg body wt). Insulin levels were measured before glucose administration and at several time points after gavage/injection. Blood samples were collected at every time point. Glucose was measured with an AlphaTRAK glucose meter (Abbott Laboratories, Abbott Park, IL) calibrated for mice. The precision and accuracy of the glucose meter readings throughout the range of measurements were confirmed with glucose standards (r² = 0.98). Plasma insulin levels were measured using the Ultra Sensitive Mouse Insulin ELISA kit (Crystal Chem, Downers Grove, IL).

**Adenovirus construction.** The VAMP2 localization sequence was inserted in a previously described mVenus:mCerulean fusion protein (44) using a two-step process. VAM2 (4, 45) was amplified by PCR with the sense primer 5′-TTT-GCTACGCCCATGTGGTATGGC-3′ and the antisense primer 5′-TTTACGGTCCCCCGCCCTTGCCGC-3′, and cloned into the mVenus:mCerulean3 vector using NheI and AgeI restriction sites to create CAM15:mCerulean3. mVenus (48) was then reinserted in the vector from the original plasmid using fragments from an AgeI digest. Proper construction was verified by DNA sequencing. Adenoviruses were produced by the University of Maryland, Baltimore, Gene Therapy Research Facility. Adenovirus construction is described previously (10).

**Total internal reflection fluorescence microscopy.** Islets were isolated using a collagenase digestion protocol described previously (29). Briefer a collagenase digestion solution was injected in the common bile duct, whereas mouse, rat, and human islets were identified, hand picked, and placed on extracellular matrix-coated dishes as previously described (10). Before experimentation (24 h), islets were infected with adenovirus (see above). Transfected islet cells were identified under 455-nm LED illumination with a Zeiss Axio Observer microscope using a ×100, 1.45-numer aperture α-Plan-Fluar lens. Luminal mVenus fluorescence was acquired under 10.220.32.246 on July 7, 2017 http://ajpendo.physiology.org/ Downloaded from
insulin [rabbit anti-insulin (Abcam), Alexa-590-conjugated donkey anti-rabbit secondaries (Jackson ImmunoResearch)] and fluorescence microscopy using appropriate collection conditions for yellow fluorescent protein (YFP) and Alexa-590. The vast majority (>90%) of infected cells stained positively for insulin (n > 350).

**RYGB surgery.** Surgeries were performed as previously described (23). Animals were killed 8 wk after the surgery. In the RYGB group, the stomach was divided using a GIA stapler (ETS-Flex Ethicon Endo surgery, 45 mm) to create an ~20% gastric pouch. The small bowel was divided to create a 15-cm biliopancreatic limb (measured from the ligament of Treitz), a 15-cm alimentary (Roux) limb, and a remaining 65- to 70-cm segment of small bowel forming the common channel. In the surgical controls (“sham operation”) the jejenum was fully transected and reattached at the same proximal level (15 cm distal from the ligament of Treitz). An additional longitudinal enterotomy was made at the level of the jejenum corresponding with the jejuno-jejunal anastomosis in the RYGB rats; this was reclosed with interrupted 5–0 prolene sutures and without forming an anastomosis. The stomach was not manipulated in this control procedure.

**Using chamber experiments.** Mice had ad libitum access to food and water before the experiment. After death, intestines were placed in ice-cold Krebs buffer (KRB, pH 7.2), the muscle layer was removed, and epithelial explants were mounted in modified Ussing chambers with a 9-mm intrachamber opening (Harvard Apparatus) that was reduced to allow an exposed tissue area of 1.76 mm² (74, 75). Mucosal compartments were filled with KRB containing 1.5 mM NaCl and serosal compartments were filled with KRB containing 10 mM NaCl. Chambers were maintained at 37°C and continuously oxygenated with 95% O₂-5% CO₂. Tissues were equilibrated for 40 min to achieve steady-state conditions in transepithelial potential difference (PD), with replacement of buffers after 20 min. Two pairs of Ag/Cl electrodes were used to measure transepithelial PD and current, respectively (44). Electrodes were coupled to an external six-channel electronic unit with a voltage-controlled current source. Data sampling was computer controlled via A/D D/A board (Lab NB; National Instruments) by a program developed in LabVIEW (National Instruments) (77). Direct pulses of 0.3, 1.5, 1.5, and 0 µA (2 s each) were sent across the tissue every other minute, voltage responses were measured, and the mean voltage response was calculated. Averages were then performed on the current (I)-voltage (V) relationship. PD and I-L were compared using ANOVA followed by Tukey's post hoc tests. Rates of insulin secretion were compared using ANOVA followed by Tukey's multiple-comparison post hoc tests. The means of the variables are presented with the SE (mean ± SE). A P value ≤0.05 was considered statistically significant.

**RESULTS.**

**Normal glucose and insulin homeostasis requires T1R3.** To determine if the sweet taste receptor is required for normal glucose and insulin homeostasis, we first performed 2-h OGTTs with T1R3+/+, T1R3+/−, and T1R3−/− mice. T1R3−/− mice of this line are aguesic for sweet tastants while both wild-type and heterozygous littermates exhibit normal sweet taste responsiveness (80). Mice of all three genotypes exhibited comparable food intake and weight gain (data not shown). Fasting (18-h) blood glucose levels (T1R3+/+, 133.1 ± 5.1 mg/dl; T1R3+/−, 127.1 ± 7.1 mg/dl; T1R3−/−, 127.1 ± 6.2 mg/dl; ANOVA, F = 0.90) and plasma insulin levels (T1R3+/+, 0.21 ± 0.02 ng/ml; T1R3+/−, 0.12 ± 0.05 ng/ml; T1R3−/−, 0.11 ± 0.01 ng/ml; ANOVA, F = 0.1) were identical across genotypes (Fig. 1, A and B). However, blood glucose and plasma insulin levels differed significantly during an oral glucose challenge. Blood glucose levels after an oral glucose gavage (5 g/kg) were significantly higher in T1R3−/− mice compared with controls (Fig. 1, A and B; see legend for statistics). T1R3−/− mice also had significantly lower plasma insulin levels during the OGTT than did T1R3+/+ or T1R3+/− mice and were completely missing the initial insulin peak present in control animals (Fig. 1, C and D). In contrast, no significant differences in blood glucose levels were seen across genotype in a separate cohort of mice that received an IPGTT (2.5 g/kg; Fig. 1, E and F), which bypasses the intestinal lumen and thus does not induce secretion of incretin (e.g., GLP-1) hormones. However, the plasma insulin area under the curve, although not incremental levels, was significantly lower in T1R3−/− mice compared with controls during the IPGTT (Fig. 1, G and H), suggesting an insulin secretion defect in these animals that is independent of gut hormones. Together, these data indicate that T1R3 is required for normal glycemic control and suggest that the sweet taste receptor affects both incretin secretion from the intestine and insulin secretion from the pancreas. However, mice lacking sweet taste receptor subunit T1R2 showed no evidence of dysglycemia or insulin dysregulation during an OGTT, despite demonstrated sweet taste ageusia in these knockout mice (80). Blood glucose and plasma insulin levels after an oral glucose gavage (5 g/kg) were nearly identical in T1R2+/+ and T1R2−/− mice (Fig. 1, I-L; see legend for statistics). This surprising result suggested that either T1R2 plays no role in postigestive glucose sensing or that the remaining T1R3 subunit can compensate, at least to some degree, for the loss of its heterodimeric partner.

**T1R2 and T1R3 mediate sugar-stimulated secretion of GLP-1 from small intestine.** The sweet taste receptor has been implicated in GS GS G from the intestine (5, 12, 27, 30, 31, 33, 41, 43,
56, 67), but such a role remains controversial (17, 18, 39, 55). To directly test this hypothesis, we isolated epithelial explants from duodenum, jejunum, ileum, colon, and rectum of T1R3+/+, T1R3−/−, T1R2+/+, and T1R2−/− mice and mounted them in Ussing chambers. The luminal side of the explant was exposed to buffer with or without sweet stimuli, and samples were collected from the serosal side chambers for measurements of GLP-1 release by ELISA (19). There were no significant differences in PD in the absence of applied current (an indicator of tissue viability) or in TER or short-circuit current before or after stimulation either between genotypes or in different regions of the gastrointestinal tract (data not shown). Glucose (250 mM) stimulation elicited robust GLP-1 secretion from jejenum and ileum explants obtained from T1R3+/+ mice (GLP-1 secretion was seen with glucose concentrations as low as 50 mM); this secretion was abolished in T1R3−/− mice (Fig. 2, A–C; see legend for statistics). Basal GLP-1 content was identical across T1R3 genotypes, as measured by ELISA from freshly dissected explants (T1R3+/+ ileum, 0.188 ± 0.004 pM; T1R3−/− ileum, 0.185 ± 0.002 pM). Quantitative real-time PCR (qRT-PCR) showed an equivalent level of proglucagon expression across genotypes (data not shown). Consistent with the observation that T1R3 is required for gustatory responses to both natural and artificial sweeteners (38, 50), sucrose (100 mM) and fructose (500 mM) promoted GLP-1 secretion from jejenum and/or ileum explants of T1R3+/+, but not T1R3−/−, mice (Fig. 2, B and C). Similar results were seen for T1R2+/+ and T1R2−/− mice (Fig. 2, D–F). However, while GSGL from jejenum explants of T1R2−/− mice was no higher than that seen with the buffer control (Fig. 2E), some GSGL remained in T1R2−/− ileum, albeit at levels fivefold lower than seen in wild-type tissue (Fig. 2F). These data indicate that both T1R2 and T1R3 are required for normal sweetener-dependent GLP-1 secretion in the small intestine but that T1R3 alone can partially compensate for the loss of T1R2.
GSGS from large intestine requires K<sub>ATP</sub> channels but not the sweet taste receptor. Although T1R2 expression has been reported in enteroendocrine L cells of the large intestine (30), we did not observe GSGS from either colon or rectum of T1R3<sup>+/+</sup> or T1R2<sup>+/+</sup> mice (Fig. 3). However, GSGS was robust from both colon and rectum of T1R3<sup>−/−</sup>, but not T1R2<sup>−/−</sup>, mice (Fig. 3; basal GLP-1 content was identical across T1R3 genotypes, as measured by ELISA from freshly dissected explants (<sup>−/−</sup> colon, 0.183 ± 0.055 pmol/mg protein; <sup>−/−</sup> colon, 0.191 ± 0.011 pmol/mg protein); similarly, proglucagon expression levels were equivalent, as assessed by qRT-PCR (data not shown). Neither sucralose nor fructose elicited GLP-1 secretion in large intestine explants (Fig. 3A and B), indicating that this stimulus-secretion coupling mechanism is glucose specific. Therefore, we conclude that there are T1R3-dependent and T1R3-independent pathways available in the gastrointestinal tract to regulate GLP-1 secretion.

We next investigated the molecular basis of the sweet taste receptor-independent GSGS in the gut. The K<sub>ATP</sub> channel inhibitor tolbutamide was reported to evoke modest (2- to 3-fold) increases in GLP-1 secretion from mixed primary cultures of upper small intestine or colon (55). To test whether members of the K<sub>ATP</sub> channel family couple glucose detection to GLP-1 secretion in the intestine, we measured GLP-1 secreted from ileum and colon explants of T1R3<sup>+/+</sup> and T1R3<sup>−/−</sup> mice in the presence of the K<sub>ATP</sub> channel inhibitor glibenclamide (50 μM). Glibenclamide treatment had no significant effect on GLP-1 secretion from ileum explants (Fig. 4A); see legend for statistics). However, the drug evoked robust GLP-1 secretion from colon of both T1R3<sup>+/+</sup> and T1R3<sup>−/−</sup> mice (14- to 9-fold increases, respectively; Fig. 4B). By contrast, the K<sub>ATP</sub> channel opener diazoxide (100 μM) and cromakalim (10 μM) blocked GSGS from colon explants of T1R3<sup>−/−</sup> mice, although to different extents (the mild cromakalim-dependent GSGS seen in wild-type colon may reflect off-target actions of this drug; Fig. 4C). Together, these results indicate that GSGS from the large intestine requires closure of K<sub>ATP</sub> channels.

Emergence of GSGS in hindgut is associated with changes in fecal composition. Next, we explored the intestinal changes that could contribute to the emergence of robust GSGS from the large intestines of T1R3<sup>−/−</sup>, but not T1R2<sup>−/−</sup>, mice. T1R3 regulates glucose absorption in the intestine, possibly via GLP-1-dependent modulation of glucose transporter expression and cellular localization in enterocytes (41, 43). We had noticed that the proximal colon was distended in every T1R3<sup>−/−</sup> mouse, but never in T1R3<sup>+/+</sup> mice, by large pockets of gas. Development of such gas pockets is indicative of carbohydrate malabsorption in the small intestine. Therefore, we measured the amount of total carbohydrate in the luminal contents of ileum, colon, and rectum of T1R3<sup>+/+</sup> and T1R3<sup>−/−</sup> mice. Carbohydrate levels were significantly higher in T1R3<sup>−/−</sup> mice (Fig. 5A). Additionally, the pH of feces obtained from T1R3<sup>−/−</sup> mice was significantly lower than that of wild-type controls (T1R3<sup>+/+</sup>, pH 7.50 ± 0.02; T1R3<sup>−/−</sup>, pH 6.99 ± 0.03; ANOVA, P = 0.001; n = 10 mice each), indicative of increased microbe-dependent fermentation. In contrast, T1R2<sup>−/−</sup> mice showed no significant differences in fecal carbohydrate content (Fig. 5B) or pH (T1R2<sup>−/−</sup>, pH 7.48 ± 0.02; T1R2<sup>−/−</sup>, pH 7.49 ± 0.02; n = 8 mice each) compared with wild-type controls. Together, these results indicate that T1R3<sup>−/−</sup> mice are deficient in their ability to absorb glucose and other sugars, thus exposing the hindgut to higher carbohydrate concentrations and changing the luminal environment of the gastrointestinal tract. T1R2<sup>−/−</sup> mice, which maintain some GSGS in the ileum, show no significant impairments of glucose absorption and thus apparently have a hindgut luminal environment similar to that of wild-type animals.
T1R3 increases the rate of secretory granule fusion in pancreatic islets. Changes in GLP-1 secretion do not fully explain insulin levels 40–50% lower in T1R3−/− mice than in wild-type controls during both the oral and intraperitoneal glucose challenges. These results suggested an additional defect in pancreatic islet function. The sweet taste receptor has been implicated in sweetener-stimulated insulin responses in pancreatic β-cells and in a β-cell line (49) and has been reported to mediate fructose-dependent potentiation of glucose-stimulated insulin secretion (34). Surprisingly, T1R3−/− and T1R3+/− mice showed no significant differences in insulin secretion from isolated pancreatic islets exposed to either 2.5 or 12.5 mM glucose for 30 min, as measured by ELISA (data not shown). However, T1R3−/− mice did exhibit a dramatic change in the rate of insulin secretion from pancreatic islets. We assessed the kinetics of insulin secretion by using TIRF microscopy to quantify secretory granule fusion in isolated pancreatic islets expressing a pH-sensitive YFP complex in the granule lumen upon exocytosis (Fig. 6, A and D). The granule fusion rate did not differ between T1R3+/+ and T1R3−/− islets, as assessed by the increased fluorescence seen with alkalization of the granule lumen upon exocytosis (Fig. 6, A and D). The granule fusion rate did not differ between T1R3+/+ and T1R3−/− islets after KCl stimulation (30 mM) (Fig. 6E), but the mean fusion rate after glucose stimulation was significantly slower for T1R3−/− granules (k = 0.1/s) than for T1R3+/+ granules (k = 0.4/s; Scheffé’s, P = 2 × 10−8; Fig. 6, C, D, and F). There was no change in fusion rate for insulin granules in T1R2+/− islets (Fig. 6, E and F), again suggesting that T1R3 can maintain some glucose responsive-
We hypothesized that rodents receiving RYGB would exhibit robust GSGS from the colon analogous to that seen in T1R3−/− mice. In these experiments, rats received either RYGB or sham surgery (23). Rats were killed 8 wk after surgery. Feces from RYGB rats had higher carbohydrate content (Fig. 7A) and lower pH than did that from sham-operated rats (sham, pH 6.92 ± 0.09; RYGB, pH 6.00 ± 0.2; ANOVA, P = 0.003; n = 5 each). Intestinal explants of ileum and colon were placed in Ussing chambers and stimulated with buffer or glucose. As expected, glucose was effective at eliciting GLP-1 secretion from the ileum of both sham and RYGB rats (Fig. 7B). However, while sham controls showed no GSGS from colon, RYGB colons showed robust GSGS (Fig. 7C). Together, these results strongly support a model in which upregulation of glucose-stimulated GLP-1 secretion from the colon following changes in the luminal environment [e.g., increased carbohydrate content or alterations in the microbiome (79)] and could explain the robust hormonal responses seen in the hindgut after RYGB.

**DISCUSSION**

Our results indicate that multiple mechanisms contribute to postigestive glucose sensing in the gut and associated organs (Fig. 8). We have shown that the same receptor that serves to detect sugars in the gustatory system, thus initiating the perception of sweet taste, also plays a critical role in detecting ingested sugars in the small intestine and in regulating the kinetics of glucose-stimulated GLP-1 secretion from the colon. Our results strongly support a model in which upregulation of glucose-stimulated GLP-1 secretion from the colon following changes in the luminal environment [e.g., increased carbohydrate content or alterations in the microbiome (79)] and could explain the robust hormonal responses seen in the hindgut after RYGB.
MECHANISMS OF POSTINGESTIVE GLUCOSE SENSING

Fig. 8. Distinct mechanisms of glucose-stimulated GLP-1 secretion in small and large intestine. Enteroendocrine L cells from ileum (pink) or colon (yellow) can both secrete GLP-1 but show different selectivities for sweet stimuli and utilize different mechanisms to couple glucose detection to hormone secretion. $K_{ATP}$, ATP-sensitive $K^+$ channel.

An independent mechanism mediates glucose-stimulated GLP-1 release from the hindgut and is upregulated in two rodent models that share the common phenotype of elevated fecal carbohydrate content. If continued in humans, these mechanisms could serve as new targets for T2DM therapies.

By directly assessing GSGS from intestinal explants of T1R2$^{-/-}$ and T1R3$^{-/-}$ mice, we have demonstrated that the sweet taste receptor is necessary for GSGS from the small intestine. Consistent with the known stimulus selectivity of the sweet taste receptor, the monosaccharides glucose and fructose and the synthetic halogenated disaccharide sucralose each stimulate GLP-1 secretion from the small intestine of wild-type mice. Furthermore, responses to these sugars are dramatically reduced or abolished in the small intestines of T1R2$^{-/-}$ and T1R3$^{-/-}$ mice, respectively. These results are consistent with previous studies showing that the typical GLP-1 surge seen after a glucose gavage is absent in $\alpha$-gustducin null mice and that the sweet taste receptor inhibitors lactisole and gurmarin abolish GLP-1 secretion in mice (31, 43).

The residual GLP-1 secretion and effective glucose absorption in T1R2$^{-/-}$ mice suggest that the T1R3 subunit can homodimerize (or perhaps heterodimerize with another protein) to function as a less efficacious sugar receptor. A T1R3 homodimer could retain the ability to bind sugars (51, 52) but would likely respond to sugar binding with a lower efficacy of activation (50).

Our studies also show that a sweet taste receptor-independent mechanism is present in the intestine. Specifically, this mechanism is found in the large intestine and emerges in association with changes in the luminal environment of the hindgut that accompany T1R3 deletion or RYGB surgery; whether this mechanism can also be engaged under normal physiological conditions is unclear. Furthermore, this GSGS mechanism is mimicked by the $K_{ATP}$ channel blocker glibenclamide (which had no effect in ileum of either T1R3$^{-/-}$ or T1R3$^{+/+}$ mice), even in wild-type animals, and is inhibited by the $K_{ATP}$ channel openers diazoxide and cromakalim. Thus, we conclude that enteroendocrine cells of the large intestine, but not small intestine, utilize a $K_{ATP}$ channel to facilitate GLP-1 secretion. These results are consistent with studies in acutely isolated colon enteroendocrine cells and in the enteroendocrine GLUTag cell line, which found that GSGS is mimicked by another sulfonyleurea drug, tolbutamide (54, 55). However, the sensitivity of GSGS in T1R3$^{-/-}$ colon to both diazoxide and cromakalim suggests that the $K_{ATP}$ channel subtype mediating GLP-1 secretion from colon resembles the variant found in smooth muscle, not that found in pancreatic $\beta$-cells (26).

Because glibenclamide can promote GLP-1 secretion from colon explants of T1R3$^{-/-}$ mice, we also conclude that enteroendocrine cells in this part of the gastrointestinal tract are always competent to secrete GLP-1, but normally lack robust expression of a glucose sensor or other aspects of the stimulus-secretion coupling mechanism needed to effectively transduce glucose stimulation into GLP-1 secretion. The molecular identity of the glucose-sensing apparatus in large intestine L cells remains unknown. Studies of the Na$^+$ glucose co-transporter SGLT1 in GLUTag cells (22) and in mice (21, 47) suggest that this molecule could play a role in GSGS. Even so, mechanistic studies in native enteroendocrine L cells are still needed to fully understand the molecular basis of hindgut GSGS.

The appearance of robust GSGS in the large intestines of both T1R3$^{-/-}$ mice and RYGB rats suggests a common mechanism regulating this change in glucose sensitivity. Both models exhibit significant increases in fecal carbohydrate content and significant decreases in fecal pH. These changes in the luminal environment likely result from carbohydrate malabsorption as well as from the enhanced production of short-chain fatty acids upon fermentation of excess carbohydrate by intestinal microflora. T1R3$^{-/-}$ mice exhibit decreased SGLT1 expression and diminished Na$^+$-dependent glucose uptake in brush-border membrane vesicles obtained from small intestines (43); results consistent with these mice having a glucose/galactose malabsorption phenotype. Similarly, the shortened small intestine in RYGB rats results in reduced carbohydrate absorption. Significantly, T1R2$^{-/-}$ mice do not exhibit an upregulation of GSGS in hindgut and also show no changes in fecal carbohydrate content or pH. Thus changes in the luminal environment of the hindgut may impact the expression of glucose sensors or other proteins that couple glucose detection to GLP-1 secretion in colonic and rectal L cells. The upregulation of this stimulus-secretion coupling capability may be a direct result of the presence of increased extracellular glucose. Alternatively, changes in microbial composition or activity may influence gut responses. The gut microbiota is positioned to impact nutrient availability and the function of nutrient-sensing cells in the gut through the production of microbial metabolites. Bacteria can also produce a number of peptides, some of which are homologous to human peptide hormones, and could act directly on intestinal tissues (28). Thus, shifts in the composition and/or activity of the gut microbiota could influence metabolic processes such as glucose homeostasis. For example, the gut microbiota may influence energy harvest in mice (71) and humans (37), and composition of the gut
microbial community has been shown to change after gastric bypass (79).

Bariatric surgeries in T2DM patients will often promote a return to euglycemia before significant weight loss (58). This rapid improvement in glycemic control is thought to reflect endocrine changes in the gut (9, 69). The increase in GSIS we observe in colon explants of RYGB rats is consistent with the hindgut hypothesis of T2DM remission after bariatric surgery (69), which notes that systemic GLP-1 levels are elevated in animals and human patients receiving these procedures (6, 8, 35, 36). Our data raise the interesting possibility that focal delivery of K<sub>ATP</sub> channel inhibitors to the lumen of the colon or rectum could promote euglycemia without the need for major surgery.

TIRF microscopy imaging of insulin granule exocytosis from T1R3<sup>−/−</sup> islets shows a pronounced slowing of insulin secretion kinetics that is independent of cellular depolarization. However, it is unclear which part of the signaling cascade that links glucose stimulation to insulin secretion is specifically changed in the absence of T1R3. The normal responses observed in T1R3<sup>−/−</sup> islets with KCl-mediated membrane depolarization suggest a defect in Ca<sup>2+</sup> influx that can be overcome by superphysiological depolarization. This interpretation is consistent with observations that high KCl leads to saturating Ca<sup>2+</sup> influx in islets and preferentially recruits a different granule pool than does glucose stimulation (4, 63). Voltage-dependent Ca<sup>2+</sup> influx is an essential trigger for exocytosis. Furthermore, GLP-1 regulates the activity of critical ion channels that participate in glucose-stimulated insulin secretion, including voltage-dependent Ca<sup>2+</sup> channels and K<sub>ATP</sub> channels (40). One possible explanation for the slowing of insulin secretion in T1R3<sup>−/−</sup> mice is that it results from changes in GLP-1 signaling from the gut. Alternatively, sweet taste receptors expressed in pancreatic β-cells could directly contribute to glucose sensing and response. This latter interpretation is supported by the observation that sucralose can potentiate glucose-stimulated insulin secretion in pancreatic islets and the MIN6 cell line (49) and that T1R2 deletion reduces fructose-glucose-stimulated insulin secretion in pancreatic islets and the MIN6 cell line (49) and that T1R2 deletion reduces fructose-sensitive insulin secretion in MIN6 cells (40). Furthermore, changes in incretin signaling are unlikely to explain our results, since incretin receptor double knockout mice (which lack receptors for both GLP-1 and GIP) are unresponsive to glucagon-like peptide-1 and are unresponsive to these two hormones) exhibit normal insulin secretion kinetics (24, 53).

The participation of the sweet taste receptor in the postigestive detection of glucose raises the possibility that other sweet-tasting sugars or nonnutritive sweeteners could have antiprandial metabolic effects by stimulating the release of gut or pancreatic hormones, altering glucose absorption, or affecting glucose-stimulated insulin secretion via the sweet taste receptor (13). Our results clearly show that a second mechanism, independent of the sweet taste receptor and apparently glucose-specific, is present in the gut and can contribute to glycemic control under certain conditions. Indeed, glucose and insulin levels in T1R3<sup>−/−</sup> mice are remarkably normal unless the animals are challenged with a large glucose load. It is possible that the metabolic relevance of sweet stimuli is determined in part by the differential activation of these distinct pathways. Such a model is consistent with previous studies of flavor conditioning in rodents that support the existence of a postigestive sugar sensor that is glucose-specific (1, 60–62).

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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