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

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Geraedts MC, Takahashi T, Viques 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 knockout 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 pancreatic β-cells. Glucose, fructose, and sucrose evoked GLP-1 secretion from T1R3⁻/⁻, but not T1R3⁻/⁺, ileal explants, and this secretion was not mimicked by the KATP channel blocker glibenclamide. T1R2⁻/⁻ mice showed normal glycemic control and partial small intestine GSGS, suggesting that T1R3 can mediate GSGS without T1R2. Robust GSGS that were KATP channel dependent and glucose specific emerged in the large intestine of TIR3⁻/⁻ mice and RYGB rats in association with elevated fecal carbohydrate content 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.

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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 (65). 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 glucosensing 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 metabotropic mechanism that includes the facilitative glucose transporter GLUT2, the glycolytic enzyme glucokinase, and the ATP-sensitive K⁺ (KATP) channel (76). Elevation of intracellular ATP levels as a result of increased glycolysis within the β-cell results in KATP channel closure, membrane depolarization, opening of voltage-gated Ca²⁺ channels, and exocytosis of insulin granules. A similar mechanism may also mediate glucose-stimulated GLP-1 secretion (GSGS) from the gut. Indeed, a KATP channel inhibitor promotes GLP-1 secretion from an enteroendocrine cell line and from isolated proglucagon-expressing gut enterendocrine cells (22, 54, 55).

An alternative strategy for glucose sensing is found in sweet taste cells of the gustatory system. There, glucose and other sweet-tasting compounds are detected by a heterodimeric, G protein-coupled sweet taste receptor composed of the subunits type 1 taste receptor 2 (T1R2) and type 1 taste receptor 3 (T1R3) (encoded by the genes Tas1r2 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 enteroendocrine 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 enteroendocrine cell lines (31, 43), this artificial sweetener failed to elicit GLP-1 secretion from primary cultures of mouse enteroendocrine 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, 35, 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 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).

**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). Mice were included in a subset of intraperitoneal injections (T1R2−/− mice, n = 4) to verify that the needle did not perforate the peritoneum (verified post mortem); (2) no significant differences were seen in glucose levels between T1R2−/− mice with and without the needle. Blood samples were collected at each 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 adenovirus: mCerulean fusion protein (44) using a two-step process. VAMP2 (14, 45) was amplified by PCR using primer 5′-TTT-TGGCTAGCGCCACCATGTCGGCTACCGCT-3′, and cloned into the mVenus:mCerulean vector using NotI and Ael restriction sites. Proper construction was verified by DNA sequencing. A 5′-TTT-TAGATCTGCCCAACAAGCTGGCGCTACCCTGTCG-3′ antisense primer was then introduced in the NH2-terminus of the fusion protein by PCR (5′-primer 5′-TTT-TGGCTAGCGCCACCATGTCGGCTACCGCT-3′, and cloned into the mVenus:mCerulean3 vector using NotI and Ael sites. Adenovirus was then reinserted in the vector from the original plasmid using fragment from an Agel digest. Proper construction was verified by DNA sequencing.

**Glucose tolerance tests.** Glucose tolerance tests (OGTT) were carried out at 37°C using the AdEasy system (25). Animals 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). Mice were included in a subset of intraperitoneal injections (T1R2−/− mice, n = 4) to verify that the needle did not perforate the peritoneum (verified post mortem); (2) no significant differences were seen in glucose levels between T1R2−/− mice with and without the needle. Blood samples were collected at each 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).

**Total internal reflection fluorescence microscopy.** Islets were isolated using a collagenase digestion protocol described previously (29). Briefly, a collagenase digestion solution was injected in the common bile duct, before excision of the pancreas and further digestion at 37°C. Digestion was stopped by addition of complete culture media (RPMI 1640, 5.5 mM glucose, 10% FBS, 1% l-glutamine, 1% penicillin/streptomycin), and islets were further purified by centrifugation with Histopaque 1077 (Sigma-Aldrich). Purified 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 axiofluor fluorescence filter set. Total internal reflection fluorescence (TIRF) imaging was performed as described (10) at 37°C on a Zeiss AxioObserver microscope using a ×100, 1.45-numer aperture α-Plan-Fluar lens. Luminal mVenus fluorescence was acquired under 488 nm laser excitation. Islets were placed in imaging buffer (in mM: 125 NaCl, 5.7 KCl, 2.5 CaCl2, 1.2 MgCl2, 0.1% BSA, and 10 HEPES, pH 7.4) containing 2.5 mM mGlu 3 h before stimulation with 10 mM glucose or 30 mM KCl. Vesicle fusion rate was determined by linear regression of the fluorescence intensity change from minimum to maximum for individual secretory granule fusions. Five or fewer fusions per cell were used in the calculation of fusion rates. Expression in pancreatic β-cells was confirmed by immunostaining for
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 layers were 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 ml 10 mM mannitol, and serosal compartments were filled with KRB containing 10 mM glucose. 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 every 20 min. Two pairs of Ag/Cl electrodes were used to measure transepithelial PD and current, respectively (64). Electrodes were coupled to an external six-channel electronic unit with a voltage-controlled current source. Data sampling was computer controlled via A/D to D/A board (Lab NB; National Instruments) by a program developed in LabVIEW (National Instruments). Direct currents of 3, 1.5, 1.0, 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 (64). Averages were performed on the current (I)-voltage (V) relationship: I = PD + I  0.5R. Transepithelial resistance (TER) was obtained from the slope of the I-V line and the PD from the intersection of the voltage. After the equilibration period, the mucosal side of the tissue was exposed to buffer containing stimuli (glucose, fructose, or glucose fructose channel drugs [glibenclamide, diazoxide, etomoxirim]). Glucose and fructose concentrations were equivalent to reported taste observations. Samples from the serosal side (1.5 ml) were collected (M. C. P. Geraedts, F. J. Troost, and W. H. Saris, unpublished observations) and from previous studies with the STC-1 murine hormone assays. Levels of active GLP-1 were determined by ELISA (Alpco).

**Carbohydrate content determination.** Fecal samples were collected from different parts of the intestine and were snap-frozen and stored at −80°C until measurement. Samples were thawed and equilibrated to room temperature. A 10% slurry was made by adding 90% vol/vol of phosphate-buffered saline (pH 7.4) to the feces and mixing thoroughly by vortexing. Total carbohydrate concentration was measured using a spectrophotometric method described previously (72). Briefly, the 10% slurry was mixed with Anthrone reagent (1 g Anthrone/500 ml sulfuric acid; Sigma-Aldrich), and the absorption was measured at 650 nm. Carbohydrate content of mouse chow was measured as a positive control and as a reference standard.

**Statistical analyses.** The descriptive and statistical analyses were performed with Systat 12 (San Jose, CA). Electrophysiological parameters were compared using the Wilcoxon signed rank tests. Means of blood glucose, plasma insulin, and secreted GLP-1 were compared using ANOVA followed by Scheffe’s post hoc tests. Rates of insulin granule fusion were compared by ANOVA followed by Tukey’s multiple-comparison post hoc tests. The means of the variables are presented with the SE (mean ± SE). A value ≤0.05 was considered statistically significant.

**RESULTS**

**Normal glucose and insulin homeostasis requires TIR3.** To determine if the sweet taste receptor is required for normal glucose and insulin homeostasis, we first performed 2-h OGTTs with TIR3+/+, TIR3−/+ and TIR3−/− mice. TIR3−/− mice of this line are ageusic 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 (TIR3+/+, 133.1 ± 5.1 mg/dl; TIR3−/−, 136.2 ± 7.1 mg/dl; TIR3−/+, 137.1 ± 5.4 mg/dl; ANOVA, P = 0.90) and plasma insulin levels (TIR3+/+, 0.21 ± 0.02 ng/ml; TIR3−/−, 0.17 ± 0.05 ng/ml; TIR3−/+, 0.11 ± 0.01 ng/ml; ANOVA, P = 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 TIR3−/− mice compared with controls (Fig. 1A and B; see legend for statistics). TIR3−/− mice also had significantly lower plasma insulin levels during the OGTT than did TIR3+/+ or TIR3−/+ 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 TIR3−/− 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 TIR3 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 TIR2 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 TIR2−/− and TIR2−/− mice (Fig. 1, I–L; see legend for statistics). This surprising result suggested that either TIR2 plays no role in postigestive glucose sensing or that the remaining TIR3 subunit can compensate, at least to some degree, for the loss of its heteromeric partner.

**TIR2 and TIR3 mediate sugar-stimulated secretion of GLP-1 from small intestine.** The sweet taste receptor has been implicated in GSGS from the intestine (5, 12, 27, 30, 31, 33, 41, 43,
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Fig. 1. Impaired glucose tolerance and reduced insulin responses in type 1 taste receptor 3 (T1R3)-null (−/−); but not type 1, taste receptor 2 (T1R2)-null, mice. A: blood glucose during an oral glucose tolerance test (OGTT) for T1R3−/− (n = 7; black, solid line), T1R3+/− (n = 10; gray, solid line), and T1R3+/+ (n = 11; black, broken line) mice. Repeated-measures ANOVA: P = 0.01 (glucose); ANOVA (60 min glucose): P = 0.0005; ANOVA (90 min glucose): P = 0.0007; Scheffe’s post hoc: *P < 0.05 (−/− vs. +/+ or +/+−/−); #P < 0.05 (−/− vs. +/−). B: glucose area under the curve (AUC) for A: ANOVA: P = 0.002. Scheffe’s post hoc: *P < 0.05. C: incremental plasma insulin levels during the same OGTT as in A. Repeated-measures ANOVA: P = 0.01; ANOVA (99 min insulin): P = 0.0001; ANOVA (90 min insulin): P = 0.01; Scheffe’s post hoc: *P < 0.05 (−/− vs. +/+ or +/+−/−); #P < 0.05 (−/− vs. +/−). D: insulin AUC for C: ANOVA: P = 0.0005; Scheffe’s post hoc: *P < 0.05. E: blood glucose during an intraperitoneal glucose tolerance test (IPGTT) for T1R3−/− (n = 4), T1R3+/− (n = 11), and T1R3+/+ (n = 5) mice. ANOVA: P = 0.19; F: glucose AUC for D; repeated-measures ANOVA: P = 0.003. G: incremental plasma insulin levels during the same IPGTT as in E; repeated-measures ANOVA: P = 0.47; H: insulin AUC for G: ANOVA: P < 0.01; Scheffe’s post hoc: *P < 0.05. I: blood glucose levels during an OGTT for T1R2−/− (n = 4; solid line) and T1R2+/− (n = 4; broken line) mice; repeated-measures ANOVA: P = 0.77; J: glucose AUC for I: ANOVA: P = 0.18. K: incremental plasma insulin levels during the same OGTT as I; repeated-measures ANOVA: P = 0.25; L: insulin AUC for K: ANOVA: P = 0.45. All values are means ± SE. Mice aged 12–20 weeks.

56, 67), but such a role remains controversial (67, 17, 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 excised 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 TFR 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 jejunum 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), sucralose (100 mM) and fructose (500 mM) promoted GLP-1 secretion from jejunum 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 GSGS from jejunum explants of T1R2−/− mice was no higher than that seen with the buffer control (Fig. 2E), some GSGS 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.
Fig. 2. Disruption of glucose-stimulated glucagon-like peptide-1 (GLP-1) secretion from small intestine of T1R3−/− and T1R2−/− mice. A–C: ELISA-based measurements of GLP-1 secretion from intestinal explants of duodenum (A), jejunum (B), and ileum (C) of T1R3+/+ (black) or T1R3−/− (white) mice upon stimulation with buffer (B), 250 mM glucose (G), 100 mM sucralose (S), or 500 mM fructose (F). D–F: ELISA-based measurements of GLP-1 secretion from intestinal explants of duodenum (D), jejunum (E), and ileum (F) of T1R2+/+ (black) or T1R2−/− (white) mice upon stimulation with buffer or 250 mM glucose. Each bar, n = 4 mice. Data are presented as means ± SE. *P < 0.01 vs. buffer for that genotype, except for comparison of buffer and glucose treatments in T1R2−/− ileum (F; P = 0.01).

GSGS from large intestine require KATP 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+/+ or T1R2+/+ mice (Fig. 3). However, GSGS was robust from both colon and rectum of T1R3−/−, but not T1R2−/−, mice (Fig. 3; basal GLP-1 content was identical across T1R3 genotypes, as measured by ELISA from freshly dissected explants (+/+ colon, 0.13 ± 0.05 pM; −/− colon, 0.19 ± 0.01 pM); 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 KATP channel inhibitor tolvaptamide was reported to evoke modest (∼ twofold) increases in GLP-1 secretion from mixed primary cultures of upper small intestine or colon (55). To test whether members of the KATP channel family couple glucose detection to GLP-1 secretion in the intestine, we measured GLP-1 secreted from ileum and colon explants of T1R3+/+ and T1R3−/− mice in the presence of the KATP 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+/+ and T1R3−/− mice (14- and 9-fold increases, respectively; Fig. 4B). By contrast, the KATP channel opener diazoxide (100 μM) and cromakalim (10 μM) blocked GSGS from colon explants of T1R3−/− 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 KATP 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−/−, but not T1R2−/−, 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−/− mouse, but never in T1R3+/+ 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+/+ and T1R3−/− mice. Carbohydrate levels were significantly higher in T1R3−/− mice compared with wild-type controls (Fig. 5A). Additionally, the pH of feces obtained from T1R3−/− mice was significantly lower than that of wild-type controls (T1R3+/+, pH 7.50 ± 0.02; T1R3−/−, pH 6.99 ± 0.03; ANOVA, P = 0.001; n = 10 mice each), indicative of increased microbe-dependent fermentation. In contrast, T1R2−/− mice showed no significant differences in fecal carbohydrate content in Fig. 5B or pH (T1R2+/+, pH 7.48 ± 0.02; T1R2−/−, pH 7.49 ± 0.02; n = 8 mice each) compared with wild-type controls. Together, these results indicate that T1R3−/− 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−/− 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.
**TIR3** 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 TIR3−/− 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 gastric C-cells and in a β-cell line (49) and has been found to stimulate GLP-1 secretion in colon, but not ileum (34). Surprisingly, TIR3−/− 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, TIR3−/− 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 (10) (Fig. 6, A and D). Glucose (10 mM) promoted granule fusion from both TIR3+/+ and TIR3−/− islets, as assessed by the increased fluorescence seen with alkalization of the granule lumen upon exocytosis (Fig. 6, C and D). The granule fusion rate did not differ between TIR3+/+ and TIR3−/− islets after KCl stimulation (30 mM) (Fig. 6E), but the mean fusion rate after glucose stimulation was significantly slower for TIR3−/− granules (k = 0.1/s) than

![Fig. 3. Upregulation of glucose-stimulated GLP-1 secretion from ileal explants of TIR3−/− but not TIR2−/− mice. A: ELISA-based measurements of GLP-1 secretion from intestinal explants of colon (A) or rectum (B) of TIR3+/+ (black) or TIR3−/− (white) mice upon stimulation with buffer (B), 250 mM glucose (G), 100 mM sucralose (S), or 300 mM fructose (F). C and D: ELISA-based measurements of GLP-1 secretion from intestinal explants of colon (C) or rectum (D) of TIR3+/+ (black) or TIR2−/− (white) mice upon stimulation withbuffer and 250 mM glucose. Each bar, n = 4 mice. Data are presented as means ± SE. *P < 0.01 vs. buffer for that genotype. #P < 0.01 vs. same treatment in TIR3+/+ mice.](http://ajpendo.physiology.org/)

![Fig. 4. ATP-dependent K+ (KATP) channel closure is required for glucose-stimulated GLP-1 secretion in colon but not ileum. A and B: ELISA-based measurements of GLP-1 secretion from intestinal explants of ileum (A) and colon (B) of TIR3−/− (black) and TIR3−/− (white) mice upon treatment with buffer (B), 250 mM glucose (G), 100 mM glibenclamide (Gb). Each bar, n = 4 mice. Data are presented as means ± SE. *P < 0.01 vs. buffer for that genotype. C: ELISA-based measurements of GLP-1 secretion from colon explants of TIR3+/+ (black) and TIR3−/− (white) mice upon stimulation with buffer, 250 mM glucose, or 250 mM glucose in combination with 50 mM glibenclamide (G + Gb), 100 mM diazoxide (G + Dz), or 10 mM cromakalin (G + Cr). Each bar, n = 4 mice. Data are presented as means ± SE. *P < 0.01 vs. buffer for that genotype, except for comparison between buffer and glucose + cromakalin treatments in TIR3+/+ colon (P = 0.03, B). #P < 0.01 vs. glucose treatment for that genotype.](http://ajpendo.physiology.org/)

![Fig. 5. Carbohydrate malabsorption in TIR3+/−, but not TIR2−/−, mice. A: carbohydrate content of feces from ileum, colon, and rectum of TIR3+/− (black) and TIR3−/− (white) mice. Each bar, n = 10 mice. *P < 0.01. B: carbohydrate content of feces from ileum, colon, and rectum of TIR2+/− (black) and TIR2−/− (white) mice. Each bar, n = 8 mice. No significant differences were found.](http://ajpendo.physiology.org/)
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 colonies 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 postprandial 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 insulin secretion from pancreatic islets. Furthermore, we found that a sweet taste receptor-
Mechanisms of postigestive 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.
mechanisms of post-igestive glucose sensing

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 GSSG 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_ATP 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_ATP 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-dependent potentiation of insulin secretion from pancreatic islets (44). 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 glucose-dependent insulinotropic peptide and are unresponsive to these two hormones) exhibit normal insulin secretion kinetics (24, 53).

The participation of the sweet taste receptor in the post-igestive detection of glucose raises the possibility that other sweet-tasting sugars or nonnutritive sweeteners could have attenuated 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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