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(54) **Title:** REGULATING GLP-1 AND SGLT-1 IN GASTROINTESTINAL CELLS

(57) **Abstract:** The present invention relates to methods involving administering sweet taste inhibitors/sweet taste potentiators. Specifically disclosed are methods for decreasing or inhibiting carbohydrate absorption by a mammal, increasing or inducing carbohydrate absorption by a mammal, treating a disorder associated with insufficient carbohydrate absorption in a patient, regulating blood sugar levels in a mammal, promoting insulin secretion in a mammal, promoting weight loss in a mammal, promoting weight gain in a mammal, treating obesity in a patient, and treating diabetes in a patient.

REGULATING GLP-1 AND SGLT-1 IN GASTROINTESTINAL CELLS

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/965,463, filed August 20, 2007.

[0002] This invention was made with government support under grant numbers DC003055 and DC003155 awarded by the National Institutes of Health/National Institute on Deafness and Other Communication Disorders. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to the regulation of GLP-1 and SGLT-1 in gastrointestinal cells.

BACKGROUND OF THE INVENTION

[0004] Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretins – peptide hormones secreted from enteroendocrine L and K cells, respectively, that augment insulin secretion after oral intake of glucose (*1*). How carbohydrates in the gut lumen elicit the release of GLP-1 from L cells and GIP from K cells is unknown (*2*). Because intravenous glucose administration does not induce secretion of GLP-1 (*3*) it appears that glucose within the lumen of the gut acts on the luminal surface to stimulate secretion. Thus we sought to determine what glucose-sensing mechanism in the gut lumen might underlie this L cell response.

[0005] One mechanism for sensing glucose is by sweet taste receptors in taste receptor cells of the lingual epithelium (*4*). Sweet compounds bind to and activate specific G-protein coupled receptors (GPCRs) that couple through the G-protein gustducin (*5*) to specific second messenger cascades (*4, 6*). Two type 1 taste GPCRs (T1Rs) heterodimerize to form the T1R2+T1R3 sweet taste receptor (*7–11*). Key elements of the taste transduction pathways are the α, β, and γ subunits of gustducin (γ-gustducin, $G\gamma_3$ and $G\gamma_{13}$) (*5, 12*), phospholipase Cγ2 (PLCγ2) (*13*), and transient receptor potential channel type M5 (TRPM5) (*14*), a Ca^{2+}-activated cation channel (*15–17*). α-Gustducin has been detected in brush cells of the stomach, duodenum and pancreatic ducts in rat (*18, 19*), T1R2 and T1R3 are present in rodent gut and the enteroendocrine STC-1 cell line (*20*), and α-gustducin and GLP-1 are present in enteroendocrine cells of the human colon (*21*). However, the functional

significance of expression of taste signaling elements in cells of the gastrointestinal (GI) tract had been unclear.

[0006] To date, the only identified sugar sensors in the mammalian gastrointestinal tract are those involved in taste transduction (*4*). Although the gut epithelium senses luminal sugars and modulates its glucose absorptive capacity accordingly, the nature of the sugar-sensing molecule(s) and downstream events remain unknown. Several studies have shown that expression of the intestinal sodium-dependent glucose transporter 1 (SGLT1) is directly regulated by monosaccharides in the lumen of the gut independently of metabolism and appears to involve a G protein linked second messenger pathway (*41–45*). Furthermore, the addition of membrane-impermeable glucose analogues to the lumen of the intestine stimulates SGLT1 expression, implying that a glucose sensor on luminal membranes is involved (*45*).

[0007] In taste cells, the detection of sugars and sweeteners depends on T1R2+T1R3, a heterodimer of type 1 taste receptor subunits (T1Rs) (*9, 10*). The taste receptor cells of the anterior tongue that express T1R2+T1R3 typically also express gustducin, a transducin-like heterotrimeric G protein (*5*). Gustducin's α-subunit ($G\alpha_{gust}$) has been detected in brush cells of the rat stomach, duodenum, and pancreatic ducts (*46*). $G\alpha_{gust}$ and bitter-responsive type 2 taste receptors (T2Rs) are expressed in mouse intestinal endocrine cells and in the murine enteroendocrine cell line STC-1 (*47*).

SUMMARY OF THE INVENTION

[0008] A first aspect of the present invention relates to a method of decreasing or inhibiting carbohydrate absorption by a mammal. This method involves administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the mammal under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the mammal, thereby decreasing or inhibiting carbohydrate adsorption by the mammal.

[0009] A second aspect of the present invention relates to a method of promoting insulin secretion in a mammal. This method involves administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby promoting insulin secretion by the mammal.

[0010] A third aspect of the present invention relates to a method of treating obesity in a patient. This method involves selecting a patient that is obese, and administering an

effective amount of a sweet taste inhibitor to the gastrointestinal tract of the patient under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the patient, thereby decreasing or inhibiting carbohydrate adsorption by the patient and treating obesity in the patient.

[0011] A fourth aspect of the present invention relates to a method of promoting weight loss in a mammal. This method involves administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the mammal under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the mammal, thereby decreasing or inhibiting carbohydrate adsorption by the mammal and promoting weight loss in the mammal.

[0012] A fifth aspect of the present invention relates to a method of treating diabetes in a patient. This method involves selecting a patient that has diabetes, and administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the patient under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby promoting insulin secretion by the patient and treating diabetes in the patient.

[0013] A sixth aspect of the present invention relates to a method of treating obesity in a patient. This method involves selecting a patient that is obese, and administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the patient under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby inducing satiety in the patient and treating obesity in the patient.

[0014] A seventh aspect of the present invention relates to a method of promoting weight loss in a mammal. This method involves administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby inducing satiety in the mammal and promoting weight loss in the mammal.

[0015] An eighth aspect of the present invention relates to a method of regulating blood sugar levels in a mammal. This method involves administering an effective amount of a sweet taste inhibitor or sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase or decrease the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby regulating insulin secretion by the mammal and regulating blood sugar levels in the mammal.

[0016] A ninth aspect of the present invention relates to a method of increasing or inducing carbohydrate absorption by a mammal. This method involves administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the mammal under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the mammal, thereby increasing or inducing carbohydrate adsorption by the mammal.

[0017] A tenth aspect of the present invention relates to a method of treating a disorder associated with insufficient carbohydrate absorption in a patient. This method involves selecting a patient that has a disorder associated with insufficient carbohydrate absorption, and administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the patient under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the patient, thereby increasing or inducing carbohydrate adsorption by the patient and treating the disorder in the patient.

[0018] An eleventh aspect of the present invention relates to a method of promoting weight gain in a mammal. This method involves administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the mammal under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the mammal, thereby increasing or inducing carbohydrate adsorption by the mammal and promoting weight gain in the mammal.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Figures 1A–F relate to the presence of taste signaling elements in L cells of the human duodenum. Figure 1A: Indirect immunofluorescent imaging showing co-expression of taste signaling elements (left column) with GLP-1 (center column). Nuclei in the merged images (right column) are stained blue. Scale bars, 15μm. Figure 1B: top row, cells showing α-gustducin cytosolic expression and dense apical immunostaining (arrows) projecting into the gut lumen. Scale bars, 5μm. Middle row, low-magnification fields showing immunostaining of α-gustducin, GLP-1 and GIP. Bottom row, solitary gustducin-expressing L (GLP-1) and K (GIP) cells amongst the more numerous enterocytes are shown; nuclei are stained red. Scale bars, 50μm. Figure 1C: Co-expression of T1R2 sweet taste receptor subunit with α-gustducin (α-gust), GLP-1 and T1R3 in duodenal enteroendocrine cells. Scale bars, 15μm. Figure 1D: Triple staining showing expression of both GLP-1 and GIP in an α-gustducin expressing cell (upper row, arrow). The same image, taken at a different depth, shows a cell that expresses GLP-1 and α-gustducin but not GIP (lower row,

arrowhead). Scale bars, 15μm. Figure 1E: Quantitation of cells expressing α-gustducin, GLP-1, or GIP, statistically significant results determined by Student's t-test, values are means ± s.e.m. Figure 1F: RT-PCR amplification of α-gustducin mRNA in the indicated sub-populations of laser-captured cells.

[0020] Figures 2A–E relate to altered secretion of GLP-1, GIP, and insulin in response to gavage-administered glucose in α-gustducin null (*α-gust*$^{-/-}$) mice. Figure 2A: Plasma GLP-1 (top panel), GIP (middle panel) and insulin (lower panel) levels after glucose gavage (5g/Kg body weight). Figure 2B: Plasma glucose after glucose gavage (2g/Kg body weight). Figure 2C: Plasma glucose after post-fasting feeding on chow. Figure 2D: Plasma GLP-1 responses from surgically isolated duodenum in vivo: the duodenum was ligated away from the stomach and rest of the intestines, and circulatory contact maintained. 10% glucose was infused directly into the isolated duodenum. Figure 2E: GLP-1 secretory responses to 10% glucose from minced proximal duodenum. For in vivo experiments n = 6–12 animals per genotype; in vitro experiments were carried out in triplicate and replicated at least twice. Statistical significance determined by ANOVA, values are means ± s.e.m; *p<0.05, **p<0.01, ***p<0.001.

[0021] Figures 3A–D relate to secretion of GLP-1 in response to glucose, sucrose, and sucralose in NCI-H716 cells. Figures 3A–B: Glucose- sucrose- and sucralose-mediated GLP-1 secretion from NCI-H716 cells. The sweet receptor inhibitor lactisole inhibited sucralose-mediated GLP-1 secretion. Figure 3C: siRNA-mediated diminution of both α-gustducin protein levels (by immunoblotting) and glucose-induced (but not basal (BSL)) GLP-1 secretion from NCI-H716 cells. Figure 3D: Immunoblotting of ERK and pERK phosphorylated in from NCI-H716 cells in response to increasing concentrations of glucose and sucralose. The inhibitor of Erk phosphorylation, PD98059, inhibited sucralose-mediated Erk phosphorylation. The sweet receptor inhibitor lactisole diminished Erk phosphorylation. BSL = basal. Experiments were carried out in triplicate and replicated at least twice. Statistical significance determined by ANOVA, values are means ± s.e.m; *p<0.05, ***p<0.001.

[0022] Figure 4 relates to the coupling of taste receptors to G-protein α-subunits in NCI-H716 cells. Membranes from NCI-H716 cells were preincubated with the indicated concentrations of glucose and sucrose for 10 min at 25°C in the presence of 5–10μCi [^{32}P] GTP-22 azidoanilide, then irradiated to cross link the GTP analog to G-proteins. G-protein specific immune complexes (anti-Gα-gustducin, anti-G$\alpha_{i1,2}$, anti-Gα_s, and control IgG) were separated by SDS-PAGE, transferred to membranes and autoradiographically imaged.

[0023] Figures 5A–E are indirect immunofluorescent images of various gastrointestinal tissues. Figure 5A: There is no fluorescent staining of enteroendocrine cells in human duodenum sections when the primary antibody was omitted. Scale bars, top row, 50μm: bottom row, 15μm. Figure 5B: Indirect immunofluorescence of duodenum showing co-expression of α-gustducin (α-gust) with GLP-1 and GLP-2. Intrinsic fluorescence of green fluorescent protein (GFP) showing co-expression of GLP-2 in the α-gustducin-expressing cells of α-gustducin-GFP mice. Figure 5C: Duodenal sections from α-gustducin-GFP transgenic mice show colocalization of GFP with α-gustducin (α-gust), GLP-1, and GLP-2. Occasional GFP-positive cells (arrow) do not co-localize with L cell products. Figures 5D–E: Indirect immunofluorescence of jejunum and ileum, respectively, showing co-expression of α-gustducin with GLP-1, GLP-2, and PYY. Scale bars, 15μm.

[0024] Figures 6A–C show that treatment of wild type (*α-gust*$^{+/+}$) and homozygous null (*α-gust*$^{-/-}$) mice with exendin-4, a GLP-1 receptor agonist, and systemic glucose (in order to bypass the bowel) elicited similar insulin secretory responses in both sets of mice. Figure 6A: Exendin-4 (0.4 nM/Kg) was given intravenously, blood was taken at the times indicated and plasma insulin assayed by ELISA. Figure 6B: Intraperitoneal injection of glucose (1g/kg) in wild type (*α-gust*$^{+/+}$) and homozygous null (*α-gust*$^{-/-}$) mice elicited similar insulin secretory responses; blood was taken at the times indicated and plasma insulin assayed. Figure 6C: GLP-1 secretory responses to 5% glucose from isolated duodenal villi.

[0025] Figure 7 shows that age-matched wild type (*α-gust*$^{+/+}$) and homozygous null (*α-gust*$^{-/-}$) mice display differences in islet size. Immunochemistry of paraffin sections (5 μm) from pancreata showing insulin-stained (brown) tissue.

[0026] Figure 8 is a stained image of isolated villi of mice.

[0027] Figures 9A–B show that NCI-H716 cells express taste signaling components. Figure 9A: Indirect immunofluorescence showing presence in NCI-H716 cells of multiple taste elements, GLP-1 (nucleus stained red), GLP-1 along with PYY. Scale bars, 30μm. Figure 9B: RT–PCR products showing that NCI-H716 cells express multiple taste elements, a fatty acid receptor (*Gpr40*) and the bile acid receptor (*Tgr5*). Numbers are product sizes. CHO cells and tongue are negative and positive α-gustducin controls.

[0028] Figure 10 relates to GLP-1 secretion from stimulated NCI-H716 cells. PD98059 (50 μM), an ERK inhibitor, did not inhibit glucose-responsive GLP-1 secretion. PLC inhibition by U73122 decreased secretion in a concentration-dependent manner, while the inactive control, U73343, had no such effect. Results are means ± s.e.m., $n = 3$, ***$p<0.001$.

[0029] Figures 11A–D show that glucose causes Ca^{2+} entry and taste receptors are coupled to G protein α-subunits in NCI-H716 cells. Figure 10A: Glucose-potentiated receptor-operated Ca^{2+} entry, assessed by depleting Ca^{2+} stores with thapsigargin (TG, 10μM) in the absence of exogenous Ca^{2+}, then replenishing with 2mM Ca^{2+}. Thapsigargin was added to both cells at the first arrow (labeled TG). Glucose was added to one set of cells (closed triangles) at the point indicated by the arrow. The experiment was initiated in cells maintained in buffer without calcium. Calcium (2 mM final) was restored to the buffer at the time indicated. Figure 10B: Representative traces show changes in $[Ca^{2+}]_i$ after the addition (indicated by arrow) of glucose or 2-deoxyglucose (non-metabolizable glucose). Figure 10C: Representative images of $[Ca^{2+}]_i$ levels are depicted on a pseudo-color scale, acquired prior to, (i), and after, (ii) and (iii), exposure to glucose. Figure 10D: Glucose-stimulated Ca^{2+} mobilization in NCI-H716 cells depends upon PLC. The traces show that the increase in $[Ca^{2+}]_i$ in NCI-H716 cells after the addition of 10% glucose (indicated by arrow) is inhibited by U73122, a specific inhibitor of PLC, but not by U73343, the inactive analog.

[0030] Figure 12 relates to G protein activation by ligand, positive control. Treatment with GLP-1 led to a dose-dependent increase in cross-linked $G\alpha_s$ (GTP-azidoanilide cross-linked subunit) in a GLP-1 receptor-expressing cell line, CHO-GLP-1R cells, due to coupling of the activated receptor to $G\alpha_s$.

[0031] Figures 13A–C relate to increased SGLT1 expression in response to dietary carbohydrate in wild-type, but not in $G\alpha_{gust}$ or T1R3 knockout, mice. Wild-type (WT), $G\alpha_{gust}$, and T1R3 knockout mice were given low (L) or high (H) carbohydrate diets for two weeks. Figure 13A: Steady-state levels of SGLT1 mRNA determined by QPCR were normalized to β-actin mRNA. Figure 13B: SGLT1 protein from brush-border membrane vesicles (BBMV) isolated from mid small intestine was detected in Western blots (*Left*). Densitometric analysis (*Right*) of Western blots normalized SGLT1 protein expression to that of β-actin. Figure 13C: SGLT1-mediated glucose uptake was measured by Na^+-dependent D-[$U^{14}C$] glucose uptake into BBMV. Mean uptake rates are presented as arbitrary units relative to rates measured in BBMV of wild-type mice maintained on the low-carbohydrate diet (defined as 100). All values are expressed relative to SGLT1 in wild-type mice on low-carbohydrate diets as means ± SD. Data were generated in triplicate, with $n = 4$ animals in each group. Statistically significant results determined by Student's unpaired two-tailed *t* test are indicated by *, ($P < 0.05$); **, ($P < 0.005$); or ***, ($P < 0.001$).

[0032] Figures 14A–D relate to increased SGLT1 expression in response to dietary supplementation with artificial sweeteners in wild-type, but not in $G\alpha_{gust}$ or T1R3 knockout,

mice. Wild-type (WT), $G\alpha_{gust}$, and T1R3 knockout mice were given a low-carbohydrate diet without (L), or with 2 mM sucralose (L+suc) for 2 weeks. Figures 14A–C: Steady-state levels of SGLT1 mRNA (Figure 14A), SGLT1 protein (Figure 14B), and SGLT1-mediated glucose transport rates (Figure 14C), were measured (see Figures 13A–C). Figure 14D: Steady-state levels of SGLT1 mRNA were measured in wild-type (WT) mice that had been maintained for 2 weeks on a high-carbohydrate diet (H) or low-carbohydrate diet without (L) or with the indicated artificial sweeteners aspartame (L+asp), acesulfame K (L+ace-K), or saccharin (L+sac). All data are expressed relative to SGLT1 in wild-type mice on low-carbohydrate diets as means ± SD. Data were generated in triplicate, with $n = 3$ animals in each group. Statistically significant results determined by Student's unpaired two-tailed t test are indicated by *, ($P < 0.05$); **, ($P < 0.005$); or ***, ($P < 0.001$).

[0033] Figures 15A–I relate to detection and localization of T1R receptors and $G\alpha_{gust}$ along the crypt–villus axis of small intestine. Figure 15A: *In situ* hybridization with antisense riboprobes to T1R2, T1R3, and $G\alpha_{gust}$. Complementary sense probes to all targets did not hybridize to any transcripts within the tissue sections (see Figure 18). Figures 15B–C: Immunofluorescent detection of the T1R3 taste receptor subunit (red) and $G\alpha_{gust}$ (green) in serial wax sections of mouse duodenum. Figure 15D: Immunofluorescent detection of the T1R3 (red) and T1R2 (green) taste receptor subunits in a single wax section of human duodenum. Figures 15E–G: Immunofluorescent detection of the T1R2 and T1R3 taste receptor subunits (green), and $G\alpha_{gust}$ (red) in a single wax section of human duodenum. Figure 15H: Chromogenic detection of SGLT1 in wax sections of mouse small intestine. Figure 15I: Chromogenic detection of $G\alpha_{gust}$ and chromogranin in serial wax sections of mouse proximal intestine. The boxed cell expresses both $G\alpha_{gust}$ and chromogranin.

[0034] Figures 16A–B show that sucralose stimulation of endogenously expressed sweet taste receptors in GLUTag cells leads to GLP-1 and GIP release. Figure 16A: GLP-1 release into the culture medium of GLUTag cells was monitored after treatment of cells with buffer alone or buffer containing sucralose (50 mM final concentration). Addition of sucralose led to increased GLP-1 release from GLUTag cells ($P = 0.00098$). Preincubation (15 min) of the GLUTag cells with gurmarin (3 μg/ml) blocked most of the sucralose-dependent increase in released GLP-1 ($P = 0.00574$ vs. sucralose alone). $n = 4$ samples per group; the experiment was carried out in triplicate; a representative experiment is shown, and levels are expressed ± SEM. Figure 16B: GIP release into the culture medium of GLUTag cells was monitored after treatment of cells with buffer alone or buffer containing sucralose (50 mM final concentration). The addition of sucralose led to a large increase over baseline in

GIP release from GLUTag cells ($P = 0.047$). Preincubation (15 min) of the GLUTag cells with the sweet taste receptor inhibitor gurmarin (3 μg/ml) blocked most of the sucralose-dependent increase in released GIP ($P = 0.00260$ vs. sucralose alone). $n = 2–4$ samples per group; the experiment was carried out in duplicate; a representative experiment is shown, and levels are expressed ± SEM.

[0035] Figures 17A–C show that intestinal structure is not affected by diet or gene deletion. Figures 17A–B: Mean villus height (Figure 17A) and crypt depth (Figure 17B) of intestinal tissues from wild-type mice maintained on low- (L) or high- (H) carbohydrate diets or on the low-carbohydrate diet supplemented with drinking water containing 20 mM saccharin (sac), 1 mM aspartame (asp), or 10 mM acesulfame K (ace-K). There is no statistically significant change observed in the crypt depth or villus height in the intestines of mice on these various diets Figure 17C: Western blots of villin and β-actin in wild-type (WT), and $G\alpha_{gust}$ and T1R3 knockout, mice on both low- (L) and high- (H) carbohydrate diets show no significant differences in levels of these proteins between wild-type and knockout mice and no change in the level of either protein as a function of dietary carbohydrate. Villin protein gives a good indication of brush-border membrane recovery and purity, both of which are unaffected by diet or gene deletion. Note that the weaker signal for low-abundance villin vs. high-abundance β-actin is due to adjusting the exposure time so that both signals are measured within the linear range for densitometric analysis. Statistical significance determined by ANOVA, data are expressed as means ± SD.

[0036] Figure 18 relates to *in situ* hybridization controls. *In situ* hybridization histochemistry with complementary sense probes to T1R2, T1R3, $G\alpha_{gust,}$ and SGLT1 did not nonspecifically hybridize with targets within the mouse small intestine.

[0037] Figure 19 relates to immunohistochemistry controls. Omission of the primary antibodies for gustducin and T1R3 with mouse (FITC Mouse and Cy3 Mouse, respectively) and human (FITC Human and Cy3 Human, respectively) tissues showed no nonspecific immunoreactivity with targets within the mouse small intestine.

[0038] Figure 20 relates to detection of taste signaling elements in enteroendocrine cell lines. Real-time qPCR indicates expression of the T1R taste receptor subunits and $G\alpha_{gust}$ in the mouse enteroendocrine cell lines STC-1 and GLUTag. Data are expressed as means ± SD. All data were generated in triplicate, with $n = 4$ samples in each group.

[0039] Figures 21A–B show that gurmarin inhibits mouse T1R2+T1R3 sweet taste receptor activity. HEK 293 cells were transfected with plasmids encoding mouse T1R2, mouse T1R3, and Gal6/gust44 loaded with the fluorescent calcium indicator dye Fluo-4 and

then exposed to sweeteners with or without the mouse sweet taste inhibitor gurmarin. Fluorescence responses were recorded using a FlexStationII fluorimeter. Figure 21A: Fluo-4 Fluorescence, in arbitrary units (a.u.), measures calcium mobilization in response to sweet taste receptor activation by the addition of 10 mM sucralose (Suc) (open circles). Preincubation (15 min) with gurmarin (1 mg) abolishes the response (filled circles). Figure 21B: DeltaF/F measures the peak amplitude of calcium mobilization in response to addition of sweeteners: 10 mM sucralose (Suc), 10 mM saccharin (Sac) and 10 mM acesulfame-K (AceK) and 20 mM cyclamate (Cyc). The expressed mouse T1R2+T1R3 receptor does not respond to the human-specific sweetener cyclamate (see *61*). Preincubation (15 min) with gurmarin (1 mg) abolishes the responses to sucralose, saccharin, and acesulfame-K.

[0040] Figure 22 shows that gurmarin inhibits calcium mobilization in sucralose-stimulated GLUTag cells. Intracellular free Ca^{2+} in GLUTag cells transfected, to maximally enhance detection of calcium mobilization, with Gal6/gust44, YC3.60, and REEP-EI was monitored continuously by fluorescence confocal microscopy. The addition of sucralose (20 mM final concentration, arrow) led to an increase in intracellular free Ca^{2+} ((*Upper*) solid line; (*Lower*) top row of cells). Preincubation (15 min) of the transfected GLUTag cells with gurmarin (1 μg/ml) blocked the Ca^{2+} response to sucralose ((*Upper*) dashed line; (*Lower*) middle row of cells). Control GLUTag cells transfected with YC3.60 and REEP-EI, but lacking Gal6/gust44, gave no Ca^{2+} response above baseline to sucralose ((*Upper*) dotted line; (*Lower*) bottom row of cells). Confocal images shown depict intracellular calcium concentrations at time points 0, 40 s, and 100 s (sucralose was added at t = 10 s). (Scale bars, 10 μm). Traces are from representative single cells.

DETAILED DESCRIPTION OF THE INVENTION

[0041] A first aspect of the present invention relates to a method of decreasing or inhibiting carbohydrate absorption by a mammal. This method involves administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the mammal under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the mammal, thereby decreasing or inhibiting carbohydrate adsorption by the mammal.

[0042] In some embodiments, the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the mammal. Exemplary taste signaling molecules include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3

receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste inhibitors include lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

[0043] In some embodiments, the adsorption of glucose, fructose, and/or other dietary monosaccharides is decreased or inhibited.

[0044] In some embodiments, the sweet taste inhibitor is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste inhibitor.

[0045] In some embodiments, the sweet taste inhibitor is administered primarily to the stomach, small intestine, and/or large intestine of the mammal. Preferably, the sweet taste inhibitor is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor into the stomach, small intestine, and/or large intestine of the mammal. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste inhibitor to taste cells in the mouth of the mammal.

[0046] A second aspect of the present invention relates to a method of promoting insulin secretion in a mammal. This method involves administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby promoting insulin secretion by the mammal.

[0047] In some embodiments, the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal. Suitable taste signaling proteins include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sweet tasting compounds selected from the group of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0048] In some embodiments, the sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste potentiator.

[0049] In some embodiments, the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal. Preferably, the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

[0050] A third aspect of the present invention relates to a method of treating obesity in a patient. This method involves selecting a patient that is obese, and administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the patient under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the patient, thereby decreasing or inhibiting carbohydrate adsorption by the patient and treating obesity in the patient.

[0051] Preferably, the patient is selected from the group of a human, a dog, and a non-ruminant livestock.

[0052] In some embodiments, the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the patient. Exemplary taste signaling molecules include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste inhibitors include lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

[0053] In some embodiments, the adsorption of glucose, fructose, and/or other dietary monosaccharides is decreased or inhibited.

[0054] In some embodiments, the sweet taste inhibitor is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste inhibitor.

[0055] In some embodiments, the sweet taste inhibitor is administered primarily to the stomach, small intestine, and/or large intestine of the patient. Preferably, the sweet taste inhibitor is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor into the stomach, small intestine, and/or large intestine of the patient. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste inhibitor to taste cells in the mouth of the patient.

[0056] A fourth aspect of the present invention relates to a method of promoting weight loss in a mammal. This method involves administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the mammal under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the mammal, thereby decreasing or inhibiting carbohydrate adsorption by the mammal and promoting weight loss in the mammal.

[0057] Preferably, the mammal is selected from the group of a human, a dog, and a non-ruminant livestock.

[0058] In some embodiments, the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the mammal. Exemplary taste signaling molecules include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste inhibitors include lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

[0059] In some embodiments, the adsorption of glucose, fructose, and/or other dietary monosaccharides is decreased or inhibited.

[0060] In some embodiments, the sweet taste inhibitor is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste inhibitor.

[0061] In some embodiments, the sweet taste inhibitor is administered primarily to the stomach, small intestine, and/or large intestine of the mammal. Preferably, the sweet taste inhibitor is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor into the stomach, small intestine, and/or large intestine of the mammal. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste inhibitor to taste cells in the mouth of the mammal.

[0062] A fifth aspect of the present invention relates to a method of treating diabetes in a patient. This method involves selecting a patient that has diabetes, and administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the patient under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby promoting insulin secretion by the patient and treating diabetes in the patient.

[0063] Preferably, the patient is selected from the group of a human, a dog, and a non-ruminant livestock.

[0064] In some embodiments, the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the patient. Suitable taste signaling proteins include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sweet tasting compounds selected from the group of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0065] In some embodiments, the sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste potentiator.

[0066] In some embodiments, the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the patient. Preferably, the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the patient. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste potentiator to taste cells in the mouth of the patient.

[0067] A sixth aspect of the present invention relates to a method of treating obesity in a patient. This method involves selecting a patient that is obese, and administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the patient under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby inducing satiety in the patient and treating obesity in the patient.

[0068] Preferably, the patient is selected from the group of a human, a dog, and a non-ruminant livestock.

[0069] In some embodiments, the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the patient. Suitable taste signaling proteins include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sweet tasting compounds selected from the group of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0070] In some embodiments, the sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste potentiator.

[0071] In some embodiments, the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the patient. Preferably, the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the patient. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste potentiator to taste cells in the mouth of the patient.

[0072] A seventh aspect of the present invention relates to a method of promoting weight loss in a mammal. This method involves administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby inducing satiety the mammal and promoting weight loss in the mammal.

[0073] Preferably, the mammal is selected from the group of a human, a dog, and a non-ruminant livestock.

[0074] In some embodiments, the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal. Suitable taste signaling proteins include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sweet tasting compounds selected from the group of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0075] In some embodiments, the sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste potentiator.

[0076] In some embodiments, the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal. Preferably, the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

[0077] An eighth aspect of the present invention relates to a method of regulating blood sugar levels in a mammal. This method involves administering an effective amount of a sweet taste inhibitor or sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase or decrease the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby regulating insulin secretion by the mammal and regulating blood sugar levels in the mammal.

[0078] Preferably, the mammal is selected from the group of a human, a dog, and a non-ruminant livestock.

[0079] In some embodiments, the sweet taste inhibitor or sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste inhibitor or sweet taste potentiator.

[0080] In some embodiments, the sweet taste inhibitor or sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal. Preferably, the sweet taste inhibitor or sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor or sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste inhibitor or sweet taste potentiator to taste cells in the mouth of the mammal.

[0081] In some embodiments when a sweet taste potentiator is administered, the sweet taste potentiator activates one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the mammal. Exemplary taste signaling molecules include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0082] Preferably, when a sweet taste potentiator is administered, insulin secretion in the mammal is downregulated, thereby increasing blood sugar levels in the mammal.

[0083] In some embodiments when a sweet taste inhibitor is administered, the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the mammal. Exemplary taste signaling molecules include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste inhibitors include lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

[0084] Preferably, when a sweet taste inhibitor is administered, insulin secretion in the mammal is upregulated, thereby decreasing blood sugar levels in the mammal.

[0085] In some embodiments, the sweet taste inhibitor or sweet taste activator is administered under conditions effective to maintain healthy blood sugar levels in the mammal.

[0086] A ninth aspect of the present invention relates to a method of increasing or inducing carbohydrate absorption by a mammal. This method involves administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the mammal under

conditions effective to increase expression and/or induce upregulation of SGLT-1 in the mammal, thereby increasing or inducing carbohydrate adsorption by the mammal.

[0087] In some embodiments, the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal. Suitable taste signaling proteins include heterotrimeric gustducin, αα-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sweet tasting compounds selected from the group of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0088] In some embodiments, the sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste potentiator.

[0089] In some embodiments, the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal. Preferably, the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

[0090] A tenth aspect of the present invention relates to a method of treating a disorder associated with insufficient carbohydrate absorption in a patient. This method involves selecting a patient that has a disorder associated with insufficient carbohydrate absorption, and administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the patient under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the patient, thereby increasing or inducing carbohydrate adsorption by the patient and treating the disorder in the patient.

[0091] Preferably, the patient is selected from the group of a human, a dog, and a non-ruminant livestock.

[0092] In some embodiments, the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the patient. Suitable taste signaling proteins include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sweet tasting compounds selected from the group of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0093] In some embodiments, the sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste potentiator.

[0094] In some embodiments, the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the patient. Preferably, the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the patient. The pharmaceutical carrier can be formulated to substantially prevent administration of the sweet taste potentiator to taste cells in the mouth of the patient.

[0095] Disorders that can be treated according to this aspect of the present invention include anorexia, bulimia, intestinal malabsorption syndromes, and celiac disease.

[0096] An eleventh aspect of the present invention relates to a method of promoting weight gain in a mammal. This method involves administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the mammal under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the mammal, thereby increasing or inducing carbohydrate adsorption by the mammal and promoting weight gain in the mammal.

[0097] Preferably, the mammal is selected from the group of a human, a dog, and a non-ruminant livestock.

[0098] In some embodiments, the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal. Suitable taste signaling proteins include heterotrimeric gustducin, α-gustducin, $G\gamma_{13}$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A. Suitable sweet taste potentiators include sweet tasting compounds selected from the group of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

[0099] In some embodiments, the sweet taste potentiator is administered in a pharmaceutical formulation. Preferably, the pharmaceutical formulation consists essentially of the sweet taste potentiator.

[00100] In some embodiments, the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal. Preferably, the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal. The pharmaceutical

carrier can be formulated to substantially prevent administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

[0100] Suitable pharmaceutical formulations for use in the methods of the present invention include the sweet taste inhibitor/potentiator and any pharmaceutically acceptable adjuvants, carriers, excipients, and/or stabilizers, and can be in solid or liquid form, such as tablets, capsules, powders, solutions, suspensions, or emulsions. The percentage of the sweet taste inhibitor/potentiator in these compositions may, of course, be varied and may conveniently be from about 0.01% to about 99% by weight, preferably between about 0.1% to about 99% percent, more preferably from about 2% to about 60%, of the weight of the unit together with the adjuvants, carriers and/or excipients. Other suitable amounts of the sweet taste inhibitor/potentiator include about 10%, about 20%, about 30%, about 40%, about 50%, about 70%, about 80%, and about 90% of the weight of the unit. The amount of the sweet taste inhibitor/potentiator in such therapeutically useful compositions is such that a suitable dosage will be obtained.

[0101] For oral therapeutic administration, the sweet taste inhibitor/potentiator may be incorporated with excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, and the like. The tablets, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch, or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, or alginic acid; and a lubricant such as magnesium stearate. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a fatty oil.

[0102] Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac. A syrup may contain, in addition to active ingredient(s), methyl and propylparabens as preservatives, a dye, and flavoring such as cherry or orange flavor.

[0103] Solutions or suspensions of the sweet taste inhibitor/potentiator (for example, for parenteral administration) can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solutions (to the extent they do not interfere with the effect of the sweet taste inhibitor/potentiator), and glycols such as propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0104] Pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0105] Administration of the sweet taste inhibitor/potentiator can be accomplished either via systemic administration to the subject or via targeted administration to affected tissues, organs, and/or cells. Typically, the therapeutic agent (*i.e.*, a sweet taste inhibitor/potentiator) will be administered to a patient in a vehicle that delivers the therapeutic agent(s) to the target cell, tissue, or organ. Typically, the therapeutic agent will be administered as a pharmaceutical formulation, such as those described above.

[0106] Exemplary routes of administration include, without limitation, orally, topically, transdermally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intraventricularly, and intralesionally; by intratracheal inoculation, aspiration, airway instillation, aerosolization, nebulization, intranasal instillation, oral or nasogastric instillation, intraperitoneal injection, intravascular injection, intravenous injection, intra-arterial injection (such as via the pulmonary artery), intramuscular injection, and intrapleural instillation; by application to mucous membranes (such as that of the nose, throat, bronchial tubes, genitals, and/or anus); and by implantation of a sustained release vehicle.

[0107] For use as aerosols, a peptide of the present invention in solution or suspension may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The peptides of the present invention also may be administered in a non-pressurized form.

[0108] Exemplary delivery devices include, without limitation, nebulizers, atomizers, liposomes (including both active and passive drug delivery techniques) (*63–70*), transdermal patches, implants, implantable or injectable sweet taste inhibitor/potentiator depot compositions, and syringes. Other delivery systems which are known to those of skill in the

art can also be employed to achieve the desired delivery of the sweet taste inhibitor/potentiator to the desired organ, tissue, or cells.

[0109] Administration can be carried out as frequently as required and for a duration that is suitable to provide the desired effect. For example, administering can be carried out once or multiple times, and can be carried out with a single sustained-release dosage formulation or with multiple (*e.g.*, daily) doses.

[0110] The amount to be administered will, of course, vary depending upon the particular conditions and treatment regimen. The amount/dose required to obtain the desired effect may vary depending on the agent, formulation, disease or condition, the duration for which treatment is desired, and the individual to whom the agent is administered.

[0111] Effective amounts can be determined empirically by those of skill in the art. For example, determination of effective amounts for *in vivo* administration may involve *in vitro* assays in which varying doses of the sweet taste inhibitor/potentiator is administered to cells in culture and the concentration effective for achieving the desired result is determined in order to calculate the concentration required *in vivo*. Effective amounts may also be based on *in vivo* animal studies.

[0112] The present invention may be further illustrated by reference to the following examples.

EXAMPLES

[0113] The following Examples are intended to illustrate, but by no means are intended to limit, the scope of the present invention as set forth in the appended claims.

Example 1 — Gut-expressed Gustducin and Taste Receptors Regulate Secretion of Glucagon-like Peptide-1.

[0114] Glucagon-like peptide-1 (GLP-1), released from gut endocrine L cells in response to glucose, regulates appetite, insulin secretion, and gut motility. How glucose given orally, but not systemically, induces GLP-1 secretion is unknown. We show that human duodenal L cells express sweet taste receptors, the taste G-protein gustducin, and several other taste transduction elements. Mouse intestinal L cells also express α-gustducin. Ingestion of glucose by α-gustducin null mice revealed deficiencies in secretion of GLP- 1 and the regulation of plasma insulin and glucose. Isolated small bowel and intestinal villi from α-gustducin null mice showed markedly defective GLP-1 secretion in response to glucose. The human L cell line NCI-H716 expresses α-gustducin, taste receptors and several other taste

signaling elements. GLP-1 release from NCI-H716 cells was promoted by sugars and the non-caloric sweetener sucralose, and blocked by the sweet receptor antagonist lactisole or siRNA for α-gustducin. We conclude that L cells of the gut "taste" glucose through the same mechanisms used by taste cells of the tongue. Modulating GLP-1 secretion in gut "taste cells" may provide an important treatment for obesity, diabetes and abnormal gut motility.

[0115] The data presented in Examples 1–16 indicate that T1R3 and gustducin have a role in glucose-mediated incretin release and may serve as the previously unknown gut lumen glucose sensor.

[0116] We examined L cells of the gut for the presence of taste receptors and elements of taste transduction pathways. In human duodenal biopsy sections α-gustducin was detected by immunofluorescence (IF) at the apical (luminal) projections and/or cytoplasm of cells with shapes characteristic of enteroendocrine cells (Figures 1A–D; see Figure 5A for negative controls). Several other taste signaling elements were also detected by IF in human duodenum: T1R2, T1R3, $G\beta_3$, $G\gamma_{13}$, PLCβ2 and TRPM5 (Figures 1A and 1C).

[0117] The numbers of cells per thousand nucleated epithelial cells (Figure 1B, bottom row) immunopositive for α-gustducin, 44±5, was higher than that of the entire GLP-1- expressing L cell population (15±2) (Figure 1E); in addition, there were some α-gustducin containing cells that did not stain for other components characteristic of L cells. GIP was detected in 13±1 cells per 1000 in the gut biopsies (Figure 1E) including some of the α-gustducin-containing cells (Figure 1D). More than 90% of human duodenal L cells contained α-gustducin, but fewer than 50% of K cells did so. Five to ten percent of incretin cells in human duodenum contain both GIP and GLP-1 ("K/L" cells) (*22*), and those cells also contained α-gustducin (Figure 1D). However, some α-gustducin-containing cells did not appear to contain either peptide, consistent with their number per thousand nucleated epithelial cells being greater than the sum of L cells and K cells and suggesting that α-gustducin is present in at least one non-enterocyte intestinal cell subpopulation besides L and K cells.

[0118] To independently confirm the presence of α-gustducin in human enteroendocrine cells, human gut cells immunostained for GLP-1 or GIP were laser-captured and their RNA subjected to reverse transcriptase polymerase chain reaction (RT-PCR). Captured cells containing GIP or GLP-1 also expressed α-gustducin, whereas captured enterocytes were devoid of α-gustducin (Figure 1F).

[0119] Mouse duodenal GLP-1-containing cells also frequently expressed α-gustducin and α-gustducin promoter driven green fluorescent protein (GFP) (*12, 23*), along with GLP-2 and peptide YY (PYY) typically present in L cells (Figures 5B and 5C). This was also true for L cells of the mouse jejunum and ileum (Figures 5D and 5E). In contrast to our results with human duodenum, mouse K cells rarely expressed α-gustducin.

[0120] To test whether gut-expressed α-gustducin plays a role in regulating postprandial secretion of GLP-1 and other gut hormones we gavage-administered glucose by feeding needles directly into the stomachs of homozygous mice lacking the gene for α-gustducin (*α-gust*$^{-/-}$) (*6*) and of their wild-type (*α-gust*$^{+/+}$) littermates, then compared their plasma concentrations of GLP-1, GIP, and insulin. In *α-gust*$^{-/-}$ mice the αβγ-gustducin heterotrimer does not form and therefore any signals mediated by gustducin or its coupled receptors are lost (*24*). Plasma concentrations of GLP-1 in *α-gust*$^{-/-}$ mice did not rise in response to glucose (Figure 2A). Rapid insulin secretion in response to glucose displayed by *α-gust*$^{+/+}$ mice was absent from *α-gust*$^{-/-}$ mice, although they eventually achieved peak insulin concentrations higher than those of *α-gust*$^{+/+}$ mice (Figure 2A). Ten min after gavage-administration of glucose, GIP concentrations in *α-gust*$^{-/-}$ mice were greater than those in wild-type animals (Figure 2A). For the next 30 min the *α-gust*$^{-/-}$ mice maintained their GIP concentrations at a near constant level. In contrast, the wild-type mice showed a higher and more transient rise in GIP concentrations, peaking at 20 min after glucose administration. Glucose homeostasis was also altered in the *α-gust*$^{-/-}$ mice: plasma glucose concentrations after gavage-administration of glucose (Figure 2B) or after eating lab chow following an 18h fast (Figure 2C) were higher in the *α-gust*$^{-/-}$ mice than in their wild-type littermates, and were maintained at this elevated level for more than 2hrs.

[0121] Thus, insulin secretion was deficient in *α-gust*$^{-/-}$ mice in response to glucose in the gut lumen; this deficit could be due to failure of either the glucose-sensing arm or the effector arm of the insulin secretory pathway. The *α-gust*$^{-/-}$ mice did increase insulin secretion in response to the GLP-1 receptor agonist exendin-4, or to intraperitoneal administration of glucose (Figures 6A and 6B), indicating that GLP-1 receptors and glucose sensing in beta cells of the pancreas functioned normally in the absence of α-gustducin. We examined both L and K cell numbers as well as GLP-1 and GIP content in duodenum, jejunum, ileum and colon of both sets of mice, and found no significant differences in this regard between *α-gust*$^{-/-}$ and wild type mice.

[0122] Gavage-administration of glucose might stimulate α-gustducin-expressing brush cells of the stomach, depending upon what receptors are expressed in these cells.

Conceivably, these brush cells might contribute to duodenal release of GLP-1. To bypass such effects we examined GLP-1 secretion in *α-gust*$^{-/-}$ and *α-gust*$^{+/+}$ mice in which glucose was infused directly into the duodenum that had been isolated from the stomach and the rest of the small intestine, but remained in circulatory contact. In *α-gust*$^{+/+}$ mice plasma concentrations of GLP-1 peaked 10 min after duodenal infusion of 10% glucose, and then returned to baseline within 20 min (Figure 2D). In contrast, in *α-gust*$^{-/-}$ mice, plasma concentrations of GLP-1 did not increase. To further eliminate any other external effects that might affect release of GLP-1 from duodenal cells we harvested the proximal duodena of mice and then examined glucose-dependent release of GLP-1 from minced tissues and isolated villi. Culture medium with 10% glucose led to a greater increase in release of GLP-1 from duodenal tissue from *α-gust*$^{+/+}$ mice (~3.5-fold baseline) than it did in tissue from *α-gust*$^{-/-}$ mice (~2-fold baseline) (Figure 2E). Unstimulated amounts of GLP-1 secreted per gram of tissue ranged from 127pg in *α-gust*$^{+/+}$ to 172pg in *α-gust*$^{-/-}$ mice; glucose-stimulated amounts ranged from 445pg in α-gust$^{+/+}$ to 322pg in *α-gust*$^{-/-}$ mice. Similar differences were observed with release of GLP-1 from isolated villi in response to 5% glucose (see Figure 6C and Figure 8 for histology of the villi). Thus the gustducin-dependent increase in GLP-1 release in vivo in response to luminal glucose appears to be intrinsic to the gustducin-expressing duodenal cells themselves. That glucose stimulated GLP-1 release from the tissue and villi of *α-gust*$^{-/-}$ mice indicates that one or more gustducin-independent mechanisms also contribute to this effect: possibilities include effects on L cell channels (e.g. closure of K_{ATP} channels or transporter associated electrogenic currents), enhanced L cell metabolism, or sweet receptor coupling to other L cell-expressed G proteins.

[0123] By IF microscopy and RT-PCR we confirmed the expression of GLP-1 and PYY in NCI-H716 cells, a human enteroendocrine L cell line, and detected the presence in this cell line of several taste signaling elements: α-gustducin, $G\beta_3$, $G\gamma_{13}$, PLCβ2, TRPM5, T1R1, T1R2, T1R3, IP_3 type III receptor (IP_3R-3), numerous phosphodiesterases (*Pde*s) including *Pde1a,* which has been described in taste cells (*4*), the fatty acid receptor *Gpr40*, and the bile acid receptor *Tgr5* (*25*) (Figures 9A–B). Stimulation of NCI-H716 cells with sucrose, glucose, or sucralose (a high potency non-caloric sweetener) led to a concentration-dependent release of GLP-1 into the medium (Figures 3A–B). However, 2-deoxy-glucose, a non-metabolizable non-sweet sugar used as a control for osmotic effects, did not increase GLP-1 release. Lactisole, a sweetness-antagonizing inhibitor of T1R3 (*26*), inhibited the sucralose-responsive release of GLP-1 (Figure 3B). We used siRNA to decrease expression

of α-gustducin in NCI-H716 cells, and found diminished glucose-mediated GLP-1 secretion whereas basal secretion was unaffected (Figure 3C). Thus, T1R3 and gustducin are both implicated in mediating sweetener-induced GLP-1 secretion from NCI-H716 cells.

[0124] Release of GLP-1 from the mouse enteroendocrine cell line STC-1 in response to stimulation by free fatty acids (FFAs) correlates with increased phosphorylation of extracellular signal-regulated kinase (ERK) (*2*). We found that phosphorylation of ERK in NCI-H716 cells was activated by glucose and sucralose, and inhibited by the sweet receptor antagonist lactisole or the ERK kinase inhibitor PD98059 (Figure 3D). Additionally, glucose-mediated GLP-1 secretion from NCI-H716 cells was inhibited by the phospholipase C inhibitor U73122 (Figure 10).

[0125] Many taste stimuli elicit Ca^{2+} release from internal stores or promote Ca^{2+} entry or both (*27, 28*). We therefore used single-cell imaging to monitor changes in the concentrations of intracellular Ca^{2+} ($[Ca^{2+}]_i$) in NCI-H716 cells loaded with a Ca^{2+} indicator dye. Addition of glucose to the cells maintained in PBS led to an increase in $[Ca^{2+}]_i$, whereas 2-deoxy-glucose had no such effect (Figure 11A). The phospholipase C inhibitor U73122 inhibited glucose-stimulated Ca^{2+} mobilization in NCI-H716 cells (Figure 11D).

[0126] To examine functional coupling of taste receptors to G-proteins in membranes from NCI-H716 cells we used the radioactive nonhydrolyzable GTP analog [^{32}P] GTP-azidoanilide. In untreated control membranes there was constitutive GTP-azidoanilide incorporation into $G\alpha_{i1,2}$ but not into α-gustducin or $G\alpha_s$ (Figure 4). Treatment with glucose and sucrose led to a consistent increase in association of GTP-azidoanilide with α-gustducin and a decreased association with $G\alpha_{i1,2}$ (Figure 4).

Example 2 — Immunofluorescence and Confocal Microscopy.

[0127] The human paraffin-embedded duodenal sections (n = 3) were from anonymous post-mortem samples. Tissue processing and immunofluorescence were performed as described previously (*22*). For double immunofluorescent staining sections were incubated overnight at 4°C with both primary antibodies (Table 1), washed and incubated with secondary antibodies (Alexa 568 donkey anti-goat antibody for GLP-1, Alexa 488 donkey anti-rabbit antibody for others and Cy3-conjugated goat anti-rabbit antibody for GLP-1 and GLP-2 in the gustducin- GFP mice) for 1h, incubated with TOPRO®-3 (Molecular Probe) for nuclear staining, washed and mounted with fluorescence mounting medium, Vectashield® (Vector Laboratories). Images were acquired using an inverted confocal microscope LSM-410 (Carl Zeiss MicroImaging) with a 63x oil-immersed objective, and

processed using the MetaMorph® software (Universal Imaging). No fluorescent staining was observed when the primary antibody was omitted (Figures 5A–E). The NCI-H716 cells grown on coverslips were fixed in fresh 4% PFA in PBS for 10 min and permeabilized in 0.1% Triton X-100 in PBS for 10 min at room temperature. The cells were treated with blocking buffer (8% BSA in PBS) for 20 min, washed in PBS supplemented with 0.5% BSA and 0.05% Tween 20, and incubated with antibodies (Table 1) for 16h at 4°C. After washing, cells were stained with Alexa Fluor® secondary antibody as described above. Pancreata were fixed in 4% paraformaldehyde, embedded in paraffin and sections were incubated with antiguinea pig insulin antibody, stained with ABC/HRP (Dako) and islet diameter was quantified using MetaMorph 4.6.3 software (Universal Imaging): all methods are as previously described (*37*).

Table 1. Details of the Antibodies Used for Immunocytochemistry.

Antigen	No.	Raised Against	Raised in	Dilution	Source
$G\alpha_{-gustducin}$	sc-395	Human α-gustducin	Rabbit	1:200	Santa Cruz Biotechnology
$G\beta_3$	sc-381	Mouse, Rat, Human $G\beta_3$	Rabbit	1:200	Santa Cruz Biotechnology
Gγ13		Mouse Gγ13	Rabbit	1:200	(*12*)
PLC-β2	sc-9018	Mouse, Rat, Human PLC-β2	Rabbit	1:200	Santa Cruz Biotechnology
TRPM5		Mouse TRPM5	Rabbit	1:80	(*14*)
T1R2		Human T1R2	Rabbit	1:1000	Alpha Diagnostics
T1R3		Human T1R3	Rabbit	1:200	(*7*)
GLP-1(C-17)	sc-7782	Mouse, Rat, Human GLP-1	Goat	1:200	Santa Cruz Biotechnology
GLP-1	GLP15-A	Human GLP-1	Rabbit	1:500	Alpha Diagnostics
PYY		Human PYY	Rabbit	1:200	Dr. Godon Ohning (*37*)
GIP		Human GIP	Rabbit	1:500	Dr. Dariush Elahi
GLP-2	g-028-14	Human GLP-2	Rabbit	1:200	Phoenix Pharmaceuticals
GFP	A21311	GFP isolated directly from *Aequorea victoria*	Rabbit	1:1000	Molecular probes
Insulin	4011-01F	Human Insulin	Guinea pig	1:500	Linco

Example 3 — Laser capture microdissection of single cells and PCR.

[0128] Laser capture microdissection and image acquisition was performed as we described previously (*22*). We designed human α-gustducin primer, 370 bp (Table 2).

Table 2. Sequence Information for PCR Primers.

Gene	Product Size (bp)	Upstream Primer	Downstream Primer	Annealing Temp (°C)	Ref
h*α-gustducin*	386	5′-tctgggtatgtgccaaatga-3′	5′-ggcccagtgtattctggaaa-3′	50	*1
h*PLC-β2*	585	5′-gcaggacccactcatagca-3′	5′-ggagggccctcagccagg-3′	55	*38*
h*Trpm5*	300	5′-aagtcctgtgacctggaggaggtgatgg-3′	5′-cctggtagaagcctcggcaggcgtc-3′	55	*2
h*Pde1a*	646	5′-agcaagtggagagcatagtctg-3′	5′-tagggccatggtccaccgataatg-3′	55	*39*
h*Pde1c*	740	5′-tggaggccctggaagtgggatac-3′	5′-acattgtccagcgatgatggagg-3′	55	*3
h*Pde3a*	405	5′-ctggccaaccttcaggaatc-3′	5′-ccctcttggtttccctttctc-3′	55	*40*
h*Pde3b*	515	5′-caggaaggattctcagtcag-3′	5′-gtattctgggcgagaaagat-3′	55	*40*
h*Pde4a*	882	5′-tgcactagatgcagtgttcacgga-3′	5′-cagagcttcctcgactcctgaca-3′	55	*39*
h*Pde4d*	721	5′-ggcctatcacaacaatatccat-3′	5′-acgattgtcctccaaagtgtcc-3′	55	*4
h*T1r1*	271	5′-acggtcctcggttcctccacatggt-3′	5′-ggatctgtggggacaccactccagt-3′	50	*5
h*T1r2*	290	5′-tgtgttccaagaggtgccagtcag-3′	5′-gtgacgacgaccaccgtatgtac-3′	50	*6

h*T1r3*	403	5′-tgacaaccagaagcccgtgtcc-3′	5′-cgaaaccccgacaagcaagtgg-3′	50	*7
h*Gpr40*	570	5′-gcctttgcgctgggcttc-3′	5′-gcccggaggcagcccac-3′	55	*8
h*Gpr120*	590	5′-gtcaagggcgaccaccgg-3′	5′-taactgatcacaatgacc-3′	55	*9
h*Tgr5*	537	5′-ggctgcttcttcctgagc-3′	5′-ggctccagcctgtgccc-3′	55	*10
h*GAPDH*	226	5′-gaaggtgaaggtcggagtc-3′	5′-gaagatggtgatgggatttc- 3′	50	*11

*1 *Homo sapiens* α-gustducin, mRNA: GenBank Accession No. XM_294370
*2 *Homo sapiens* TRPM5, mRNA: GenBank Accession No. NM_014555
*3 *Homo sapiens* PDE1C, mRNA: GenBank Accession No. NM_005020
*4 *Homo sapiens* PDE4D, mRNA: GenBank Accession No. NM_006203
*5 *Homo sapiens* T1R1, mRNA: GenBank Accession No. NM_177541
*6 *Homo sapiens* T1R2, mRNA: GenBank Accession No. NM_152232
*7 *Homo sapiens* T1R3, mRNA: GenBank Accession No. BK000152
*8 *Homo sapiens* GPR40, mRNA: GenBank Accession No. NM_005303
*9 *Homo sapiens* GPR120, mRNA: GenBank Accession No. AB_115768
*10 *Homo sapiens* TGR5, mRNA: GenBank Accession No. AB_089307
*11 *Homo sapiens* GAPDH, mRNA: GenBank Accession No. NM_002046

Example 4 — Animals.

[0129] The design and production of α-gustducin null (*α-gust*$^{-/-}$) mice (*6*) and the mice expressing GFP from the 7.6-kb promoter region upstream from the α-gustducin gene (gustducin-GFP mice) have been described (*12, 23, 24*). The genetic background of all mice is C57BL/6. For comparisons of wild type and α-gustducin null mice *α-gust*$^{+/+}$, *α-gust*$^{+/-}$ and *α-gust*$^{-/-}$ littermate mice were generated from *α-gust*$^{+/-}$ x *α-gust*$^{+/-}$ matings.

Example 5 — Gavage-administration of Glucose Load in Wild Type and Transgenic Mice.

[0130] We carried out gastric gavages of glucose (2–5 g/Kg body weight) on fasted 8 week old, wild type and α-gustducin null mice (n = 7–12 animals per group). Gavage-administration of glucose was by syringe attached to feeding needles (20Gx1-1/2, Popper and Sons, Inc) inserted through the mouth into the stomach. Blood samples (100 μl), to which was added dipeptidyl peptidase IV (DPPIV) inhibitor (Linco Research, 10 μl per ml blood), were obtained before gavage (time 0) and 10, 20 and 40 min after gavage for determination of blood glucose by Glucometer Elite (Bayer), plasma insulin by ELISA (Crystal Chem Inc) and plasma N-terminal GLP-1 and total GIP by ELISA (Linco Research).

Example 6 — Tissue Measurements of Incretin Levels.

[0131] GLP-1 and GIP content of duodenum, jejunum, ileum, and colon were measured in extracts of wild type (n = 5) and α-gustducin null mice (n = 5), as previously described (*38, 39*).

Example 7 — Blood Glucose in Wild Type and Transgenic Mice After Alimentation.

[0132] Wild type and α-gustducin null mice (n = 6 animals per group) were fasted for 18h and then allowed access to their regular food (Picolab rodent diet 20, Purina Mills, cat #

5053) ad libitum. The blood glucose concentration was measured from tail vein by Freestyle glucometer (Therasense): measurements were taken immediately before feeding (time 0) and 15, 30, 45, 60, 90, and 120 min after feeding.

Example 8 — Secretion of GLP-1 from Intact Duodenum in Vivo.

[0133] Wild type and α-gustducin null mice (n = 12) were fasted overnight and anesthetized by intraperitioneal (IP) injection of avertin (2-2-2 Tribromoethanol, 250mg/kg). Median laparotomy was performed. The duodenum was physically isolated from the stomach and rest of intestinal tract by clamps placed at the gastroduodenal junction and within the duodenum ~5cm distal to gastroduodenal junction. Care was taken to maintain circulatory contact. Glucose (10%) was injected directly into the lumen of the duodenum. Blood samples were collected from the posterior vena cava before glucose infusion, and at 10 and 20 min after glucose infusion. DPPIV inhibitor was added to the blood samples, and plasma GLP-1 was measured.

Example 9 — Secretion of GLP-1 from Minced Duodenum and Duodenal Villi.

[0134] To acquire the duodenum for mincing median laparotomy was done as above, then an incision was made in the antimesenteric side of the duodenum near the gastroduodenal junction, and the duodenum was flushed with 20ml of HBSS (calcium and magnesium free). The proximal 5cm of duodenum were dissected out, the serosal layer stripped off, then minced pieces of tissue (~1-2 mm^2) were placed in culture medium (*40*) (DMEM with or without 10% glucose with 10% fetal bovine serum, 100U/ml penicillin, 100mg/ml streptomycin and 20µl/ml DPPIV inhibitor). Tissues were incubated in 5% CO_2 for 1h at 37°C, the media was collected and GLP-1 levels assayed to determine unstimulated (baseline control) levels as well as that released into media. At the completion of the experiment cell viability was confirmed by exclusion of trypan blue. Duodenal villi were obtained from the proximal duodenum by scraping with mild pressure from the short edge of a glass slide. The isolated tissue was allowed to settle at the bottom of a tube on ice and than was washed three times with HBSS. After the final wash purified villi were resuspended in DMEM as above for minced tissue, aliquoted and incubated as above but with 5% glucose to determine GLP-1 release.

Example 10 — Culture of NCI-H716 Cells and Secretion Studies.

[0135] Human enteroendocrine NCI-H716 cells were maintained in suspension culture as described by the American Type Culture Collection. Two days before experiments,

cells were seeded into 24-well culture plates precoated with Matrigel as described (*41*). On the day of the experiments, supernatants were replaced by PBS containing 1mM $CaCl_2$ and DPP IV inhibitor. The solutions were adjusted to pH 7.2. Cells were incubated for 1h at 37°C with or without different test agents and inhibitors. GLP-1 was measured by ELISA and normalized to protein content. Glucose, sucrose, 2-deoxy-glucose and lactisole were from Sigma and sucralose was from Toronto Chemical Co. U73122 and U73343 were from Calbiochem.

Example 11 — RNA Isolation and RT-PCR of NCI-H716 Cells.

[0136] Total RNA was extracted using Triazol (Invitrogen) according to the manufacturer's instruction. RT-PCR was performed using the Qiagen OneStep RT-PCR kit (Qiagen); see Table 2 for primer sequences and annealing temperatures. Each target gene was amplified over 35 cycles in a Peltier thermal cycler PTC-225 (MJ Research).

Example 12 — siRNA preparation and NCI-H716 Cell Transfection.

[0137] The siRNA sequence targeting human α-gustducin (GenBank Accession No. XM_294370) was from position 177–195 relative to the start codon. This α-gustducin sequence was reversed and used as unspecific siRNA control. 21-nt RNAs were purchased from Dharmacon in deprotected and desalted form, and the formation of siRNA duplex (annealing) was performed according to the manufacturer's instructions (Dharmacon). Subconfluent differentiated NCI-H716 cells were transiently transfected with siRNAs using Lipofectamine 2000™ according to the manufacturer's protocol (Life Technologies). The entire mixture was then added to the cells in one dish resulting in a final concentration of 300nM for the siRNAs. Cells were usually studied 48h after transfection.

Example 13 — Calcium Imaging.

[0138] Levels of intracellular free Ca^{2+}($[Ca^{2+}]_i$) were quantified by fluorescence imaging using the calcium indicator dye fura-2 as described previously (*9*). Briefly, cells were incubated for 30 min in the presence of 2μM acetoxymethylester form of fura-2 (Molecular Probes) and then washed twice in PBS (pH 7.4, w/o Ca^{2+}, Invitrogen) and allowed to incubate an additional 20–30 min for complete deesterification of the dye. Cells were imaged on a Zeiss Axiovert microscope (x40 oil immersion objective) coupled to an Attofluor imaging system. The average $[Ca^{2+}]_i$ in 20–30 cells per microscope field was quantified in four separate cultures per treatment condition.

Example 14 — Immunoblotting.

[0139] Western blot analysis was performed as described previously (*36*). Detection of individual proteins was performed by immunoblotting with the primary antibodies, pErk (1:1000, Cell Signaling) and total Erk (1:5000, Santa Cruz) and visualized by ECL. PD98059 was from Calbiochem.

Example 15 — Membrane Preparation, Labeling and Immunoprecipitation of Membrane-associated G Proteins.

[0140] Cell membranes were purified and labeled as described previously (*36*). [^{32}P] GTP-azidoanilide was from ALT. The clarified supernatants (160µl) were transferred to tubes containing 5–10µl polyclonal rabbit antisera raised against Gα-gustducin, $G\alpha_s$ (Santa Cruz) and $G\alpha_{i1,2}$ (Affinity BioReagents). Immunoprecipitation was performed as described previously (*42*). An aliquot (20µl) of the samples was subjected to SDS-PAGE.

[0141] Gels were transferred to membrane, and band intensity was quantified by electronic autoradiography using a Storm (Molecular Dynamics). The protein concentration was determined by the Bradford method.

Example 16 — Statistical Analysis.

[0142] GLP-1 data represents means ± s.e.m. Differences between mean values for variables within individual experiments were compared statistically by ANOVA and followed by *post hoc* testing with Scheffé's test. Comparisons were performed using Graphpad Prism (GraphPad Software, Inc. San Diego, CA). $P < 0.05$ was viewed as significant.

Discussion of Examples 1–16

[0143] The presence in enteroendocrine cells of functional gustducin-coupled sweet taste receptors responsive to glucose identifies a new signaling mechanism to regulate intestinal hormone secretion. Our data indicate that glucose leads to secretion from L cells *via* a signaling pathway quite similar to that used by taste cells in the tongue, i.e. glucose activation of gustducin-coupled sweet receptors (T1R2+T1R3).

[0144] In vivo, α-gustducin null mice are defective for secretion of GLP-1 in response to lumenal glucose. In vitro, isolated duodenum and duodenal villi from α-gustducin null mice likewise show deficient release of GLP-1 in response to glucose. In NCI-H716 enteroendocrine L cells we observed GPCR-mediated activation of α-gustducin by glucose and sucrose. Decreasing α-gustducin expression in NCI-H716 cells by siRNA resulted in

decreased glucose-mediated GLP-1 secretion. In addition, the high potency sweetener sucralose induced GLP-1 secretion from NCI-H716 cells in a concentration dependent manner, and this secretion of GLP-1 was inhibited by the sweet receptor inhibitor lactisole. These results confirm the involvement of α-gustducin coupled sweet receptors in sugar- and sweetener-stimulated secretion of GLP-1 from L cells.

[0145] The impression that there are many more L cells in the distal (ileum and colon) vs. proximal (duodenum and early jejunum) gut led to the suggestion that the early rapid rise of plasma GLP-1 following a glucose load was not from direct stimulation of the L cells. "Proximal-to-distal" models proposed that indirect neurally-mediated signaling, initiated by glucose-sensing K cells or some other non-L cell of the proximal gut, leads to release of GLP-1 from L cells of the distal gut. However, contrary to this indirect neural model there is considerable evidence that luminal glucose directly leads to GLP-1 secretion from the proximal gut. First, although in humans there are more L cells in the gut distally than proximally, there are still many L cells in the duodenum and jejunum (*22*). Indeed, in proximal gut in human the L cells are as numerous as the K cells (*22*). Second, the time course (onset, peak and duration) of glucose-elicited release of GLP-1 in humans is consistent with the time course of glucose reaching the proximal intestine (*29–32*). Third, instilling small amounts of glucose solution by catheter into the duodenum in humans leads to increased plasma GLP-1 levels (*33*). Fourth, Roux-en-Y gastric bypass surgery leads to peak plasma levels of GLP-1 that are both higher and reached earlier than before bypass because of dumping of glucose directly into the jejunum (*34*). Fifth, many animal studies show that glucose directly stimulates GLP-1 secretion even in neurally isolated ileum preparations (*35*). Finally, our studies with isolated tissues from wild-type and *α-gust*$^{-/-}$ mice strongly support a model in which direct sensing of glucose by taste signaling elements expressed in proximal L cells leads to GLP-1 release from these same proximal L cells.

[0146] GLP-1 receptor agonists and GLP-1 analogs are under intense investigation as treatments for type 2 diabetes and obesity. Exendin-4, an agonist of the GLP-1 receptor, has recently been approved for human use in type 2 diabetes because of its insulinotropic and weight-reducing properties. An alternative mode of modulating signaling through GLP-1 receptors would be to develop secretagogues to increase plasma levels of endogenous ligands, analogous to the use of sulfonylureas in increasing insulin secretion for the treatment of type 2 diabetes. It is now thought that the beneficial effects of Roux-en-Y bypass surgery on post-prandial satiety, weight loss and diabetes are due, at least in part, to the increased GLP-1 and PYY secretion seen after such surgery (*36*). If endogenous hormone levels of

these peptides could be modulated, without serious anatomic and irreversible interventions such as Roux-en-Y bypass, it would be a major break-through in diabetes and obesity treatment. Sweeteners and secretagogues that increase plasma concentrations of endogenous GLP-1 may provide such an alternative means to promote GLP-1 receptor activity as a treatment for type 2 diabetes and obesity.

Example 17 — T1R3 and Gustducin in Gut Sense Sugars to Regulate Expression of Na^+–Glucose Cotransporter 1

[0147] Dietary sugars are transported from the intestinal lumen into absorptive enterocytes by the sodium-dependent glucose transporter isoform 1 (SGLT1). Regulation of this protein is important for the provision of glucose to the body and avoidance of intestinal malabsorption. Although expression of SGLT1 is regulated by luminal monosaccharides, the luminal glucose sensor mediating this process was unknown. Examples 17–29 show that the sweet taste receptor subunit T1R3 and the taste G protein gustducin, expressed in enteroendocrine cells, underlie intestinal sugar sensing and regulation of SGLT1 mRNA and protein. Dietary sugar and artificial sweeteners increased SGLT1 mRNA and protein expression, and glucose absorptive capacity in wild-type mice, but not in knockout mice lacking T1R3 or α-gustducin. Artificial sweeteners, acting on sweet taste receptors expressed on enteroendocrine GLUTag cells, stimulated secretion of gut hormones implicated in SGLT1 upregulation. Gut-expressed taste signaling elements involved in regulating SGLT1 expression could provide novel therapeutic targets for modulating the gut's capacity to absorb sugars, with implications for the prevention and/or treatment of malabsorption syndromes and diet-related disorders including diabetes and obesity.

[0148] We reported previously that T1R taste receptors and $G\alpha_{gust}$ are expressed in the mouse small intestinal epithelium and proposed that they function as luminal sugar sensors to control SGLT1 expression in response to dietary sugar (*20*). Here, we provide three lines of evidence in favor of T1R taste receptors and $G\alpha_{gust}$ acting as luminal sugar/sweetener sensors. First, dietary sugars and artificial sweeteners increase SGLT1 mRNA and protein expression and glucose-absorptive capacity in wildtype mice, but not in T1R3 or $G\alpha_{gust}$ knockout mice. Second, T1R taste receptors and $G\alpha_{gust}$ are expressed in human and mouse enteroendocrine cells. Third, artificial sweeteners, acting on sweet taste receptors expressed on enteroendocrine GLUTag cells stimulate secretion of gut hormones implicated in SGLT1 up-regulation.

Example 18 — Regulation of Intestinal SGLT1 Expression in Wild-type and Knockout Mice in Response to Dietary Carbohydrate.

[0149] We maintained wildtype, $G\alpha_{gust}$ knockout (*6*) and T1R3 knockout (*11*) mice on diets of varied carbohydrate content and then measured intestinal expression of the SGLT1 gene. In wild-type mice kept on a high-carbohydrate (70% sucrose) diet for 2 weeks, the amount of intestinal SGLT1 mRNA was 1.6-fold higher ($P = 0.003$) than that in mice fed a low-carbohydrate (1.9% sucrose) diet (Figure 13A). However, the $G\alpha_{gust}$ and T1R3 knockout mice showed no change in SGLT1 mRNA expression on either diet; the amount of SGLT1 mRNA in both types of knockout mice was identical to that of wild-type animals on the low-carbohydrate diet. This implies that there is a constitutive pathway, independent of luminal sugar sensing by T1R3 and/or $G\alpha_{gust}$, that maintains the basal expression of SGLT1, and an inducible pathway dependent on T1R3 and $G\alpha_{gust}$.

[0150] The abundance of SGLT1 protein in brush-border membrane vesicles (BBMV) from mid small intestine was assessed by Western blotting (Figure 13B). In BBMV from wild-type mice on the high-carbohydrate diet, there was a 1.9-fold increase ($P = 0.006$) in SGLT1 protein abundance, which correlated with a 1.9-fold increase ($P = 0.0076$) in the initial rate of Na^+-dependent glucose transport into the isolated BBMV from mid small intestine (Figure 13C); a similar increase in glucose transport was observed with BBMV isolated from duodenum and ileum. $G\alpha_{gust}$ and T1R3 knockout mice had similar amounts of intestinal SGLT1 protein and Na^+-dependent glucose transport when maintained on low- or high-carbohydrate diets. Thus, whereas wild-type animals are known to respond to increased dietary carbohydrate with enhanced SGLT1 expression (*43, 48*), neither $G\alpha_{gust}$ nor T1R3 knockouts responded in this way. Morphometric analysis indicated that neither crypt depths nor villus heights differed in the intestines of mice maintained on either a low- or a high-carbohydrate diet, ruling out a trophic effect (see Figures 17A–B). Furthermore, Western blots showed no differences in the abundance of villin and β-actin structural proteins in the intestines of wild-type vs. knockout mice and that there were no changes in the abundance of these proteins as a function of dietary carbohydrate (Figure 17C). It appears, therefore, that knocking out either $G\alpha_{gust}$ or T1R3 abolishes the ability of mouse intestine to increase SGLT1 expression in response to increased dietary carbohydrate. This supports our hypothesis that $G\alpha_{gust}$ and T1R3 are part of the sugar-sensing regulatory machinery within the small intestinal mucosa that regulates SGLT1 expression in response to dietary sugars.

Example 19 — Regulation of Intestinal SGLT1 Expression in Wild-type and Knockout Mice in Response to Dietary Sweeteners.

[0151] If the T1R2+T1R3 sweet receptor is involved in intestinal sugar sensing, then artificial sweeteners that activate this receptor in taste cells of the tongue might increase intestinal expression of SGLT1. We kept wild-type and knockout mice for 2 weeks on a low-carbohydrate diet with or without supplementation with water containing the artificial sweetener sucralose. In wild-type mice consuming sucralose-sweetened water, there was a 2.1-fold increase ($P = 0.0015$) in SGLT1 mRNA, a 2.2-fold increase ($P = 0.0037$) in SGLT1 protein, and a 1.9-fold increase ($P = 0.0354$) in the initial rate of Na^+-dependent glucose transport as compared with the wild-type controls (Figures 14A–C). The magnitude of these changes was similar to those produced by the high-carbohydrate diet containing natural sugars (see Figures 13A–C). In contrast, in response to supplementation with sucralose, neither the $G\alpha_{gust}$ nor the T1R3 knockouts showed an increase in SGLT1 mRNA (Figure 14A) or protein (Figure 14B), similar to the effects of the high-carbohydrate diets on SGLT1 expression in these knockout mice (see Figures 13A–C). This suggests that T1R2+T1R3 and $G\alpha_{gust}$ are involved in sensing the presence of sugars and artificial sweeteners in the intestinal lumen.

[0152] The responsiveness of the intestinal sugar/sweetener sensor in wild-type mice to various artificial sweeteners was similar to that of the T1R2+T1R3 sweet receptor in taste cells of the tongue. In wild-type mice consuming the low-carbohydrate diet plus artificial sweetener-containing water, SGLT1 mRNA expression increased 1.8-fold in response to saccharin ($P = 0.002$) and 1.9-fold in response to acesulfame K ($P = 0.01$) but did not increase in response to aspartame (Figure 14D). The lack of response to aspartame is expected because this compound is not sweet to mice and does not stimulate expressed mouse T1R2+T1R3 (*4, 5, 9, 10*). The increased levels of SGLT1 mRNA in response to saccharin or acesulfame K were similar to those of wild-type mice on the high-carbohydrate diet, 2.2-fold ($P = 0.0001$) (see Figures 13A–C).

Example 20 — Expression of T1Rs and $G\alpha_{gust}$ in Enteroendocrine Cells.

[0153] Having implicated $G\alpha_{gust}$ and T1R3 in regulating SGLT1 expression *in vivo* in response to dietary sugars, we assessed which cells along the crypt–villus axis express the T1R taste receptors and $G\alpha_{gust}$. *In situ* hybridization showed that mRNAs for T1R2, T1R3, and $G\alpha_{gust}$ were expressed only in a subpopulation of epithelial cells along the crypt–villus axis in mouse intestine (Figure 15A). In contrast, SGLT1 mRNA was expressed in all

enterocytes along the villus (Figure 15A). There was negligible expression of T1R2, T1R3, $G\alpha_{gust}$, and SGLT1 in the crypts. None of the sense probe controls hybridized to cells of the small intestine (Figure 18). Immunodetection methods demonstrated that T1R and $G\alpha_{gust}$ proteins were expressed in a subset of cells along the crypt–villus axis of both mouse (Figures 15B–C) and human (Figures 15D–G) small intestine. SGLT1 protein, however, was present on the luminal membrane of all of the villus enterocytes in mouse intestine (Figure 15H) (*45, 49*). To investigate the colocalization of T1R taste receptor subunits and $G\alpha_{gust}$, we used serial sections of wax-embedded mouse (Figures 15B–C) and human (Figures 15D–G) proximal intestines. In mouse villi, most T1R3-expressing cells expressed $G\alpha_{gust}$ (Figure 15B) or were in close proximity to such cells (Figure 15C). In human duodenum, virtually all T1R3-expressing cells also expressed $G\alpha_{gust}$ (Figures 15F–G). Omission of the primary antibodies for gustducin or T1R3 showed no nonspecific immunoreactivity in small intestine (Figure 19). The cells that contain T1Rs and $G\alpha_{gust}$ appear to be either triangular or flask-like in shape, suggesting they are of enteroendocrine type (Figure 15G), and immunohistochemistry of serial sections of mouse intestine showed that the enteroendocrine cell marker chromogranin was indeed coexpressed with $G\alpha_{gust}$ (Figure 15I).

Example 21 — Sucralose Activates Sweet Taste Receptors on Enteroendocrine Cells to Elicit Hormone Secretion.

[0154] Having determined that T1Rs and $G\alpha_{gust}$ are expressed in enteroendocrine cells, we next examined the contribution of these cells to regulation of SGLT1 expression. Because of practical difficulties in harvesting adequate numbers of viable enteroendocrine cells of specific subtypes, we turned to intestinal endocrine cell lines. GLUTag and STC-1 cells, two mouse enteroendocrine cell lines, have been shown to secrete hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotrophic peptide (GIP), in response to glucose (*50, 51*). GLUTag cells also respond to fructose and nonmetabolizable glucose analogues (*35, 51*). STC-1 cells have been shown to express T1Rs, T2Rs, and taste G proteins (*20, 47*). We have examined GLUTag cells and found that they express T1Rs and $G\alpha_{gust}$ (Figure 20).

[0155] Using GLUTag cells, we sought to determine whether enteroendocrine cells, through their T1R2+T1R3 sweet taste receptor, respond to sweeteners with the release of hormone effectors; in particular, those hormones implicated in SGLT1 up-regulation (*52*). GLUTag cells tonically release GLP-1 and GIP into the culture medium; however, the addition of sucralose elicited increased release of GLP-1 (Figure 16A) and GIP (Figure 16B)

and an elevation of intracellular calcium (Figure 22). Gurmarin, a specific inhibitor of sweet taste in mice (*53*) that acts on the T1R2+T1R3 sweet taste receptor (Figures 21A–B), blocked the sucralose-stimulated release from GLUTag cells of GLP-1 (Figure 16A) and GIP (Figure 16B) and blocked the sucralose-dependent mobilization of calcium in GLUTag cells (Figure 22). We conclude that sweetener-dependent release of GLP-1 and GIP from GLUTag cells depends on stimulation of sweet taste receptors.

Example 22 — Mice, Diets, and Tissue Collection.

[0156] The generation of $G\alpha_{gust}^{-/-}$ and $T1R3^{-/-}$ mice has been described (*6, 11*). Six- to 8-week-old C57BL/6 $G\alpha_{gust}^{-/-}$ and $T1R3^{-/-}$ mice and their wild-type littermates of the same origin were used. Animals were placed individually in standard tub cages in a room with automatically controlled temperature, humidity, and 12-h light/12-h dark cycle. Mice were divided into three groups, with equal number of wild-type, $G\alpha_{gust}^{-/-}$, and $T1R3^{-/-}$ animals of both genders. Group one was fed a high-carbohydrate diet (High-carbohydrate Diet (70%), Testdiet #5810; Purina Mills, Richmond, IN), group two was fed a low-carbohydrate diet (Low-carbohydrate Diet (1.9%), Testdiet #590N; Purina Mills). The carbohydrate content of both diets consisted of sucrose. The two diets are equicaloric, being 3.73 and 3.86 (Kcal/g) (*42*) for the high- and low-carbohydrate diets, respectively. Group three was maintained on the low-carbohydrate diet, and 2 mM sucralose solution was supplied instead of water. A 2 mM concentration of sucralose was used because C57BL/6 wild-type mice displayed markedly increased behavioral responses to this concentration (*11*). The sucralose solution was changed every second day. All diets were provided ad libitum. An additional three groups of wild-type mice were fed the low-carbohydrate diet and given the following artificial sweetener solutions instead of water: 10 mM acesulfame K, 20 mM saccharin, and 1 mM aspartame. Saccharin and acesulfame K at these concentrations taste sweet to mice and activate expressed mouse T1R2+T1R3 (*4, 9–11*). Aspartame tastes sweet to humans but not to rodents; neither 1 mM nor 10 mM concentrations of aspartame activate the mouse sweet receptor (*5, 9, 10*). After 2 weeks, animals were killed by cervical dislocation, and intestines were immediately excised, flushed with ice-cold saline, immersed in liquid nitrogen, and stored at -80°C until use.

Example 23 — Quantitative PCR.

[0157] Total RNA isolated from intestinal tissue by using the RNeasy Mini Kit with on-column DNase 1 digestion (Qiagen, Crawley, U.K.) was used for cDNA synthesis using

Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo(dT)$_{12–18}$ primers. cDNA was cleaned up by using the Machery–Nagel Nucleospin extract kit (AB Gene, Epsom, U.K.), and 50 ng of cDNA was used per reaction. PCR primers and probes (FAM/TAMRA-labeled) for the amplification of T1R1, T1R2, T1R3, Gα_{gust}, and the Na^+/glucose cotransporter, SGLT1, (FAM/TAMRA), along with β-actin (JOE/TAMRA-labeled) were designed by using Primer Express (Applied Biosystems, Warrington, U.K.), and purchased from Eurogentec (Seraing, Belgium) (Table 3). For real-time PCRs, the enzyme was activated by heating at 95°C for 2 min. A two-step PCR procedure was used, 15 s at 95°C and 60 s at 60°C for 45 cycles in a PCR mix containing 5 ml of cDNA template, 1xJumpstart qPCR master mix (Sigma–Aldrich, Poole, U.K.), 900 nM concentrations of each primer, and 250 nM probe in a total volume of 25 ml. Where multiplex reactions were performed, the β-actin primers were primer-limiting and used at 600 nM. All reactions were performed in a Rotor-Gene 3000 (Corbett Research). Relative amounts of mRNA were normalized to β-actin mRNA within each sample.

Table 3. Primer and Probe Sequence Information for qPCR

Gene	Accession No.	Oligo	Sequence
Mouse	NM_031867	mT1R1 s	ACTCTGAGTGGCGGCTTCA
Tas1R1		mT1R1 as	GAAAGTGTTCTGTGTTGTTGAGTTCTG
		mT1R1 TM	**F**-CGGCTATTTCCTCCCTAAATGCTACGTGATT-**T**
Human	NM_177541	hT1R1 s	GGCTTCGGTGGGTATTTTCTG
TAS1R1		hT1R1 as	GGAAGTGCTCTGTGCTGTTGAG
		hT1R1 TM	**F**-ACGTGATCCTCTGCCGCCCAGA-**BHQ**
Mouse	NM_031873	mT1R2 s	GGATGGTCCCCGTGTATGTG
Tas1R2		mT1R2 as	GCAGACGGAGAAGCAAACG
		mT1R2 TM	**F**-TGTTTCTGCCGCCAGGCTTTCTTCA-**T**
Human	NM_152232	hT1R2 s	TGGCATTTATCACGGTACTCAAA
TAS1R2		hT1R2 as	AGTACGGGTGGTGGGACTGA
		hT1R2 TM	**F**-TGGTCATTGTGGTAATTGGCATGCTGG-**BHQ**
Mouse	NM_031872	mT1R3 s	AGTTCTGCTTTGGCCTGATCTG
Tas1R3		mT1R3 as	AGGGAGGTGAGCCATTGGTT
		mT1R3 TM	**F**-TTCCCAGGGCGGCCAAGCTCT-**T**
Human	BK000152	hT1R3 s	CCGCAGTGTGACTGCATCAC
TaS1R3		hT1R3 as	CTATACACAGCTGCGTAGACAGAGAA
		hT1R3 TM	**F**-AGAACGTGAGCGCAGGGCTAAATC-**BHQ**
Mouse	NM-008140	mGUST s	CCTCACCTGTTTAACAGCATATGTAATCA
Gα_{gust}		mGUST as	CCTTAGCCACTTTCTCCTGGAA
		mGUST TM	**F**-TCGCAACCACCTCCATTGTTCTGTTTCTT-**T**
Human	XM_374627	hGUST s	TTGTGCTGCACTTAGTGCCTATG
Gα_{gust}		hGUST as	GAAGGCTTTCATGCATTCTATTCA

		hGUST TM	**F**-CATGGTCCTCGTGGAAGACGAAGA-**BHQ**
Mouse	NM_019810	mSGLT1 s	CATTCCAGACGTGCACCTGTAC
SGLT1		mSGLT1 as	TCCAGGTCGATTCGCTCTTC
		mSGLT1 TM	**F**-TTGTGTTGGAGTCTACGCAACAGC-**T**
Mouse	NM_007393	mACTB s	GCTCTGGCTCCTAGCACCAT
β-actin		mACTB as	GCCACCGATCCACACAGAGT
		mACTB TM	**J**-ATCAAGATCATTGCTCCTCCTGAGCGC-**T**

Assays on Demand Taqman primer/probe mixes to human SGLT1 (FAM/MGB) and β-actin (VIC/MGB) were purchased from Applied Biosystems. **F**, FAM; **T**, TAMRA; **J**, JOE; **BHQ**, black hole quencher; s, sense; as, antisense; TM, Taqman probe.

Example 24 — Western Blotting and Glucose Transport.

[0158] BBMV were isolated from mouse small intestine by the method described (*59*) in the presence of a mixture of protease inhibitors (Roche Diagnostics, Indianapolis, IN). Western blot analysis was performed as described (*44*) with antisera to SGLT1 (*44, 45*), villin (clone 1D2C3; Abcam, Cambridge, U.K.), and β-actin (clone AC-15; Sigma–Aldrich). Immunoreactive bands were visualized by using horseradish peroxidase-conjugated secondary antibodies (DAKO, Carpenteria, CA) and enhanced chemiluminescence (Amersham Biosciences). Scanning densitometry was performed by using Phoretix 1D (Nonlinear Dynamics, Newcastle upon Tyne, U.K.). Relative levels of SGLT1 protein were normalized to β-actin levels within each sample. Glucose transport assays were performed as described (*44, 59*). D-glucose uptake was initiated by the addition of 100 μl of incubation medium containing 100 mM NaSCN (or KSCN), 100 mM mannitol, 20 mM Hepes/Tris (pH 7.4), 0.1 mM $MgSO_4$, 0.02% (wt/vol) NaN_3, and 0.1 mM D-[$U^{14}C$]glucose to BBMV (100 μg of protein). The reaction was stopped after 3 sec by addition of 1 ml of ice-cold stop buffer, containing 150 mM KSCN, 20 mM Hepes/Tris (pH 7.4), 0.1 mM $MgSO_4$, 0.02% (wt/vol) NaN_3, and 0.1 mM phlorizin (*44, 59*). A 0.9-ml portion of the reaction mixture was removed and filtered under vacuum through a 0.22-μm pore cellulose acetate/nitrate filter (GSTF02500; Millipore, Bedford, MA). The filter was washed five times with 1 ml of stop buffer, and the radioactivity retained on the filter was measured by liquid scintillation counting. All uptakes were measured in triplicate.

Example 25 — Morphometry.

[0159] Morphometry was performed on 5-μm sagittal sections prepared from paraffin-embedded intestinal samples and stained with hematoxylin and eosin. Digital images were captured with an Eclipse microscope and DXM 1200 digital camera (Nikon, East Rutherford, NJ) and analyzed by using Image J software (National Institutes of Health,

Bethesda, MD). Crypt depth was measured as the distance from crypt base to crypt–villus junction on an average of 17 well oriented crypts from 10 different sagittal sections of intestinal tissues from mice maintained on different diets and sweetener supplements. Villus height was measured as villus base to villus tip from an average of 18 well oriented villi.

Example 26 — In Situ Hybridization Histochemistry.

[0160] Tissue sections (fixed for 4 h in 4% paraformaldehyde in PBS) were paraffin wax embedded and sectioned at 5- to 7-mm thickness. After dewaxing and hydration, sections were permeabilized by a 20-min incubation in 0.2 M HCl, followed by two 3-min washes in 2xSSC, 1 h of incubation at 37°C in 50 mM Tris·HCl (pH 7.4) containing 2 μg/ml proteinase K (Sigma), and two 3-min washes in 0.2% glycine/PBS. Slides were then treated with two 10-min washes in 0.1 M triethanolamine (pH 8.0), containing 0.25% (vol/vol) acetic anhydride; postfixation in 4% paraformaldehyde/PBS; and 1-min block of endogenous alkaline phosphatase in 20% acetic acid to reduce background. Sections were prehybridized in hybridization buffer [50% deionized formamide/300 mM NaCl/20 mM Tris·HCl (pH 8.0)/5 mM EDTA/1xDenhardt's/1xRNA Protect (Sigma)/100 mg/ml dextran sulfate] for 1 h at 60°C and hybridized overnight at 25°C in hybridization buffer containing 100 μg/ml tRNA and 100 ng/ml DIG-labeled probe (Table 4). After hybridization, slides were washed for 1 h in 2xSSC, followed by 4 h at 25°C in 300 mM NaCl, 200 mM Tris·HCl (pH 8.0), 10 mM EDTA, 50% formamide, 1xDenhardt's and then 30 min in 2xSSC and 30 min in 0.2xSSC. Bound DIG-labeled probes were detected by 5-min equilibration in digoxigenin–alkaline phosphatase (DIG-AP) buffer [100 mM Tris·HCl (pH 7.5)/150 mM NaCl], followed by 30-min block in DIG-AP buffer containing 1% (wt/vol) DIG blocking reagent (Roche), overnight incubation in DIG-AP buffer containing 1% (wt/vol) DIG blocking reagent and anti-DIG AP-conjugated antibody diluted 1:1,000, 5-min wash in DIG-AP buffer, 5-min equilibration in NBT/BCIP buffer [100 mM Tris·HCl (pH 9.5)/100 mM NaCl/50 mM $MgCl_2$], 1-h incubation in the dark in NBT/BCIP buffer containing 10% polyvinyl alcohol and NBT/ BCIP mixture (Roche) diluted 1:50, and 5-min wash in 10 mM Tris·HCl (pH 8.0), 1 mM EDTA. Slides were then rinsed in tap water, counterstained in chloroform-extracted 1% methyl green for 5 min, and mounted in Vectamount (Vector Laboratories, Burlingame, CA).

Table 4. Primer Sequence Information for the Generation of Riboprobe Templates for Use in *in Situ* Hybridization.

Gene	Product size, bp	Primer Sequence
mT1R1	384	Sense AAGTGTGGGTCGCCTCAGAAG
		Antisense CTGAAGAAGCTGCCAGGGGTAG
mT1R2	254	Sense TTGCCTTCCAGGAGGTTCTGCC
		Antisense TGTGGCGCAGCTCTGTGAGGTT
mT1R3	263	Sense GCAAGTTCTTCAGCTTCTTCCT
		Antisense GGCCCTCATGTGCGATGCAG
mGα_{gust}	253	Sense AGGAGTCACCTGCATTATATT
		Anitsense AACTGGTTCTTGATGTAGTT
mSGLT1	243	Sense GGTGGCTTTGAATGGAACGC
		Antisense CCAAGTATATATCCAGGCCCAAG

Example 27 — Immunohistochemistry.

[0161] Sections of paraffin-embedded tissue (6-μm thick) were dewaxed in xylene and rehydrated; antigen retrieval was performed by incubation of tissue sections with 0.05% pronase (Roche) solution for 15 min at 37°C in a humidified chamber. Sections were washed in PBS and blocked for 30 min in 3% BSA, 0.3% Triton X-100, 2% goat serum, and 0.1% Na azide. Serial sections were incubated with primary antibody against Gα_{gust} (Santa Cruz Biotechnology, Santa Cruz, CA), 1:1,000; T1R2 (Santa Cruz Biotechnology), 1:500; or T1R3 (*11*) 1:1,000, washed in PBS three times for 5 min, and incubated with Cy3-conjugated goat anti-rabbit secondary antibody, (Jackson ImmunoResearch, West Grove, PA), and FITC-conjugated goat anti-rabbit secondary antibody (Molecular Probes, Eugene, OR). Images from serial sections were merged by using Volocity imaging software for confocal microscope (Improvision). For double-staining with Gα_{gust}/T1R2/T1R3, the Zenon Alexa Fluor Rabbit IgG Labeling kit (Molecular Probes) was used: in brief, antibodies were preincubated with Alexa Fluor 488-labeled fab fragments (green) or Alexa Fluor 594-labeled fab fragments (red) for 10 min after 10-min incubation with blocking reagent (nonspecific IgG) to absorb excess labeling reagent. Directly labeled rabbit anti- Gα_{gust}/T1R2/T1R3 antibodies were applied for 1 h. The sections were washed in PBS three times for 5 min after final incubation and mounted. Negative controls omitting the primary antibodies for gustducin and T1R3 were done with mouse and human tissues and were uniformly negative.

[0162] For chromogenic detection, after postfixation, antigen retrieval was performed by autoclaving in 10 mM Tris buffer (pH 10) for 22 min, and slides were pretreated for peroxidase blocking by incubation in 3% H_2O_2/PBS for 15 min. Sections were blocked for 1 h at room temperature in 5% BSA/PBS. The slides were then incubated at room temperature overnight with primary antibodies to SGLT1 or chromogranin A+B (Abcam), diluted 1:100 in 1% BSA/TBS, washed, and incubated with horseradish peroxidase-conjugated swine anti-

rabbit secondary antibody (DAKO) diluted 1:200 in 1% BSA/TBS for 2 h at room temperature. Another three 5-min washes were performed, and then the slides were developed in 0.05% DAB/0.03% H_2O_2/50 mM Tris·HCl (pH 7.6) for 10 min at room temperature in the dark. The slides were then counterstained in 1% chloroformextracted methyl green and mounted by using DPX (Raymond Lamb, Eastbourne, East Sussex, U.K.).

Example 28 — GLUTag Cells, GLP-1, GIP, and Ca^{2+} Imaging Assays.

[0163] For GLP-1 and GIP release assays, GLUTag cells (*60*) were incubated in PBS with or without gurmarin (3 mg/ml) for 15 min, followed by incubation (1 h) with sucralose (50 mM final concentration). The concentrations of GLP-1 and GIP (total) (active form) in the culture medium were determined by using GLP-1 and GIP ELISA kits (LincoResearch). For the Ca^{2+} imaging experiments GLUTag cells were transiently transfected by using Lipofectamine 2000 (Invitrogen) with the following plasmids: Ga16/gust44 [a plasmid encoding a Ga16- $G\alpha_{gust}$ chimeric G protein α-subunit that couples to sweet taste receptors (*61*)], YC3.60 (a ratiometric fluorescent indicator of free Ca^{2+}) (*62*), and REEP-EI (a plasmid encoding a receptor-enhancing protein that promotes function of olfactory (*62*) and gustatory receptors. Calcium responses of GLUTag cells to 20 mM sucralose were monitored 60 h after transfection with a Zeiss LSM510 laser-scanning confocal microscope (Zeiss, Oberkochen, Germany). Time-lapse fluorescence imaging of YC3.60 was achieved by appropriate filter sets using a multitracking mode. Inhibition of T1R2+T1R3 sweet taste receptors endogenous to GLUTag cells was obtained by preincubation of cells (15 min) with the sweet taste inhibitor gurmarin (1 μg/ml) (*57*), which acts on the mouse T1R2+T1R3 sweet taste receptor (Figures 21A–B).

Example 29 — Statistical Analysis.

[0164] Results are expressed as means ± SD or means ± SEM, as indicated. Data were analyzed, as appropriate for the data set, by ANOVA with Dunnett's post test, or by unpaired two-tailed Student's *t* test (GraphPad Prism). $P < 0.05$ was considered significant.

[0165] We thank Drs. A. Ellis (Consultant Gastroenterologist, Royal Liverpool and Broadgreen Hospitals, Liverpool, U.K.) for the provision of human intestinal biopsies, A. Miyawaki for the YC3.60 plasmid, D. J. Drucker for GLUTag cells, and Tate and Lyle for the provision of sucralose.

Discussion of Examples 17–29

[0166] The Na^+/glucose cotransporter SGLT1 is the major route for the transport of dietary sugars from the lumen of the intestine into enterocytes. Regulation of this protein is essential for the provision of glucose to the body and, thus, is important for maintenance of glucose homeostasis. Examples 17–29 show that T1R3 and $G\alpha_{gust}$ are expressed in enteroendocrine cells and are required for the enhanced expression of SGLT1 shown by enterocytes *in vivo* in response to luminal sugars or sweeteners. The finding that $G\alpha_{gust}$ and T1Rs are expressed in enteroendocrine cells, whereas SGLT1 is expressed in enterocytes, implies that a chemical signaling event takes place between the chemosensory enteroendocrine cells and absorptive enterocytes. Enteroendocrine cells, in response to luminal nutrients, secrete endocrine hormones including cholecystokinin (CCK), peptide tyrosine tyrosine (PYY), neurotensin, GLP-1, GLP-2, and GIP (*35, 51, 54–56*). GLP-1 and GIP, known as incretins, are secreted in response to dietary sugars, and influence glucose transport, metabolism, and homeostasis (*57*). Infusion of the intestinal lumen with glucose, galactose, fructose, 3-*O*-methyl-D-glucose and a-methyl-D-glucose causes GIP and GLP-1 secretion in rats, pigs, and humans (*54–56*). These sugars also increase SGLT1 expression when infused into the intestinal lumen (*43, 45, 58*). The addition of GIP to the serosal side of intestinal tissue *in vitro* results in increased expression of SGLT1 (*52*). Collectively, these reports imply a strong correlation among intestinal glucose sensing, subsequent endocrine secretion, and modulation of SGLT1 expression. Our observation that endogenous sweet taste receptors in GLUTag cells mediate sweetener-dependent release of GLP-1 and GIP, coupled with the finding that the presence of GIP on the serosal aspect of intestinal tissue results in increased expression of SGLT1 (*52*), suggests that one or both of these hormones could serve *in vivo* as the signal between the sensory enteroendocrine cells and absorptive enterocytes. We propose that the T1R2+T1R3 sweet taste receptor, expressed on the luminal membrane of villus enteroendocrine cells, senses the luminal glucose concentration. Luminal glucose above a threshold level may activate in enteroendocrine sensor cells a signaling pathway involving T1R2+T1R3, $G\alpha_{gust}$, and other taste signaling elements, resulting in the secretion of GLP-1, GIP, and/or other endocrine products. We infer that one or more of these hormones bind to receptors on their target cells and through a paracrine mechanism enhance SGLT1 expression. We speculate that glucose transport across the basolateral membrane facilitated by GLUT 2 may also be subject to this form of regulation.

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WHAT IS CLAIMED:

1. A method of decreasing or inhibiting carbohydrate absorption by a mammal, said method comprising:

administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the mammal under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the mammal, thereby decreasing or inhibiting carbohydrate adsorption by the mammal.

2. The method according to claim 1, wherein the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the mammal.

3. The method according to claim 2, wherein the one or more taste signaling molecules is selected from the group of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

4. The method according to claim 3, wherein the sweet taste inhibitor is selected from the group consisting of lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

5. The method according to claim 1, wherein adsorption of glucose, fructose, and/or other dietary monosaccharides is decreased or inhibited.

6. The method according to claim 1, wherein the sweet taste inhibitor is administered in a pharmaceutical formulation.

7. The method according to claim 1, wherein the sweet taste inhibitor is administered primarily to the stomach, small intestine, and/or large intestine of the mammal.

8. The method according to claim 7, wherein the sweet taste inhibitor is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor into the stomach, small intestine, and/or large intestine of the mammal.

9. The method according to claim 8, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste inhibitor to taste cells in the mouth of the mammal.

10. A method of promoting insulin secretion in a mammal, said method comprising:

administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby promoting insulin secretion by the mammal.

11. The method according to claim 10, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal.

12. The method according to claim 11, wherein the one or more taste signaling proteins is selected from the group consisting of heterotrimeric gustducin, α-gustducin, $G\gamma 13$, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

13. The method according to claim 12, wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

14. The method according to claim 10, wherein the sweet taste potentiator is administered in a pharmaceutical formulation.

15. The method according to claim 14, wherein the pharmaceutical formulation consists essentially of the sweet taste potentiator.

16. The method according to claim 10, wherein the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal.

17. The method according to claim 16, wherein the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal.

18. The method according to claim 17, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

19. A method of treating obesity in a patient, said method comprising:

selecting a patient that is obese, and

administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the patient under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the patient, thereby decreasing or inhibiting carbohydrate adsorption by the patient and treating obesity in the patient.

20. The method according to claim 19, wherein the patient is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

21. The method according to claim 19, wherein the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the patient.

22. The method according to claim 21, wherein the taste signaling molecule is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

23. The method according to claim 22, wherein the sweet taste inhibitor is selected from the group consisting of lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

24. The method according to claim 19, wherein adsorption of glucose, fructose, and/or other dietary monosaccharides is decreased or inhibited.

25. The method according to claim 19, wherein the sweet taste inhibitor is administered in a pharmaceutical formulation.

26. The method according to claim 19, wherein the sweet taste inhibitor is administered primarily to the stomach, small intestine, and/or large intestine of the patient.

27. The method according to claim 26, wherein the sweet taste inhibitor is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor into the stomach, small intestine, and/or large intestine of the patient.

28. The method according to claim 26, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste inhibitor to taste cells in the mouth of the patient.

29. A method of promoting weight loss in a mammal, said method comprising:

administering an effective amount of a sweet taste inhibitor to the gastrointestinal tract of the mammal under conditions effective to decrease expression and/or inhibit upregulation of SGLT-1 in the mammal, thereby decreasing or inhibiting carbohydrate adsorption by the mammal and promoting weight loss in the mammal.

30. The method according to claim 29, wherein the mammal is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

31. The method according to claim 29, wherein the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the mammal.

32. The method according to claim 31, wherein the taste signaling molecule is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

33. The method according to claim 32, wherein the sweet taste inhibitor is selected from the group consisting of lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

34. The method according to claim 29, wherein adsorption of glucose, fructose, and/or other dietary monosaccharides is decreased or inhibited.

35. The method according to claim 29, wherein the sweet taste inhibitor is administered in a pharmaceutical formulation.

36. The method according to claim 29, wherein the sweet taste inhibitor is administered primarily to the stomach, small intestine, and/or large intestine of the mammal.

37. The method according to claim 36, wherein the sweet taste inhibitor is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor into the stomach, small intestine, and/or large intestine of the mammal.

38. The method according to claim 36, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste inhibitor to taste cells in the mouth of the mammal.

39. A method of treating diabetes in a patient, said method comprising:

selecting a patient that has diabetes, and

administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the patient under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby promoting insulin secretion by the patient and treating diabetes in the patient.

40. The method according to claim 39, wherein the patient is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

41. The method according to claim 39, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the patient.

42. The method according to claim 41, wherein the one or more taste signaling proteins is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

43. The method according to claim 42, wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

44. The method according to claim 39, wherein the sweet taste potentiator is administered in a pharmaceutical formulation.

45. The method according to claim 44, wherein the pharmaceutical formulation consists essentially of the sweet taste potentiator.

46. The method according to claim 39, wherein the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the patient.

47. The method according to claim 46, wherein the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the patient.

48. The method according to claim 47, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste potentiator to taste cells in the mouth of the patient.

49. A method of treating obesity in a patient, said method comprising:

selecting a patient that is obese, and

administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the patient under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby inducing satiety in the patient and treating obesity in the patient.

50. The method according to claim 49, wherein the patient is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

51. The method according to claim 49, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the patient.

52. The method according to claim 51, wherein the one or more taste signaling proteins is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

53. The method according to claim 52, wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

54. The method according to claim 49, wherein the sweet taste potentiator is administered in a pharmaceutical formulation.

55. The method according to claim 54, wherein the pharmaceutical formulation consists essentially of the sweet taste potentiator.

56. The method according to claim 49, wherein the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the patient.

57. The method according to claim 56, wherein the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the patient.

58. The method according to claim 57, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste potentiator to taste cells in the mouth of the patient.

59. A method of promoting weight loss in a mammal, said method comprising:

administering an effective amount of a sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby inducing satiety in the mammal and promoting weight loss in the mammal.

60. The method according to claim 59, wherein the mammal is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

61. The method according to claim 59, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal.

62. The method according to claim 61, wherein the one or more taste signaling proteins is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

63. The method according to claim 62, wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

64. The method according to claim 59, wherein the sweet taste potentiator is administered in a pharmaceutical formulation.

65. The method according to claim 64, wherein the pharmaceutical formulation consists essentially of the sweet taste potentiator.

66. The method according to claim 59, wherein the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal.

67. The method according to claim 66, wherein the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal.

68. The method according to claim 67, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

69. A method of regulating blood sugar levels in a mammal, said method comprising:

administering an effective amount of a sweet taste inhibitor or sweet taste potentiator to a gastrointestinal endocrine cell in the gastrointestinal tract of the mammal under conditions effective to increase or decrease the synthesis and/or secretion of GLP-1 by the gastrointestinal endocrine cell, thereby regulating insulin secretion by the mammal and regulating blood sugar levels in the mammal.

70. The method according to claim 69, wherein the mammal is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

71. The method according to claim 69, wherein the sweet taste inhibitor or sweet taste potentiator is administered in a pharmaceutical formulation.

72. The method according to claim 71, wherein the pharmaceutical formulation consists essentially of the sweet taste inhibitor or sweet taste potentiator.

73. The method according to claim 69, wherein the sweet taste inhibitor or sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal.

74. The method according to claim 73, wherein the sweet taste inhibitor or sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste inhibitor or sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal.

75. The method according to claim 74, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste inhibitor or sweet taste potentiator to taste cells in the mouth of the mammal.

76. The method according to claim 69, wherein a sweet taste potentiator is administered.

77. The method according to claim 76, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal.

78. The method according to claim 77, wherein the one or more taste signaling proteins is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

79. The method according to claim 78, wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

80. The method according to claim 76, wherein insulin secretion in the mammal is downregulated, thereby increasing blood sugar levels in the mammal.

81. The method according to claim 69, wherein a sweet taste inhibitor is administered.

82. The method according to claim 81, wherein the sweet taste inhibitor inhibits activation of one or more taste signaling molecules contained within a gastrointestinal endocrine cell in the mammal.

83. The method according to claim 82, wherein the one or more taste signaling proteins is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

84. The method according to claim 83, wherein the sweet taste inhibitor is selected from the group consisting of lactisole, gurmarin, gymnemic acid, substituted sulfamates, and substituted cyclamate sulfamates.

85. The method according to claim 81, wherein insulin secretion in the mammal is upregulated, thereby decreasing blood sugar levels in the mammal.

86. The method according to claim 69, wherein in the sweet taste inhibitor or sweet taste activator is administered under conditions effective to maintain healthy blood sugar levels in the mammal.

87. A method of increasing or inducing carbohydrate absorption by a mammal, said method comprising:

administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the mammal under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the mammal, thereby increasing or inducing carbohydrate adsorption by the mammal.

88. The method according to claim 87, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal.

89. The method according to claim 88, wherein the one or more taste signaling molecules is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

90. The method according to claim 89, wherein the wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

91. The method according to claim 87, wherein the sweet taste potentiator is administered in a pharmaceutical formulation.

92. The method according to claim 91, wherein the pharmaceutical formulation consists essentially of the sweet taste potentiator.

93. The method according to claim 87, wherein the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal.

94. The method according to claim 93, wherein the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal.

95. The method according to claim 94, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

96. A method of treating a disorder associated with insufficient carbohydrate absorption in a patient, said method comprising:

selecting a patient that has a disorder associated with insufficient carbohydrate absorption, and

administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the patient under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the patient, thereby increasing or inducing carbohydrate adsorption by the patient and treating the disorder in the patient.

97. The method according to claim 96, wherein the patient is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

98. The method according to claim 96, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the patient.

99. The method according to claim 98, wherein the one or more taste signaling molecules is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

100. The method according to claim 99, wherein the wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

101. The method according to claim 96, wherein the sweet taste potentiator is administered in a pharmaceutical formulation.

102. The method according to claim 101, wherein the pharmaceutical formulation consists essentially of the sweet taste potentiator.

103. The method according to claim 96, wherein the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the patient.

104. The method according to claim 103, wherein the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the patient.

105. The method according to claim 104, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste potentiator to taste cells in the mouth of the patient.

106. The method according to claim 96, wherein the disorder is selected from the group consisting of anorexia, bulimia, intestinal malabsorption syndromes, and celiac disease.

107. A method of promoting weight gain in a mammal, said method comprising:

administering an effective amount of a sweet taste potentiator to the gastrointestinal tract of the mammal under conditions effective to increase expression and/or induce upregulation of SGLT-1 in the mammal, thereby increasing or inducing carbohydrate adsorption by the mammal and promoting weight gain in the mammal.

108. The method according to claim 107, wherein the mammal is selected from the group consisting of a human, a dog, and a non-ruminant livestock.

109. The method according to claim 107, wherein the sweet taste potentiator activates one or more taste signaling proteins in the gastrointestinal endocrine cell of the mammal.

110. The method according to claim 109, wherein the one or more taste signaling molecules is selected from the group consisting of heterotrimeric gustducin, α-gustducin, Gγ13, $G\beta_3$, $G\beta_1$, T1R2, T1R3, T1R2+T1R3 dimeric receptor, Trpm5, Trpm4, PLCβ2, IP_3 receptor type 3, Type I PDEs, and PDE-1A.

111. The method according to claim 110, wherein the wherein the sweet taste potentiator is a sweet tasting compound selected from the group consisting of sugars, peptides, proteins, natural non-sugar sweeteners, glycyrrhizic acid, stevioside, and artificial non-sugar sweeteners.

112. The method according to claim 107, wherein the sweet taste potentiator is administered in a pharmaceutical formulation.

113. The method according to claim 112, wherein the pharmaceutical formulation consists essentially of the sweet taste potentiator.

114. The method according to claim 107, wherein the sweet taste potentiator is administered primarily to the stomach, small intestine, and/or large intestine of the mammal.

115. The method according to claim 114, wherein the sweet taste potentiator is encapsulated in a pharmaceutical carrier that releases the sweet taste potentiator into the stomach, small intestine, and/or large intestine of the mammal.

116. The method according to claim 115, wherein the pharmaceutical carrier substantially prevents administration of the sweet taste potentiator to taste cells in the mouth of the mammal.

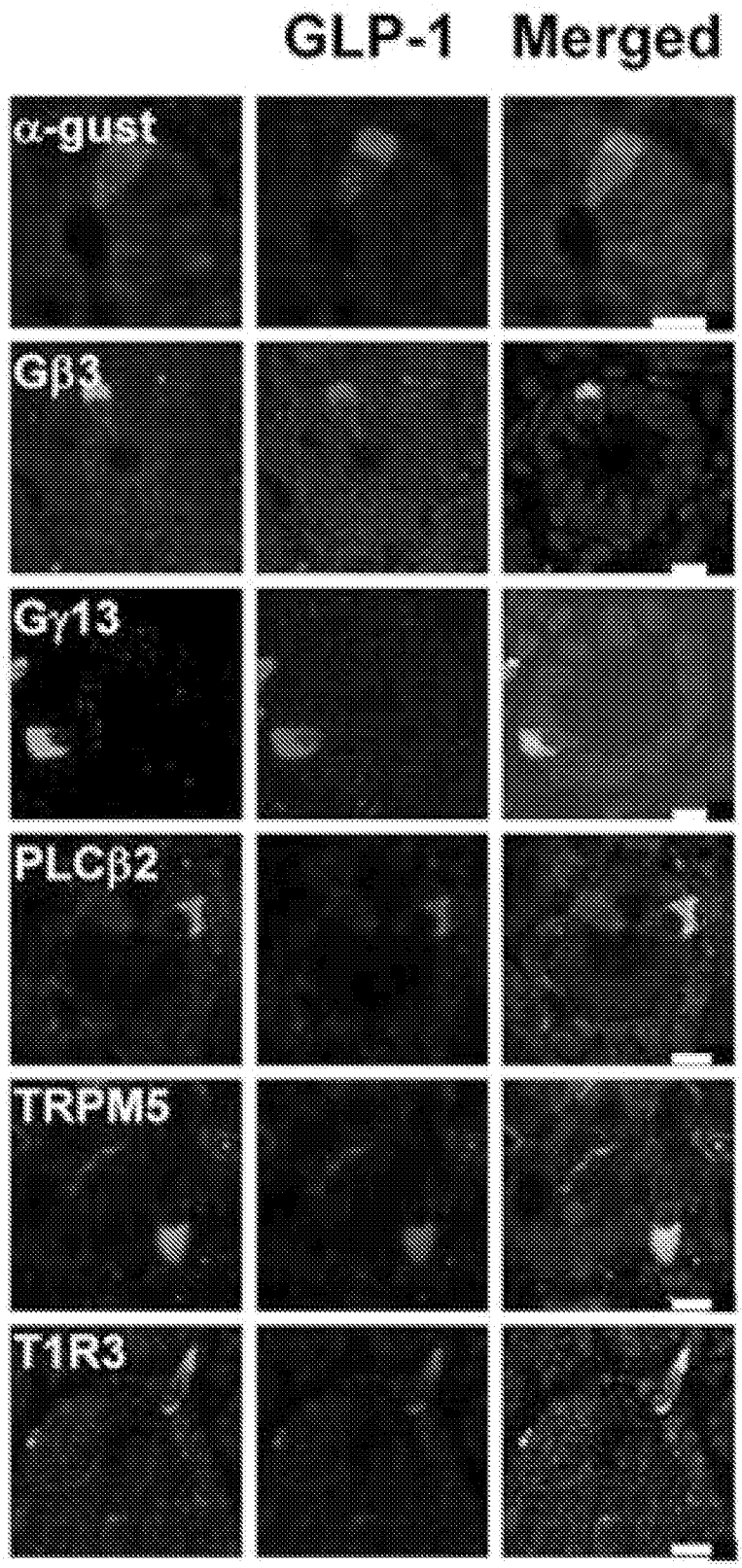

Figure 1A

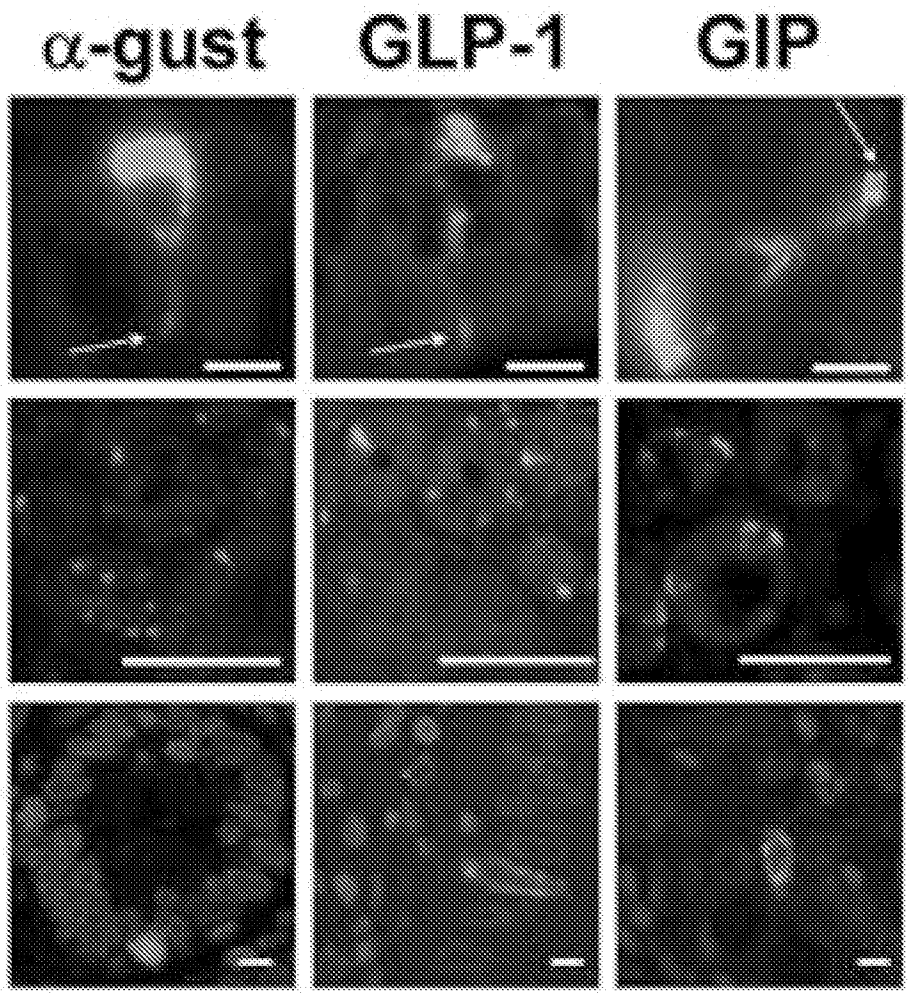

Figure 1B

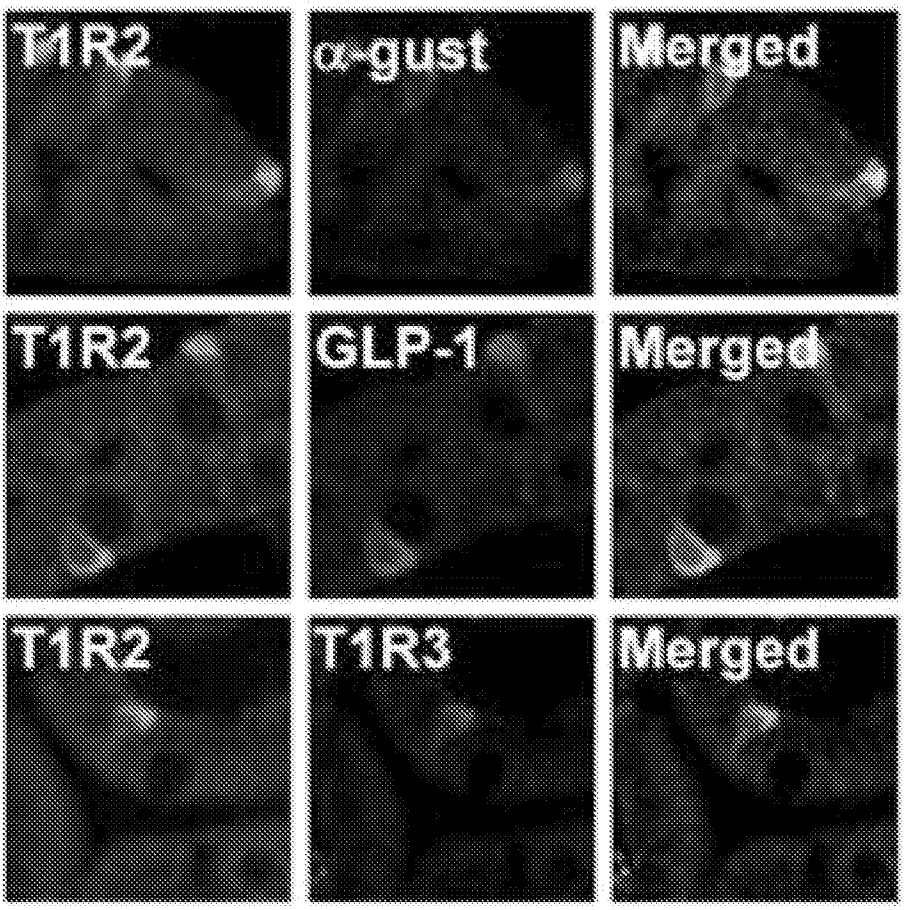

Figure 1C

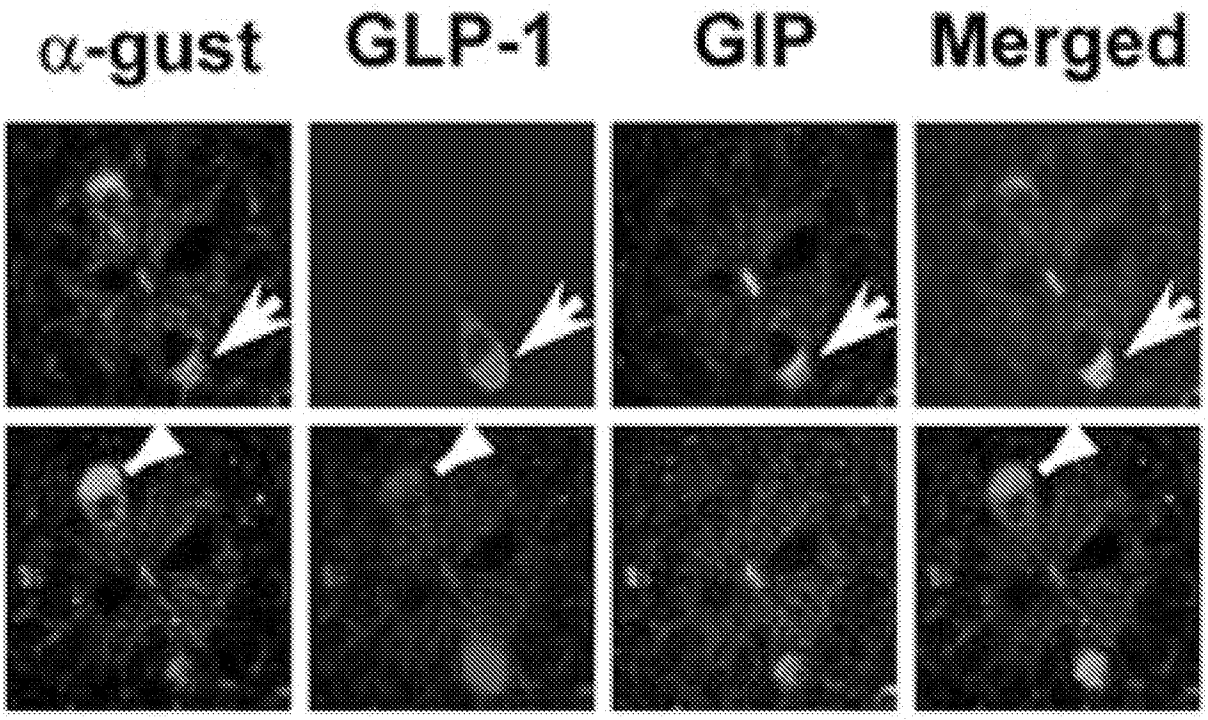

Figure 1D

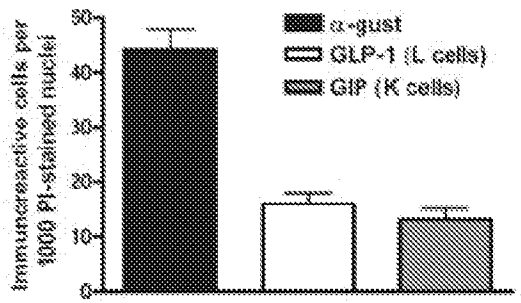

Figure 1E

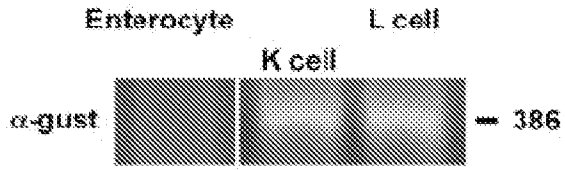

Figure 1F

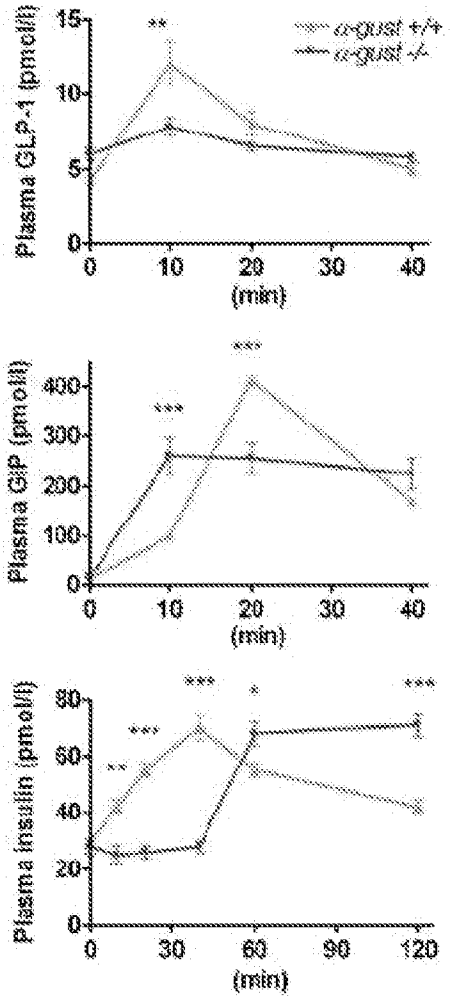

Figure 2A

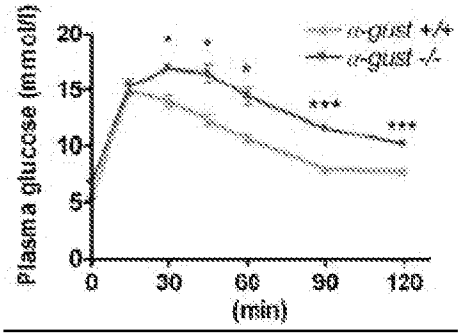

Figure 2B

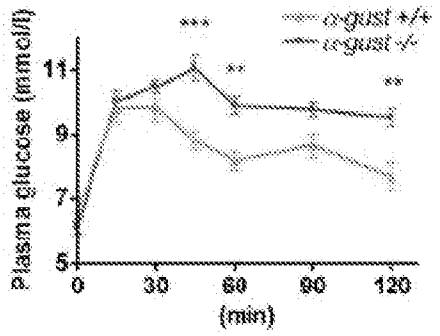

Figure 2C

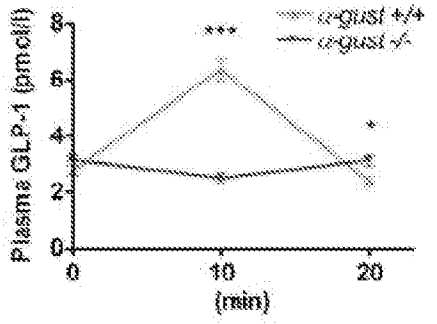

Figure 2D

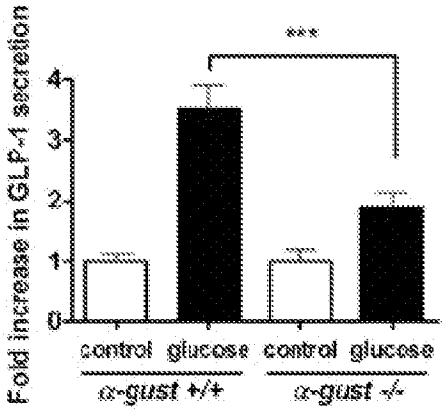

Figure 2E

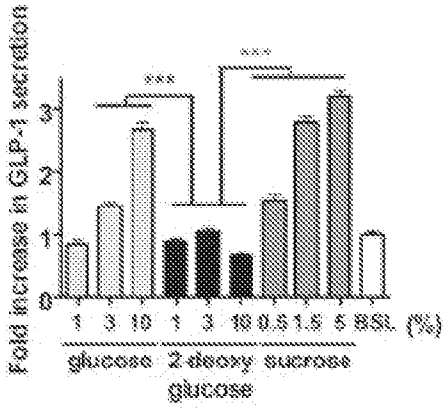

Figure 3A

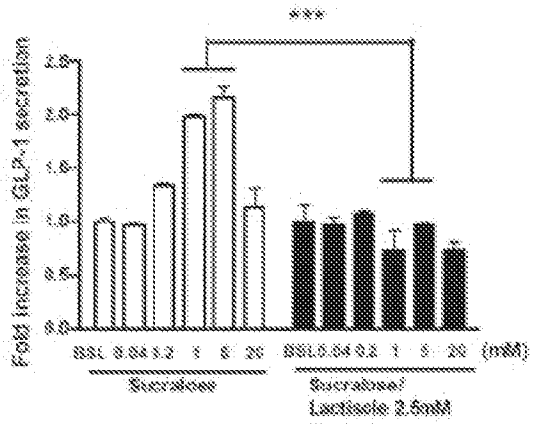

Figure 3B

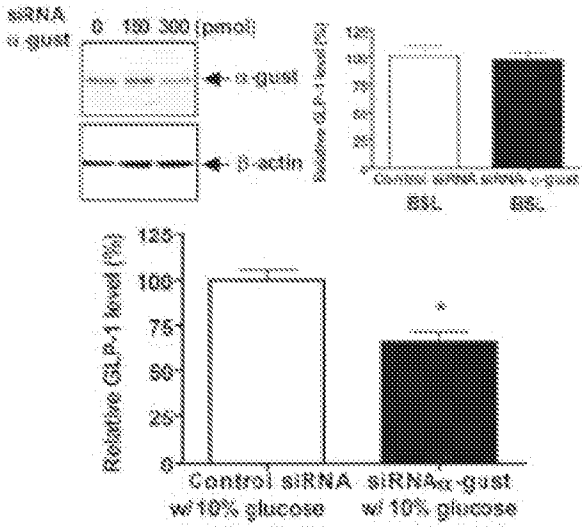

Figure 3C

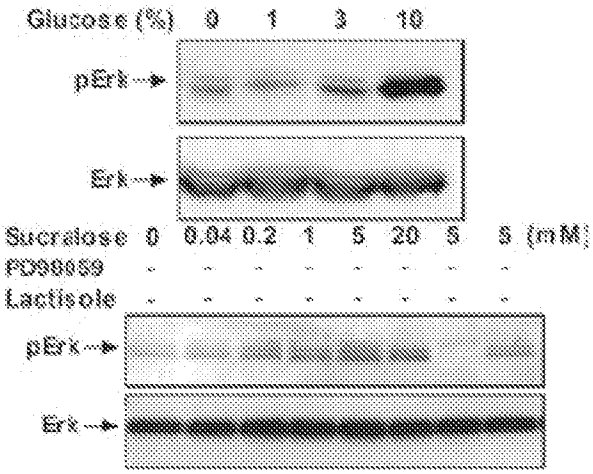

Figure 3D

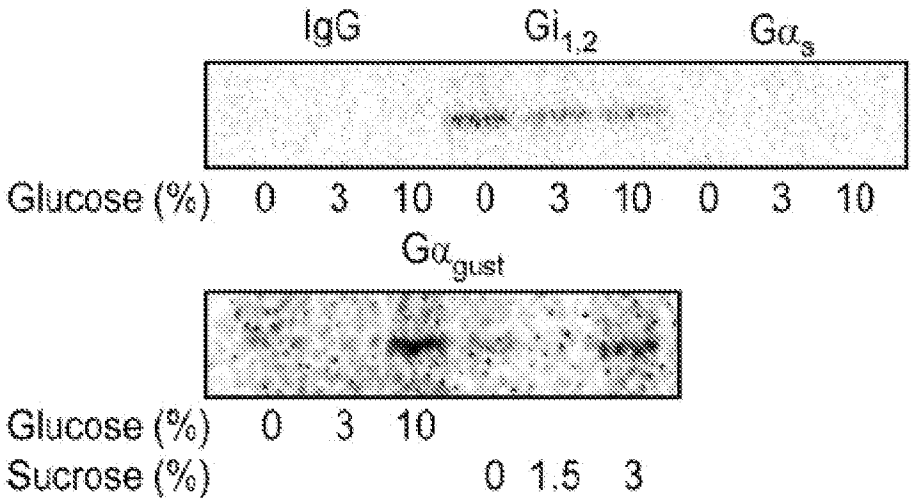

Figure 4

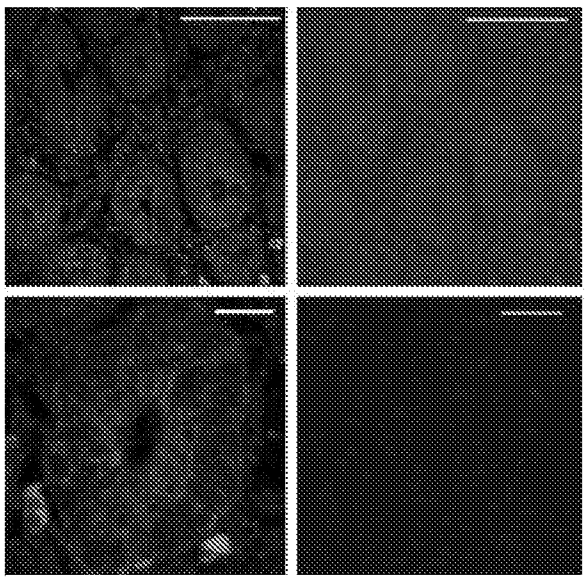

Figure 5A

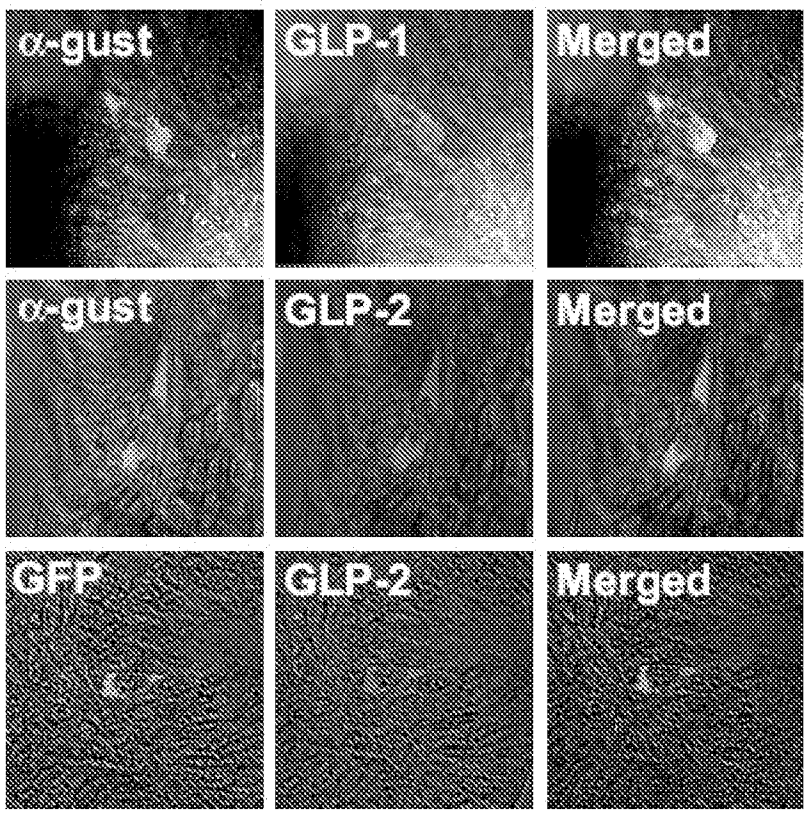

Figure 5B

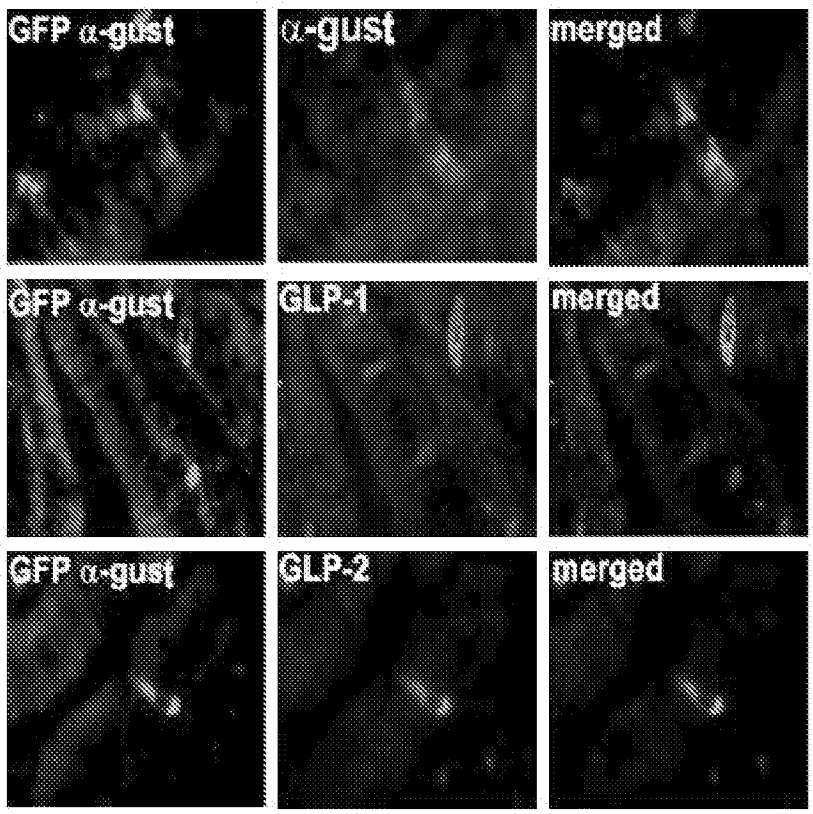
GFP α-gust
α-gust
merged
GFP α-gust
GLP-1
merged
GFP α-gust
GLP-2
merged

Figure 5C

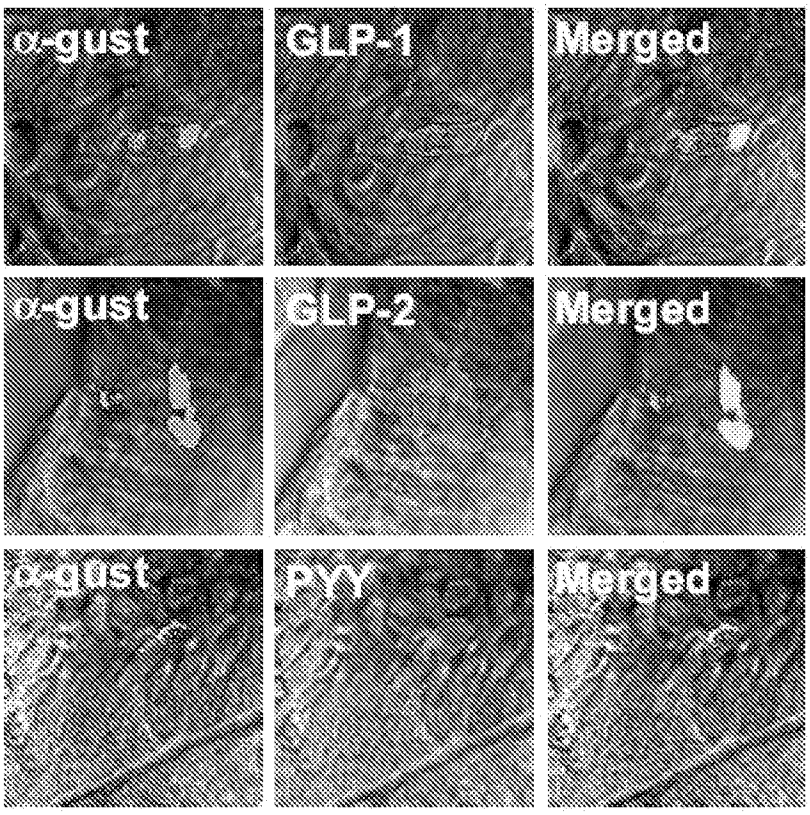

Figure 5D

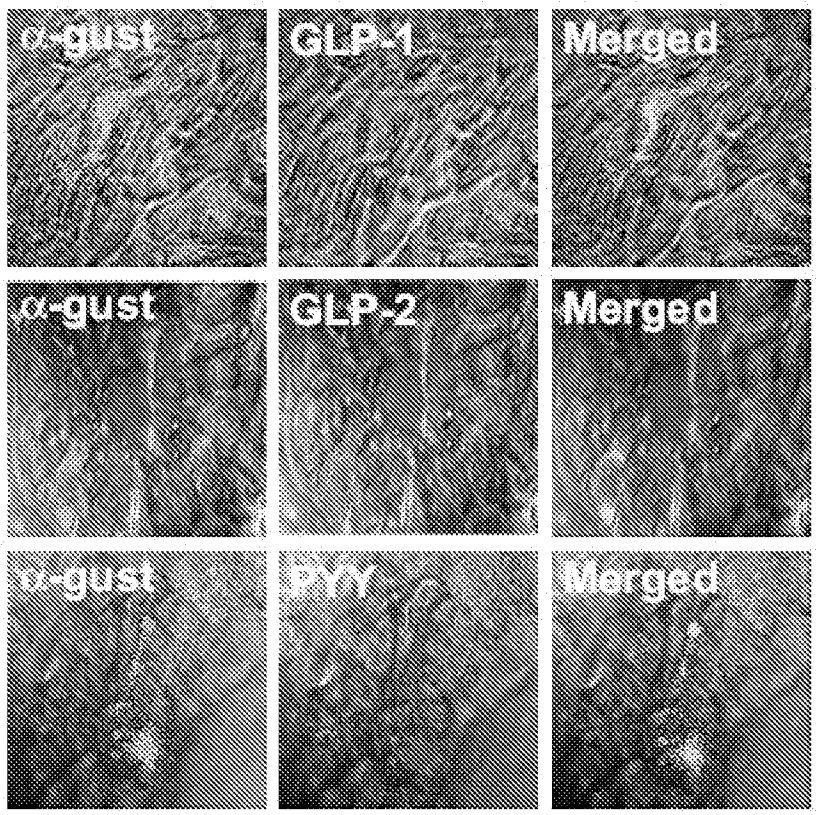

Figure 5E

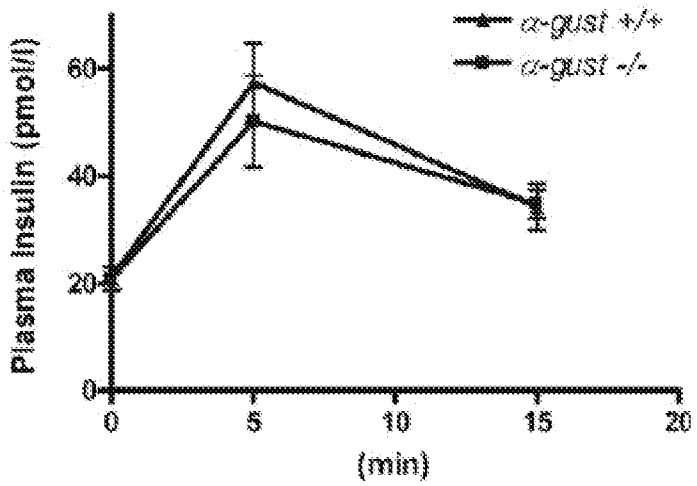

Figure 6A

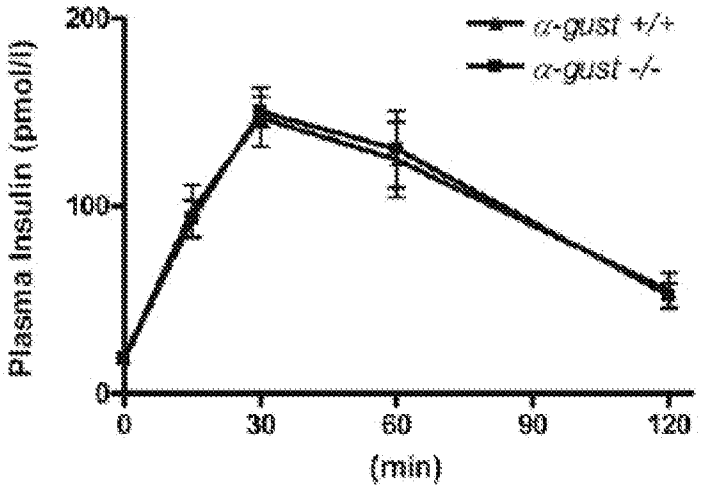

Figure 6B

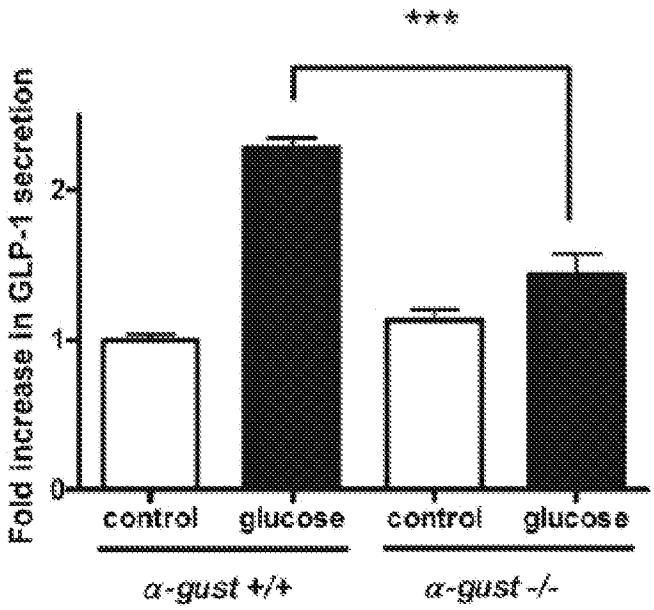

Figure 6C

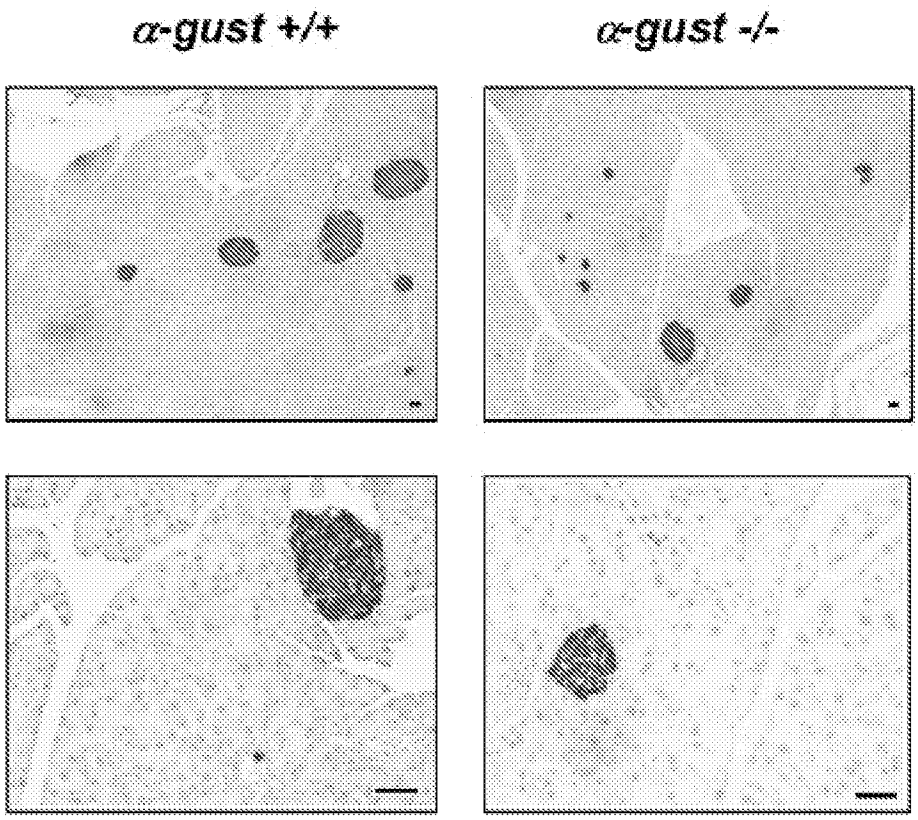

Figure 7

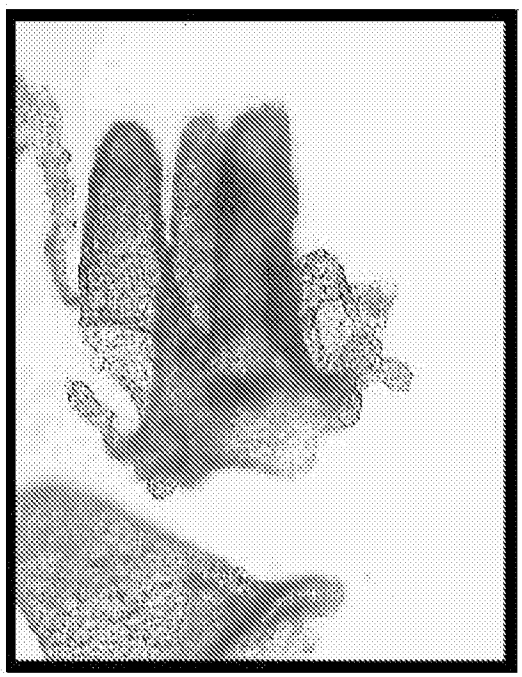

Figure 8

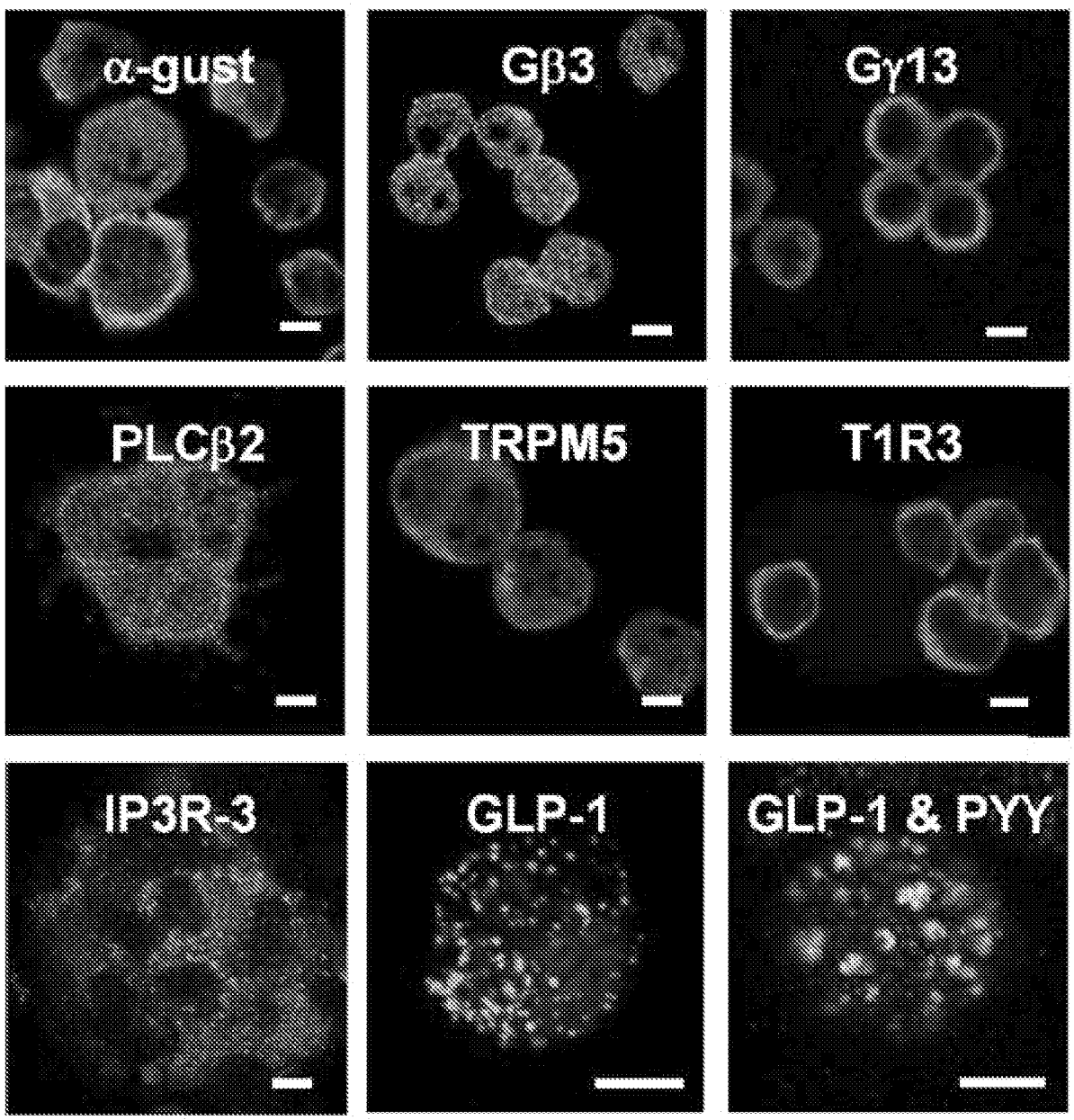

Figure 9A

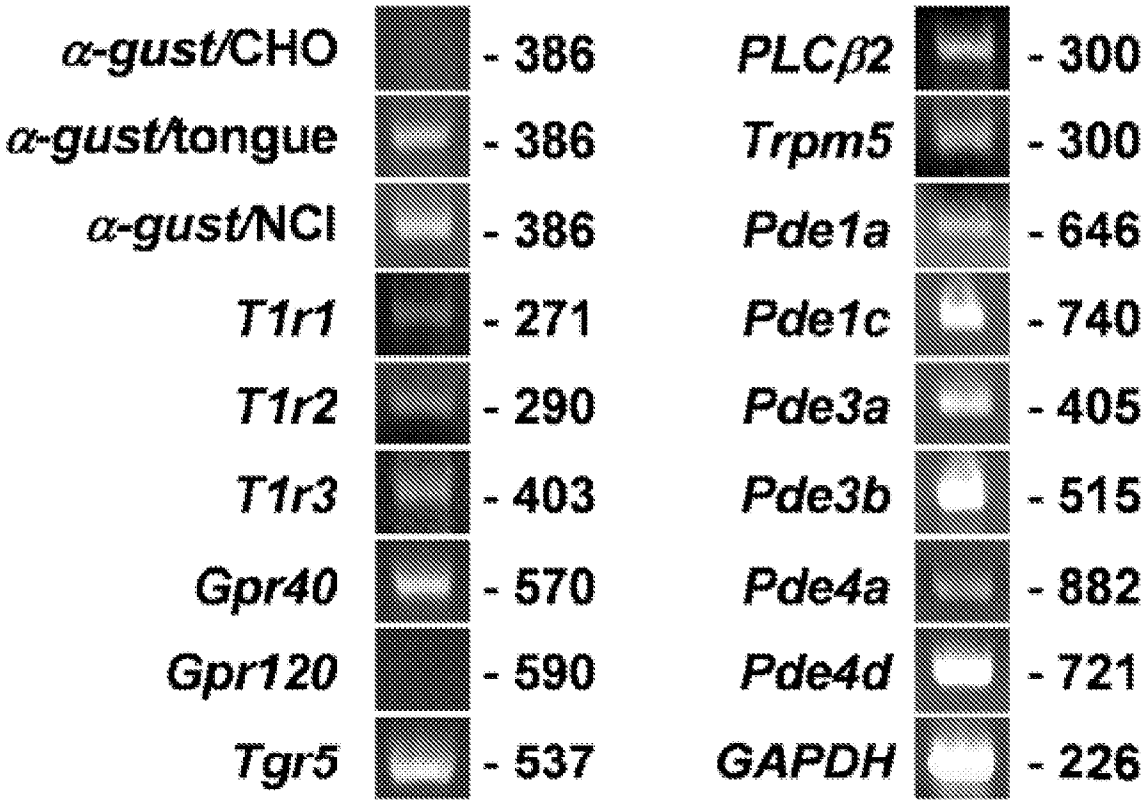

Figure 9B

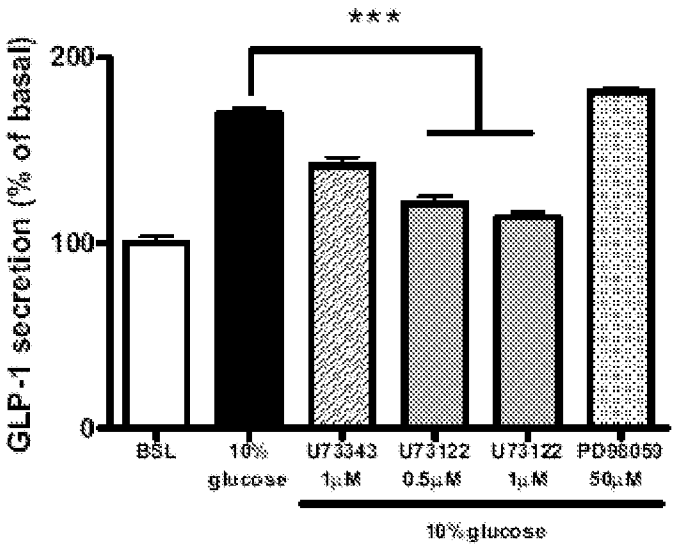

Figure 10

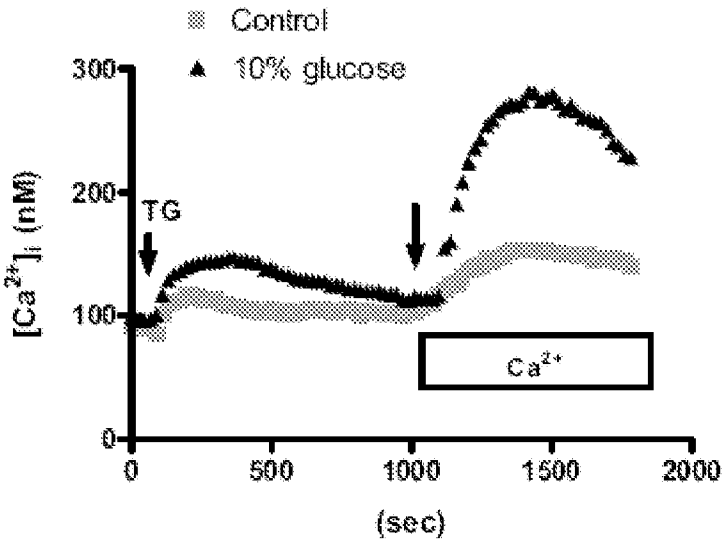

Figure 11A

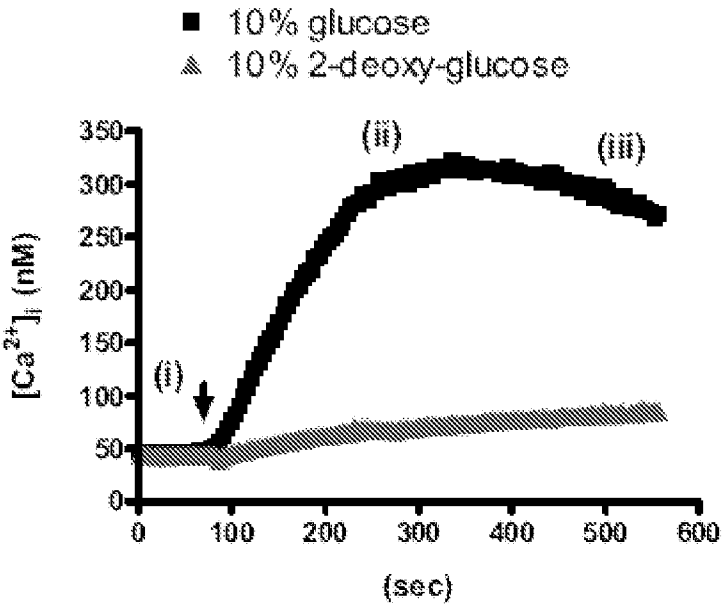

Figure 11B

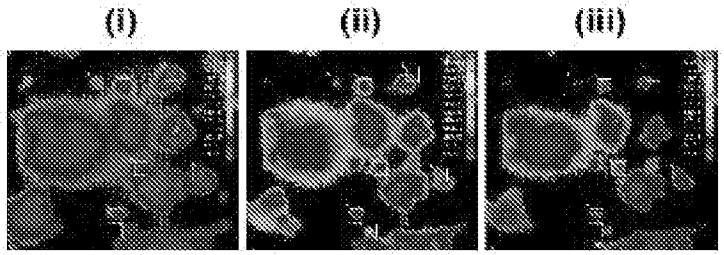

Figure 11C

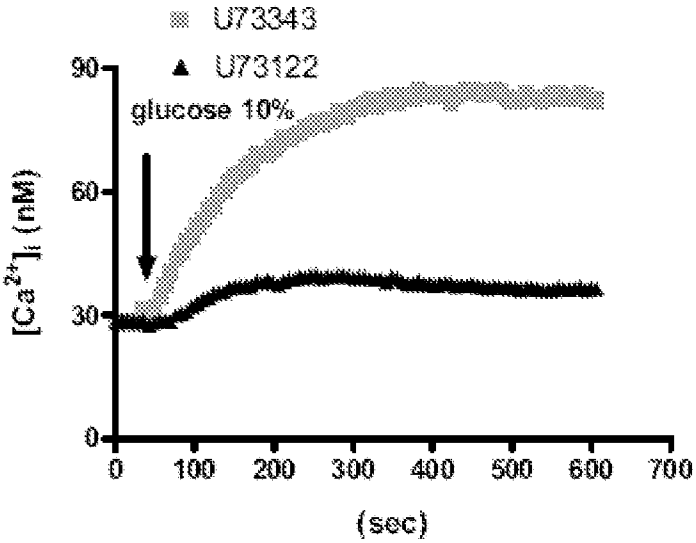

Figure 11D

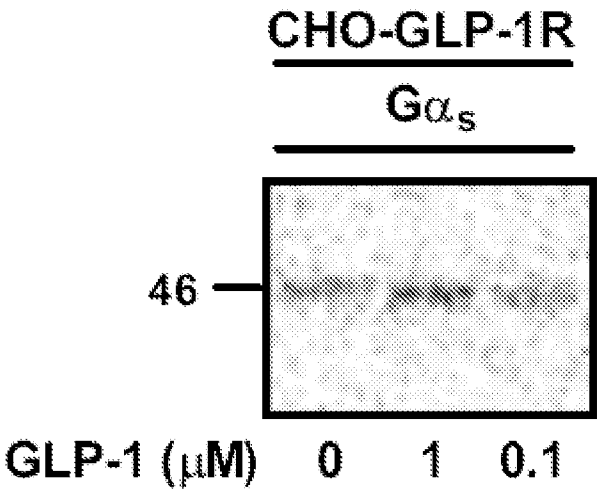

Figure 12

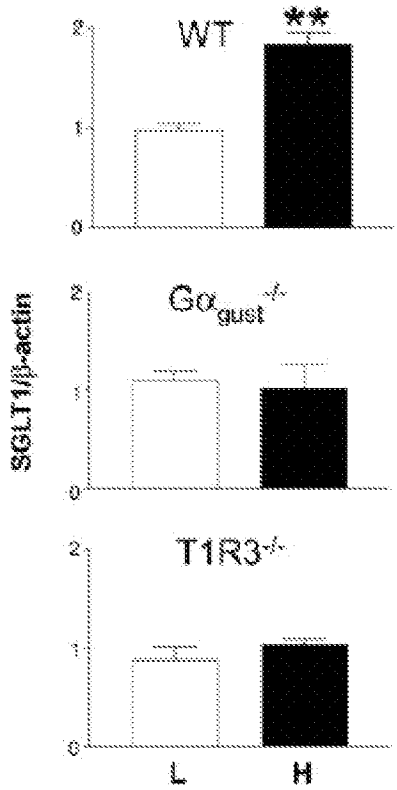

Figure 13A

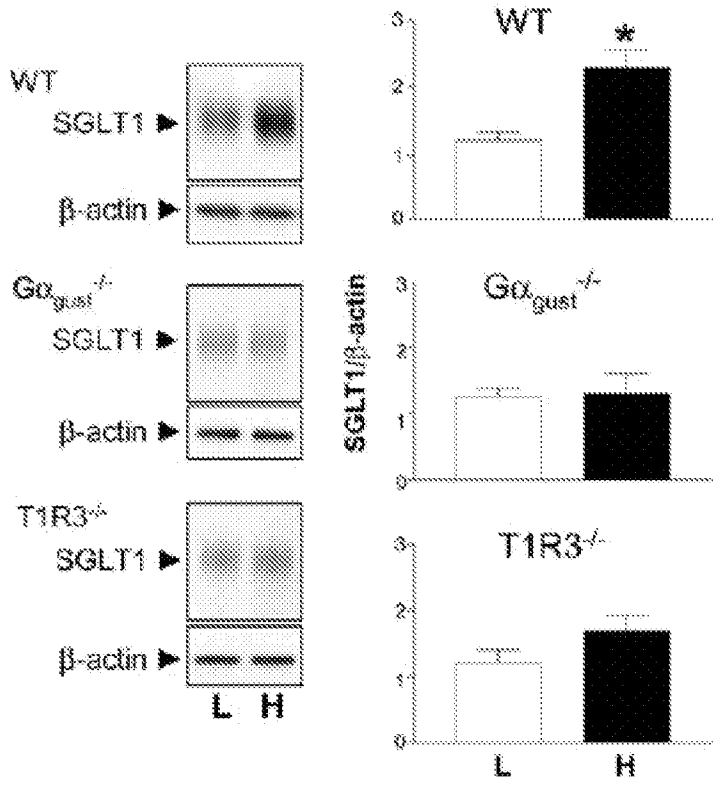
WT
SGLT1
β-actin
Gα gust -/-
SGLT1
β-actin
T1R3 -/-
SGLT1
β-actin
L H
WT
*
Gα gust -/-
T1R3 -/-
SGLT1/β-actin
0
1
2
3
L
H

Figure 13B

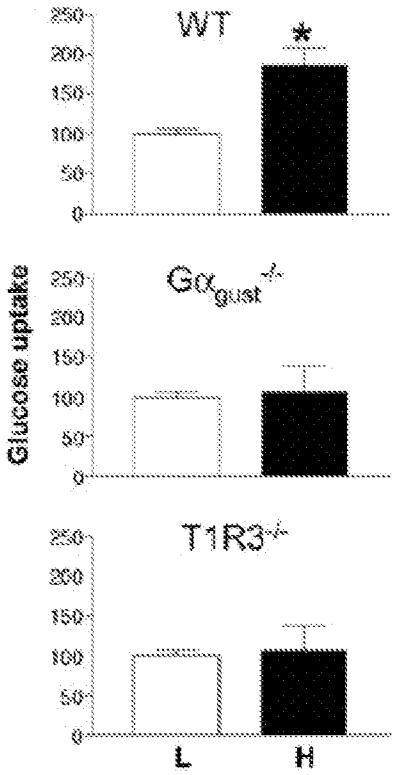

Figure 13C

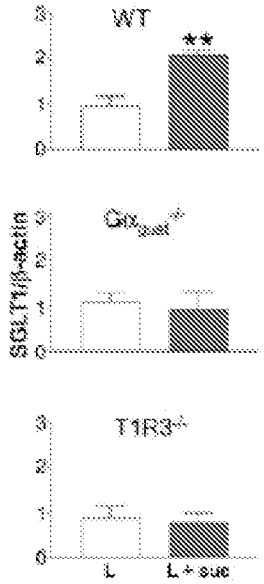

Figure 14A

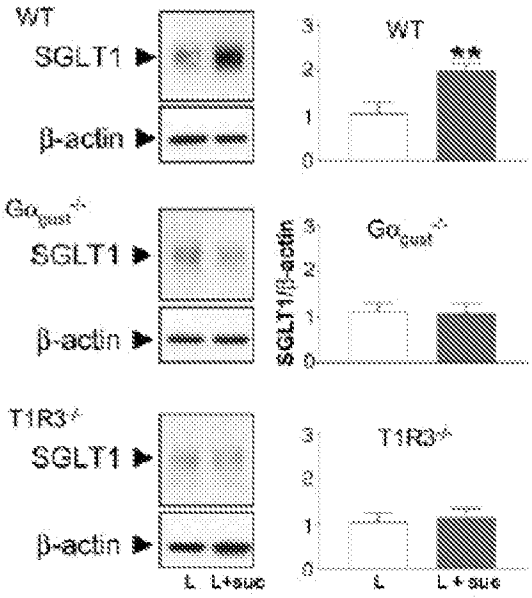

Figure 14B

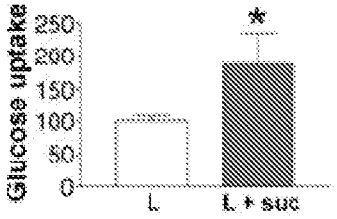

Figure 14C

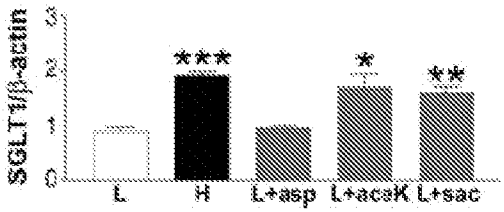

Figure 14D

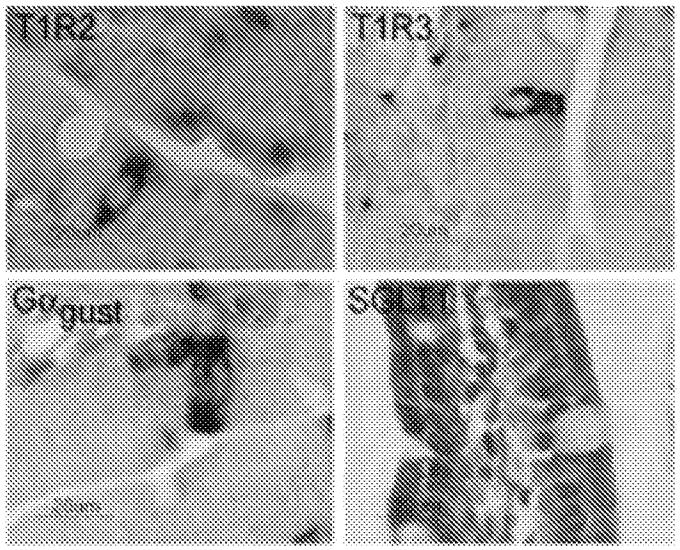

Figure 15A

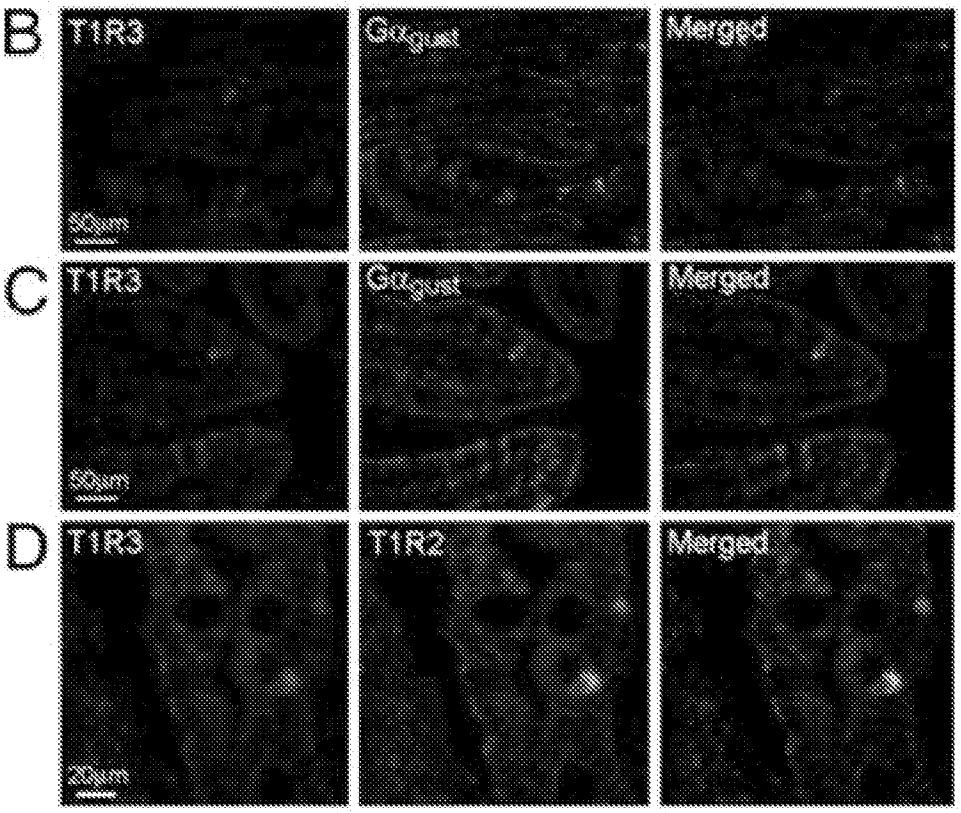

Figure 15B–D

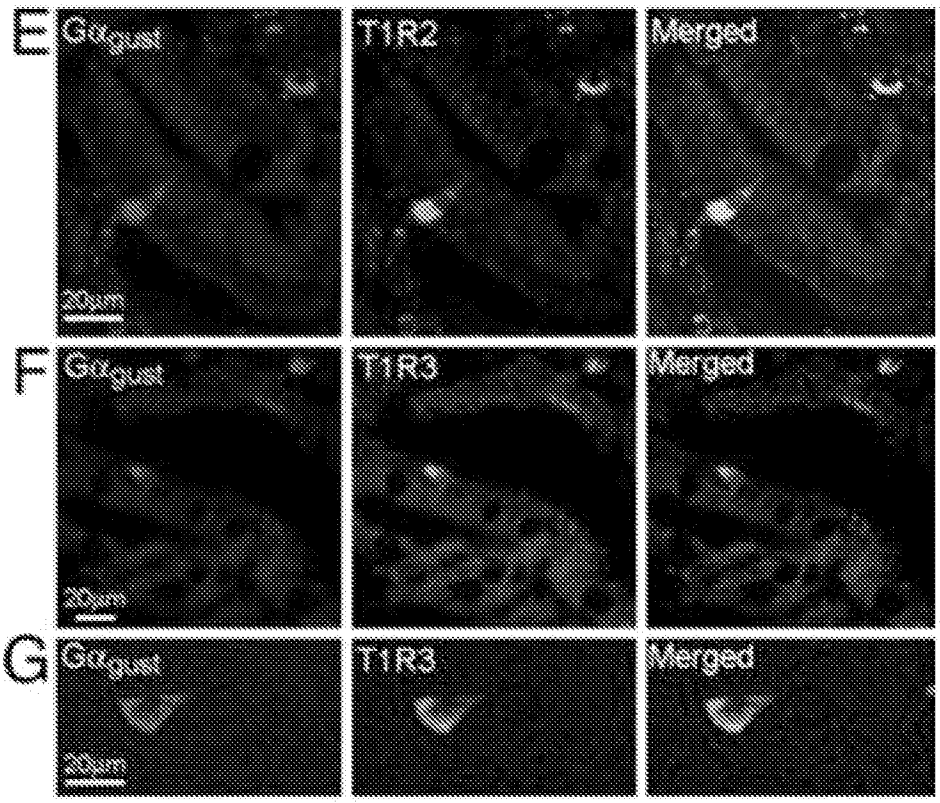

Figure 15E–G

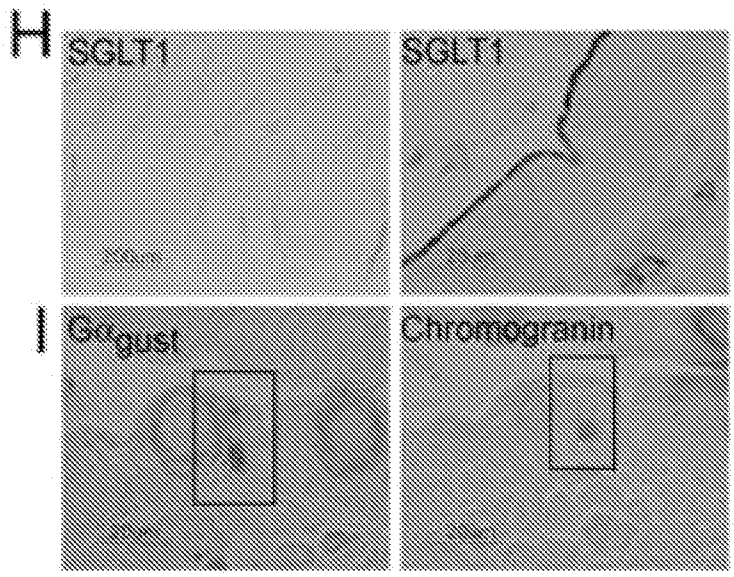

Figure 15H–I

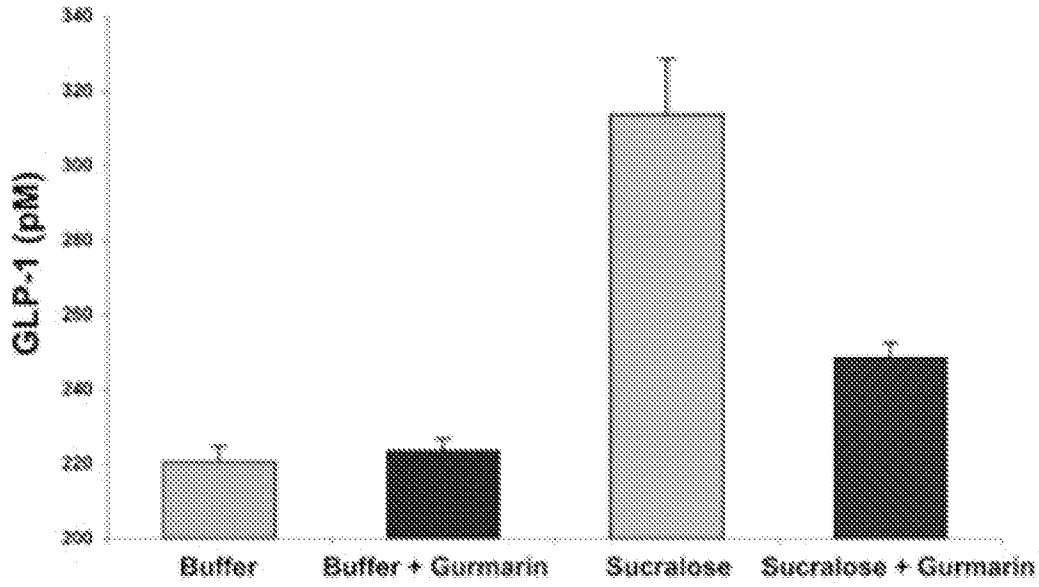

Figure 16A

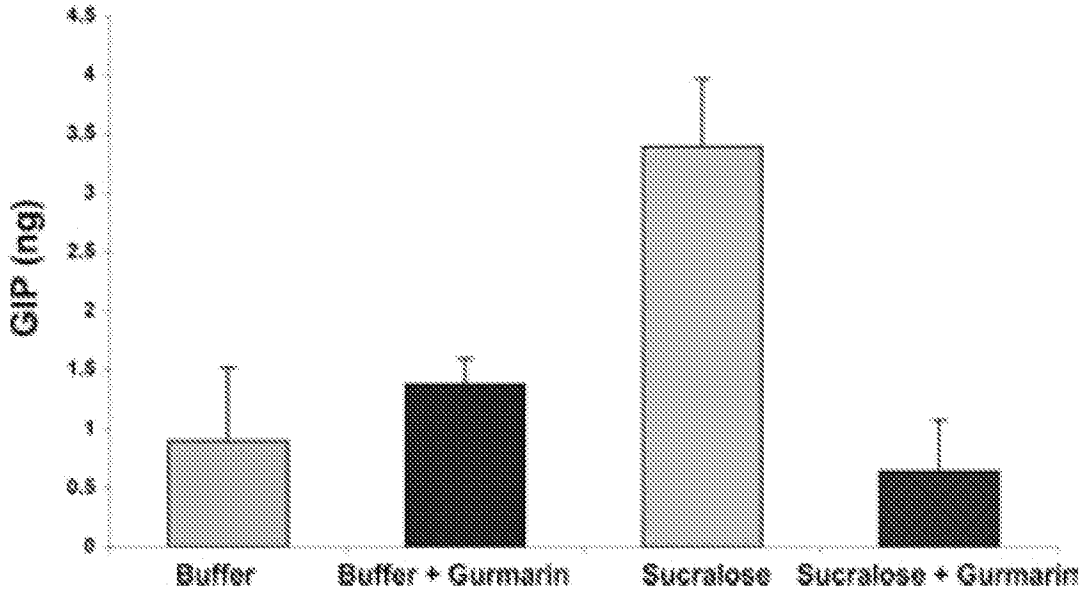

Figure 16B

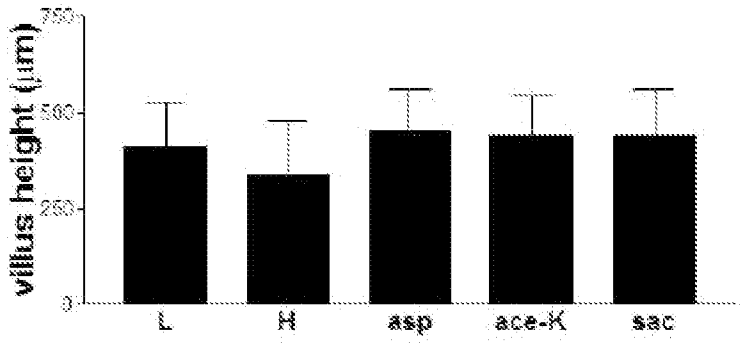

Figure 17A

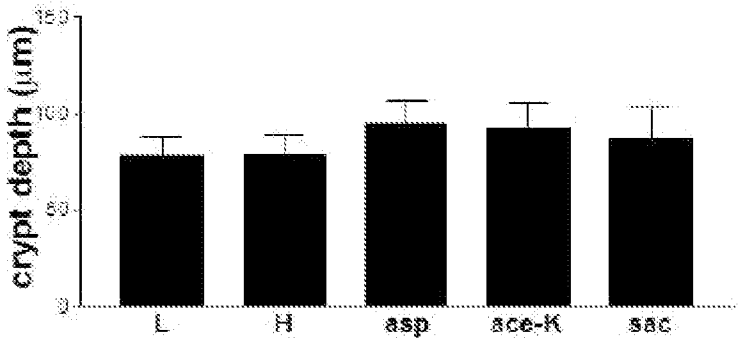

Figure 17B

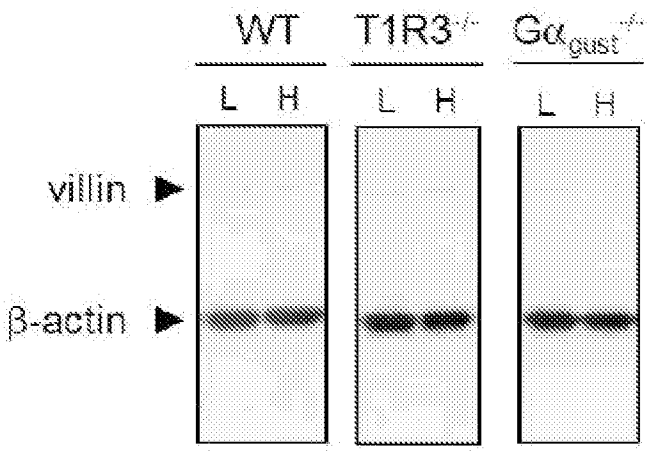

Figure 17C

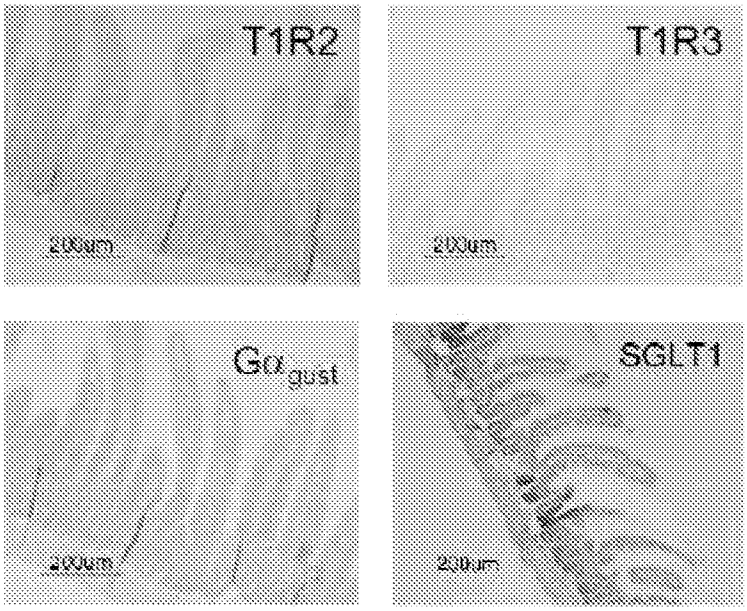

Figure 18

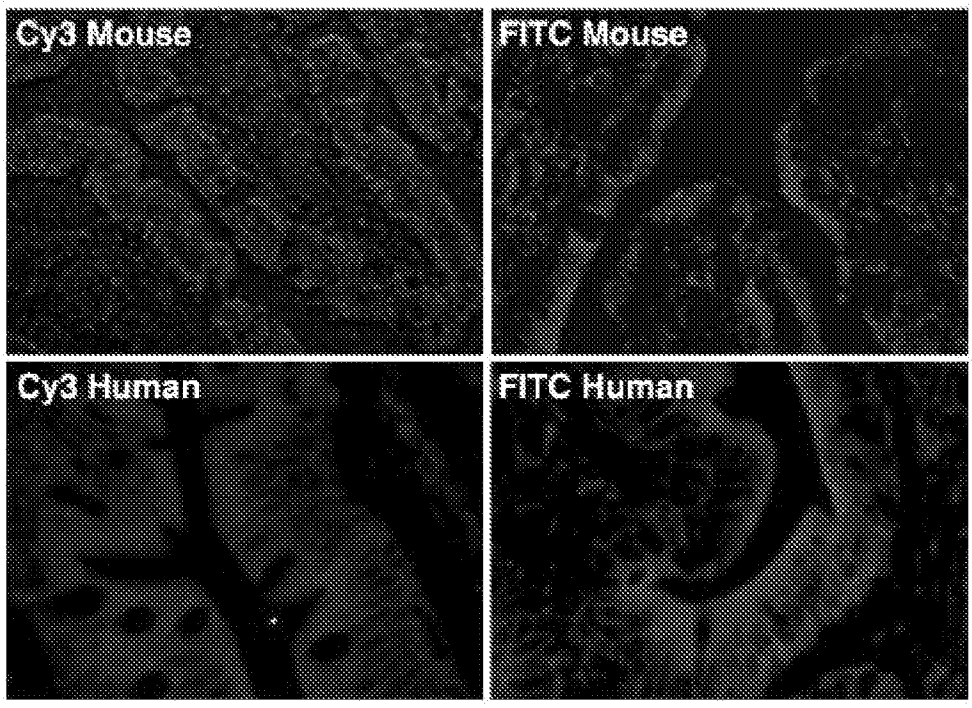

Figure 19

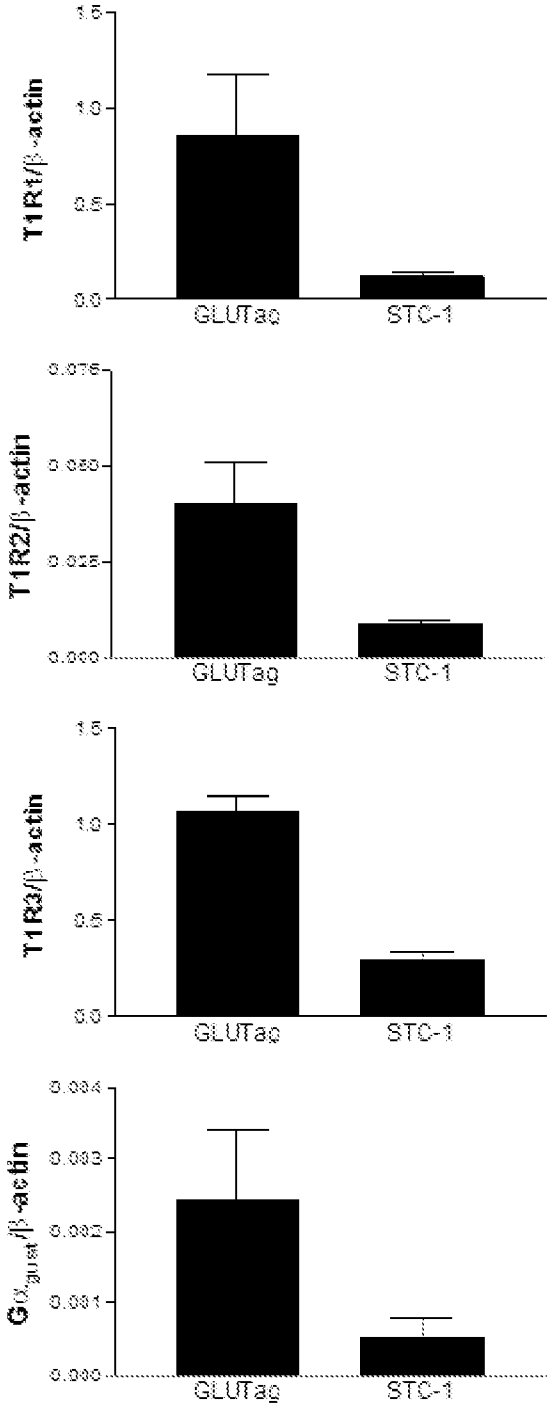
T1R1/β-actin
1.5
1.0
0.5
0.0
GLUTag
STC-1
T1R2/β-actin
0.075
0.050
0.025
0.000
GLUTag
STC-1
T1R3/β-actin
1.5
1.0
0.5
0.0
GLUTag
STC-1
Gα gust/β-actin
0.004
0.003
0.002
0.001
0.000
GLUTag
STC-1

Figure 20

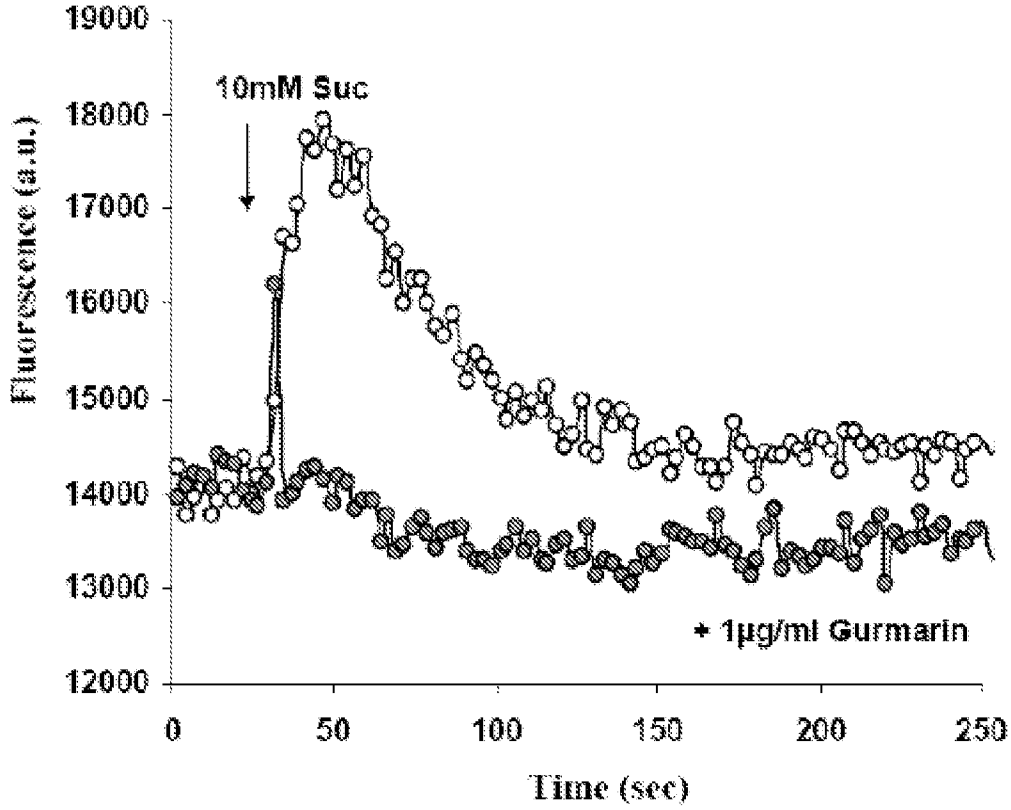

Figure 21A

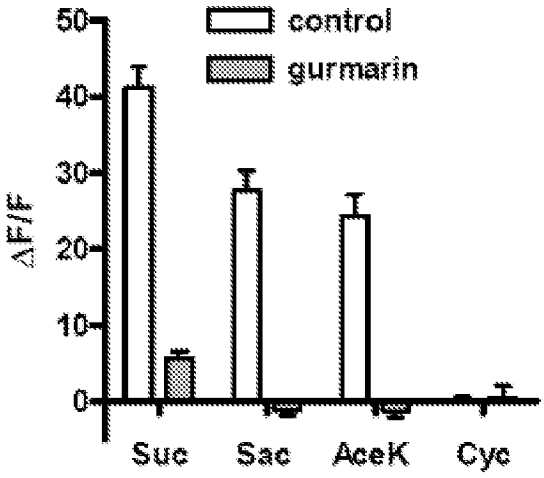

Figure 21B

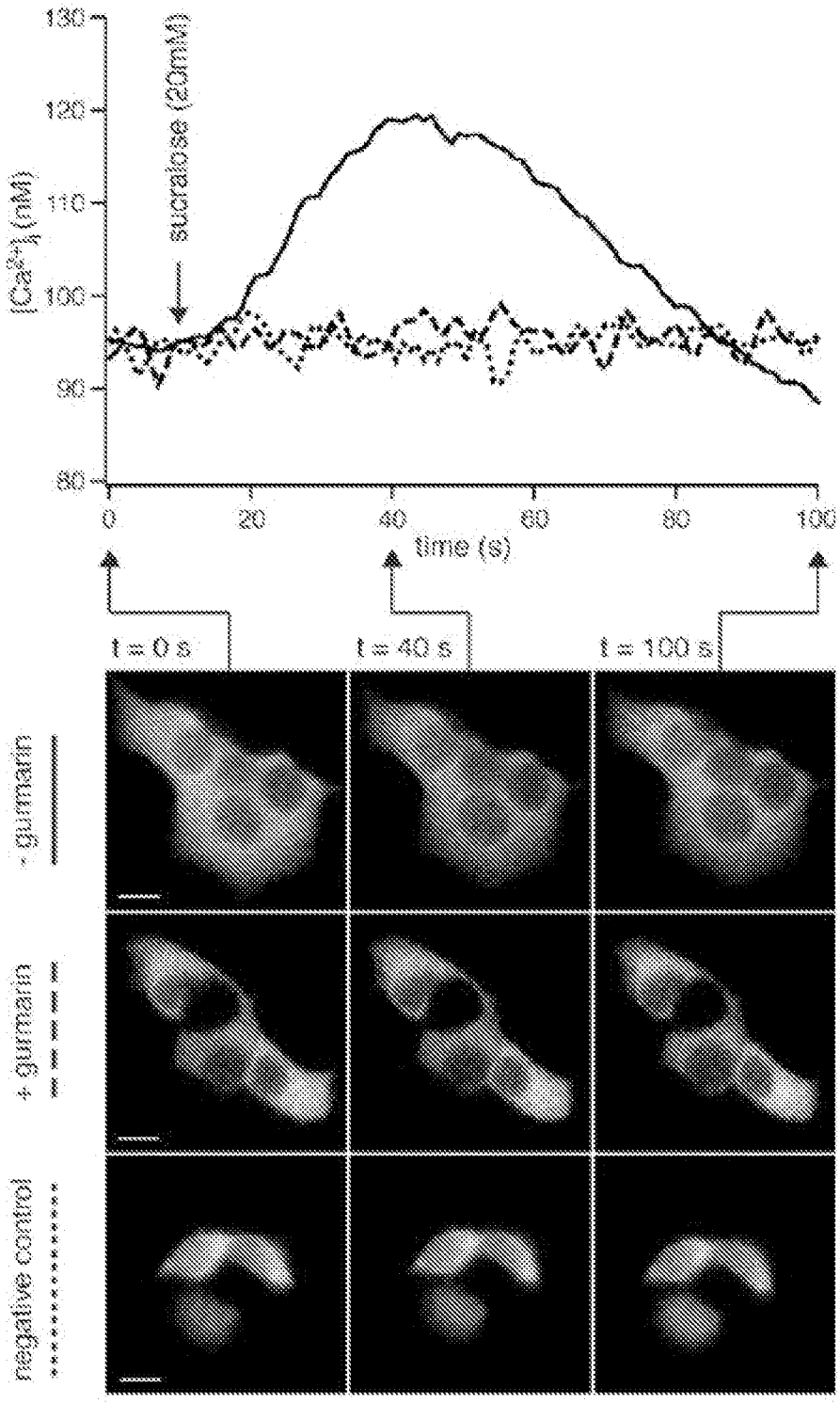
130
120
110
100
90
80
$[Ca^{2+}]_i$ (nM)
sucralose (20mM)
0
20
40
60
80
100
time (s)
t = 0 s
t = 40 s
t = 100 s
- gurmarin
+ gurmarin
negative control

Figure 22