Characterization of the taste receptor-related G-protein, α-gustducin, in pancreatic β-cells

Haruhide Udagawa1,2, Masaki Hiramoto3, Miho Kawaguchi1, Takashi Uebano4, Mica Ohara-Imaizumi2, Takao Nammo1, Wataru Nishimura5,6, Kazuki Yasuda1,7,

1Department of Metabolic Disorder, Diabetes Research Center, Research Institute, National Center for Global Health and Medicine, Tokyo, Japan, 2Department of Cellular Biochemistry, Kyorin University School of Medicine, Tokyo, Japan, 3Department of Biochemistry, Tokyo Medical University, Tokyo, Japan, 4Department of Preventive Environment and Nutrition, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Japan, 5Department of Molecular Biology, International University of Health and Welfare School of Medicine, Chiba, Japan, 6Division of Anatomy, Jichi Medical University, Bio-imaging and Neuro-cell Science, Shimotsuke, Japan, and 7Department of Diabetes, Endocrinology and Metabolism, Kyorin University School of Medicine, Tokyo, Japan

Keywords
α-Gustducin, Insulin secretion, Taste receptor

ªCorrespondence
Kazuki Yasuda
Tel.: +81-3-3202-7181
Fax: +81-3-3202-7364
E-mail address: kyasuda@ri.ncgm.go.jp

J Diabetes Invest 2020; 11: 814–822
doi: 10.1111/jdi.13214

ABSTRACT

Aims/Introduction: Taste receptors, T1rs and T2rs, and the taste-selective G-protein, α-gustducin, are expressed outside the taste-sensing system, such as enteroendocrine L cells. Here, we examined whether α-gustducin also affects nutrition sensing and insulin secretion by pancreatic β-cells.

Materials and Methods: The expression of α-gustducin and taste receptors was evaluated in β-cell lines, and in rat and mouse islets either by quantitative polymerase chain reaction or fluorescence immunostaining. The effects of α-gustducin knockdown on insulin secretion and on cyclic adenosine monophosphate and intracellular Ca2+ levels in rat INS-1 cells were estimated. Sucralose (taste receptor agonist)-induced insulin secretion was investigated in INS-1 cells with α-gustducin suppression and in islets from mouse disease models.

Results: The expression of Tas1r3 and α-gustducin was confirmed in β-cell lines and pancreatic islets. Basal levels of cyclic adenosine monophosphate, intracellular calcium and insulin secretion were significantly enhanced with α-gustducin knockdown in INS-1 cells. The expression of α-gustducin was decreased in high-fat diet-fed mice and in diabetic db/db mice. Sucralose-induced insulin secretion was not attenuated in INS-1 cells with α-gustducin knockdown or in mouse islets with decreased expression of α-gustducin.

Conclusions: α-Gustducin is involved in the regulation of cyclic adenosine monophosphate, intracellular calcium levels and insulin secretion in pancreatic β-cells in a manner independent of taste receptor signaling. α-Gustducin might play a novel role in β-cell physiology and the development of type 2 diabetes.

INTRODUCTION

Taste cells aid in the detection of nutrients, such as sugars, sweeteners and umami compounds, through heterodimers of type 1 taste receptor subunits (T1rs). T1r2 and T1r3 form a heterodimer in taste cells, which responds widely to sweetness elicited by compounds, such as glucose, sucrose and artificial sweeteners1,2. A heterodimer of T1r1 and T1r3 recognizes umami compounds, such as glutamate, monosodium glutamate and inosinic acid1,3. Sweet and bitter compounds bind to and activate specific G-protein coupled receptors that interact with G-proteins, such as α-gustducin4.

α-Gustducin is also expressed in brush cells of the stomach, duodenum and pancreatic duct in rats5-7. It has been reported that T1rs, T2rs and α-gustducin are expressed in intestinal endocrine L cells, and are involved in glucagon-like peptide-1 (GLP-1) secretion signaling in response to glucose8-10. As pancreatic β-cells secrete insulin in response to glucose and other nutrients, taste receptors and/or α-gustducin could also be implicated in nutrition sensing in these cells. However, just a few studies have explored the expression and function of these taste-related molecules in pancreatic β-cells11-14.

In the present study, we focused on the taste signaling-related molecule, α-gustducin, and examined its role in insulin secretion by pancreatic β-cells. We examined the expression of...
α-gustducin in rat and mouse islets, and the rat pancreatic β-cell line INS-1, and investigated the role of α-gustducin in INS-1 cells. We also examined whether α-gustducin is involved in the regulation of cyclic adenosine monophosphate (cAMP) and intracellular calcium levels within these cells.

METHODS

Cell culture

INS-1 cells were cultured in RPMI1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 50 μmol/L 2-mercaptoethanol, 1 mmol/L sodium pyruvate, 10 mmol/L HEPES, 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen) at 37°C in a 5% CO₂-enriched atmosphere. MIN6 cells were gifted by Dr J Miyazaki (Osaka University, Osaka, Japan). They were cultured at 37°C in a 5% CO₂-enriched atmosphere in Dulbecco’s modified Eagle’s medium (Sigma-Aldrich) with 15% heat-inactivated fetal bovine serum (Sigma-Aldrich), 50 μmol/L 2-mercaptoethanol, 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen).

Animals

Male C57BL/6J mice and male BKS.Cg-Leprdb/+ Leprdb/jcl (db/db) mice were purchased from CLEA Japan Inc. (Tokyo, Japan). C57BL/6J mice were maintained under standard conditions and fed with a standard diet (STD; CE-2, 12 kcal%; CLEA Japan Inc., Tokyo, Japan) ad libitum. At 10 weeks of age, one group of mice of each strain was fed with a high-fat diet (HFD; D12492, 60 kcal%; Research Diets Inc., New Brunswick, NJ, USA), whereas other strain- and age-matched control groups were fed with STD during the experimental period. All experimental procedures were carried out in accordance with the guidelines of the animal care and experimentation committee at the National Center for Global Health and Medicine. Male Wistar rats were purchased from CLEA Japan Inc.

Islet isolation and batch incubation

Islets of Langerhans were isolated from the pancreas of 11-week-old male C57BL/6J mice and 26-week-old STD mice, HFD mice or 8-week-old Wistar rat through collagenase digestion. The isolated islets were then washed and preincubated at 37°C for 30 min in Krebs-Ringer bicarbonate (KRB) buffer (129 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 2 mmol/L CaCl₂, 5 mmol/L NaHCO₃, 10 mmol/L HEPES (pH 7.4) and 0.1% bovine serum albumin [BSA]). The islets were incubated in buffer containing 2.8 mmol/L glucose + 10 mmol/L sucrose, 2.8 mmol/L glucose + 0.1 μmol/L GLP-1 and 16.7 mmol/L glucose for 30 min. Cellular insulin was extracted with acid-ethanol. Insulin secreted into the Krebs-Ringer-HEPES (KRH) buffer and total insulin content was measured using the Ultra-Sensitive Mouse Insulin ELISA Kit (Morinaga Institute of Biological Science Inc., Yokohama, Japan).

Quantitative polymerase chain reaction primers

TaqMan gene expression assay probes for rat guanine nucleotide-binding protein alpha transducing 3 (Gnat3; α-gustducin), taste receptor type 1 member 1 (Tas1r1), taste receptor type 1 member 2 (Tas1r2), taste receptor type 1 member 3 (Tas1r3) and β-actin (Actb) were obtained from Thermo Fisher Scientific (Waltham, MA, USA; catalog numbers Rn00597619, Rn01516038, Rn01515491, Rn00590759 and 4352931E, respectively). TaqMan gene expression assay probes for mouse Gnat3 (α-gustducin), Tas1r1, Tas1r2 and Tas1r3 were obtained from Ambion (Mm01165313, Mm00473433, Mm00499716 and Mm00473459, respectively).

Immunohistochemistry

Pancreata were excised, fixed in 4% paraformaldehyde, embedded in paraffin and immunostained, as described previously. Primary antibodies against rabbit anti-α-gustducin (sc395; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and guinea pig anti-insulin (Takara Bio Inc., Shiga, Japan) were used. DyLight 594-conjugated anti-guinea pig immunoglobulin G (1:100) or fluorescein isothiocyanate-conjugated anti-rabbit (1:100; Jackson ImmunoResearch, West Grove, PA, USA) were used as secondary antibodies. TOPRO3 (1:1000) was used to label the nuclei (Molecular Probes, Eugene, OR, USA). Immunofluorescence images were obtained using a Zeiss LSM510 confocal microscope (Carl Zeiss Co., Tokyo, Japan) in confocal mode.

Immunocytochemistry

INS-1 cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.25% (w/v) Triton X-100 in phosphate-buffered saline (PBS) for 15 min and immunostained, as described previously. After blocking with PBS containing 10% (w/v) BSA for 30 min, the cells were incubated overnight with the primary antibody in PBS containing 3% (w/v) BSA, washed with PBS and then incubated with secondary antibody in PBS containing 3% (w/v) BSA for 1 h, followed by washing with PBS and mounting. Immunofluorescence images were obtained using a Zeiss LSM510 confocal microscope in confocal mode.

Transfection of small interfering ribonucleic acids

Small interfering ribonucleic acids (siRNA) targeting rat α-gustducin (2 different siRNA), and a non-targeting negative control siRNA were obtained from Thermo Fisher Scientific (Gust siRNA1: s141856, Gust siRNA2: s141857 and AM4642, respectively).

siRNA was transiently transfected with Lipofectamine 2000 reagent (Invitrogen), according to the manufacturer’s instructions. α-Gustducin messenger RNA (mRNA) expression was assayed 48 h after siRNA transfection, and transcriptional silencing was validated using at least three independent experiments.

RNA preparation and quantitative real-time polymerase chain reaction

Total RNA was extracted from INS-1 cells or mouse pancreatic islets using the RNeasy Mini Kit (Qiagen, Cologne, Germany).
Complementary deoxyribonucleic acid was prepared from 1 μg of total RNA using reverse transcriptase (SuperScript II<sup>TM</sup>; Invitrogen), according to the manufacturer’s instructions. Quantitative polymerase chain reaction amplification was carried out using the TaqMan universal polymerase chain reaction master mix core reagent kit and analyzed using an ABI Prism 7900 (Applied Biosystems). The mRNA levels were evaluated and normalized against Actb expression level using the 2<sup>ΔΔCt</sup> method<sup>21</sup>.

**Western blot analysis**
Protein fraction of INS-1 cells was extracted using a Subcellular Protein Fractionation Kit (Thermo Fisher Scientific Inc.). The protein samples were resolved using 4–20% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and gels were transferred to polyvinylidenedifluoride membrane filters (BioRad Laboratories, Hercules, CA, USA). Western blot analyses were carried out using antibodies against α-gustducin (Santa Cruz Biotechnology), Actb, α-tubulin, lamin A/C (Cell Signaling Technology, Danvers, MA, USA; #4967, #2144, #2032, respectively) and E-cadherin (BD Transduction Laboratories, Franklin Lakes, NJ, USA; #610181), and detected using Immobilon Western Chemiluminescent HRP Substrate (Millipore Co., Billerica, MA, USA).

**Measurement of insulin level and secretion from INS-1 cells**
After the indicated culture periods, INS-1 cells were washed with KRB buffer supplemented with 0.1 mmol/L glucose and 0.5% BSA, preincubated at 37°C for 30 min in KRB buffer containing 3 mmol/L glucose, and incubated at 37°C for 60 min with the indicated concentration of glucose and/or several stimulants. Insulin secreted into the KRB buffer was quantified using the Mouse Insulin ELISA KIT (T-Type; AKRIN-011T; Shibayagi, Gunma, Japan). Cellular insulin was extracted using acid-ethanol overnight at 4°C, and insulin level was determined using enzyme-linked immunosorbet assay, after dilution.

**Measurement of cAMP levels**
cAMP levels in INS-1 cells were determined using the cAMP Biotrak enzyme immunoassay system (GE Healthcare UK Ltd., Amersham, UK), according to the manufacturer’s instructions.

**Intracellular calcium analysis**
Intracellular calcium concentration ([Ca<sup>2+</sup>]) in INS-1 cells was measured with a Calcium kit Fluo-4 (#CS22; Dojindo Molecular Technologies, Inc., Kumamoto, Japan). INS-1 cells were placed on a 95-mm glass bottom culture dish. Growth medium was replaced with loading buffer containing 3 mmol/L glucose and Fluo4-AM, and the culture dish was incubated at 37°C for 1 h. Loading buffer was then replaced with recording buffer containing 3 mmol/L glucose. The Fluo-4 fluorescent signal over the INS-1 cell area was detected through excitation at 488 nm using an LSM 880 Live microscope (Carl Zeiss, Oberkochen, Germany).

---

**Figure 1** | Expression of taste receptors and α-gustducin (Gust) in islets and cell lines. (a) The expression of taste receptors, T1r1, T1r2 and T1r3, and G-protein, α-gustducin, in isolated rat, mouse islets, and in INS-1 and MIN6 cells were analyzed using quantitative polymerase chain reaction. Values are shown as the mean ± standard error of the mean (n = 3). (b) Portions of each extract in INS-1 cells were analyzed by western blotting using specific antibodies against α-gustducin or endogenous control proteins, including cytoplasm (Cyto; α-tubulin), plasma membrane (Memb; E-cadherin) and nuclear soluble fraction (Nuc; lamin A/C). The expression of α-gustducin in mouse tongue and isolated mouse islets extracted by RIPA buffer was analyzed using western blotting. (c) INS-1 cells were immunostained to detect fluorescin isothiocyanate using anti-α-gustducin antibodies (green) and insulin (red). The specificity of α-gustducin antibody was confirmed using its blocking peptide (BP) in immunocytochemistry; the pre-immune antibody showed no staining at the same concentrations used in these experiments. mRNA, messenger ribonucleic acid; ND, not detected; PBS, phosphate-buffered saline.
Statistical analysis
The data are presented as the mean ± standard error of the mean. Statistical significance was determined using two-tailed unpaired Student’s t-test. A two-way analysis of variance (ANOVA) was carried out to analyze: (i) insulin secretion data after α-gustducin knockdown and sucralose treatment; and (ii) cAMP concentration data after α-gustducin knockdown and measurement time.

RESULTS
Expression of taste receptor-related protein α-gustducin in islets and pancreatic β-cells
To investigate whether taste receptors and α-gustducin are involved in nutrition sensing in pancreatic β-cells, we first examined mRNA expression of these molecules in rat and mouse islets, and INS-1 and MIN6 cells. As a control, we also measured taste receptors and α-gustducin expression in the tongue tissue of each species. Quantitative polymerase chain reaction results showed the expression of Tas1r3 and α-gustducin in rat and mouse islets, and in INS-1 and MIN6 cells (Figure 1a). Interestingly, α-gustducin and Tas1r3 expression in rat islets was markedly higher than in the tongue. Expression of Tas1r1 mRNA was detected in rat islets, INS-1 cells, rat tongue, MIN6 cells and mouse tongue, but not in mouse islets. Tas1r2 expression was detected only in the tongue tissue. Furthermore, we examined α-gustducin protein expression by western blot analysis in the cytoplasm, membrane or nuclear extract of INS-1 cells, isolated mouse islets and mouse tongue. The expression of α-gustducin protein was observed in the cytoplasm and membrane fractions of INS-1 cells, mouse isolated islets and mouse tongue (Figure 1b). Fluorescence immunostaining showed that α-gustducin was localized in cytoplasm (Figure 1c). We carried out α-gustducin knockdown experiments using two kinds of siRNAs. Transfection of
α-gustducin siRNA led to a downregulation of α-gustducin mRNA expression to approximately 10%, as compared with the negative control siRNA (Figure 2a). Reduction in α-gustducin protein levels by transfection of siRNA in INS-1 cells was detected by western blotting and fluorescence immunostaining (Figure 2b,c).

**Effect of α-gustducin knockdown on levels of cAMP, intracellular Ca²⁺ and insulin secretion in INS-1 cells**

We examined whether a reduction in expression of α-gustducin affects insulin secretion from INS-1 cells. Interestingly, basal insulin secretion from INS-1 cells, cultured with 3 mmol/L glucose, was significantly increased by α-gustducin knockdown compared with that in the control siRNA transfected cells (Figure 2d). However, insulin secretion was not affected by 10 mmol/L glucose, 25 mmol/L glucose or 30 mmol/L KCl stimulation (Figure 2d). These results suggested that α-gustducin is involved in basal, but not in high-glucose- or depolarization-stimulated, insulin secretion by INS-1 cells. Next, we determined whether α-gustducin knockdown mediated enhancement of basal insulin secretion was caused by normal exocytosis. Epinephrine stimulation has been reported to suppress insulin secretion by decreasing cAMP through the alpha-2A-adrenoceptor. Epinephrine stimulation completely abolished the increase in basal insulin secretion by α-gustducin knockdown (Figure 2e). Clapp et al. reported that α-gustducin maintains a tonically low cAMP level in taste cells to ensure adequate Ca²⁺ signaling. As cAMP and [Ca²⁺]i are also critical for insulin secretion pathway in pancreatic β-cells, we speculated that α-gustducin plays an important role in the regulation of basal insulin secretion by regulating cAMP and calcium levels. First, we examined whether knockdown of α-gustducin causes changes in cAMP concentration. As shown in Figure 2f, the cAMP level in INS-1 cells was significantly increased after α-gustducin knockdown compared with that in cells treated with negative control siRNA. Thus, α-gustducin was found to retain Gα-protein-specific functions in pancreatic β-cells. The potentiation of insulin secretion by cAMP is strictly dependent on the extracellular glucose concentration, and the threshold is not reached at 3 mmol/L glucose. We measured [Ca²⁺]i levels in INS-1 cells by α-gustducin knockdown. α-Gustducin knockdown in INS-1 cells significantly increased the amount of basal [Ca²⁺]i (Figure 2g.h). These results suggested that the increase in basal insulin secretion in INS-1 cells by α-gustducin knockdown was caused by an increase in [Ca²⁺]i levels.

**Expression of α-gustducin in HFD-fed mice and diabetic db/db mice**

α-Gustducin is expressed in INS-1 cells, and affects basal insulin secretion by INS-1 cells. However, whether α-gustducin is involved in the pathogenesis of diabetes is still unclear.

![Figure 3](http://wileyonlinelibrary.com/journal/jdi)
Therefore, we examined changes in expression of α-gustducin in diabetic db/db mice and HFD-fed mice. α-Gustducin was weakly expressed in both STD-fed mice (4 weeks STD) and HFD-fed mice islets (4 weeks HFD), and co-localized with insulin (Figure 3a). A similar observation was made after comparison between 6-week-old and 12-week-old db/m mice (Figure 3c,d). In mice fed with HFD for 52 weeks, the expression of α-gustducin in islets was reduced compared with that in 52-week STD-fed mice (Figure 3b). In addition, in diabetic obesity db/db mice, α-gustducin expression was decreased in pancreatic islets compared with that in control db/m mice (Figure 3c,d). These results suggested that the expression of α-gustducin in pancreatic islets increases with age and decreased with HFD feeding and obesity.

**Involvement of α-gustducin in insulin secretion through the sucralose–taste receptor axis**

Although basal insulin secretion levels were enhanced by α-gustducin knockdown in INS-1 cells, glucose-induced insulin secretion was not significantly affected. To elucidate whether α-gustducin is implicated in the detection of sweetness in pancreatic β-cells through taste receptors, we examined levels of basal cAMP and basal insulin secretion under low glucose concentrations induced by the taste signal agonist, sucralose, in pancreatic β-cells. cAMP levels were significantly increased after α-gustducin knockdown in INS-1 cells treated with 10 mmol/L sucralose in the presence of 3 mmol/L glucose (Figure 4a).

Two-way ANOVA showed a statistically significant interaction between the effects of α-gustducin knockdown and sucralose treatment on cAMP ($P < 0.001$). Basal insulin secretion by INS-1 cells was significantly enhanced by 10 mmol/L sucralose treatment, but not by 1 mmol/L sucralose treatment (Figure 4b). Sucralose-stimulated insulin secretion was enhanced by α-gustducin knockdown when compared with that in the control. However, two-way ANOVA showed no statistically significant interaction between the effects of α-gustducin knockdown and sucralose treatment on insulin secretion ($P = 0.056$). These results suggest that α-gustducin is not mainly involved in the sucralose–taste receptor signaling axis in the regulation of insulin secretion.

Next, we investigated whether sucralose stimulation in islets isolated from HFD mice with low α-gustducin expression altered insulin secretion. As previously reported,$^{25,26}$ 16.7 mmol/L glucose stimulation of STD mouse islets significantly increased the insulin secretion compared with 2.8 mmol/L glucose, and this increase was attenuated with HFD exposure; furthermore, GLP-1 stimulation did not increase basal insulin secretion. Meanwhile, we found that sucralose-stimulated basal insulin secretion was significantly increased in HFD mouse islets compared with that in STD mouse islets (Figure 4c). These results suggested that sucralose showed even larger enhancement of basal insulin secretion with lower α-gustducin expression.
expression (Figure 3b,d). These results collectively suggested that α-gustducin is involved in insulin secretion in mouse pancreatic islets through signaling pathways independent of the taste receptor.

DISCUSSION

In the present study, we showed the expression of the taste signaling-related G-protein, α-gustducin, in both mouse islets and the rat pancreatic β-cell line, INS-1, and examined its role in insulin secretion after knockdown experiments in INS-1 cells. Although α-gustducin did not play a major role in glucose- or depolarization-stimulated insulin secretion in INS-1 cells, three interesting observations were made in this study. First, basal insulin secretion, cAMP levels and [Ca^{2+}]i levels were significantly increased after α-gustducin knockdown in INS-1 cells (Figure 2d–h). The α2 adrenoceptor is coupled with inhibitory G protein and is known to decrease the second messenger cAMP. The results of stimulation with epinephrine, an α2-adrenoceptor agonist (Figure 2e) suggested that the increase in basal insulin secretion due to α-gustducin knockdown was not a result of protein leakage, but due to a change in the exocytotic signal. The negative regulation of basal cAMP levels by α-gustducin in INS-1 cells is consistent with the report that the taste cells of α-gustducin-knockout mice show an elevated basal cAMP level. The suppression of α-gustducin in INS-1 cells significantly increased the basal [Ca^{2+}]i levels, indicating that in the basal state, α-gustducin contributes to maintain cAMP and [Ca^{2+}]i at low levels. Second, α-gustducin expressed in mouse pancreatic islets was co-localized with insulin, and the expression levels of α-gustducin were decreased in HFD-fed mice and diabetic db/db mice (Figure 4). db/db mice at 6–8 weeks-of-age usually present hyperinsulinemia and hyperlipidemia. At 10–14 weeks-of-age, the db/db mice became fully diabetic with significantly higher plasma glucose levels. We observed that the expression of α-gustducin decreased in 6-week-old or 12-week-old db/db mice. Thus, decreased α-gustducin expression levels are speculated to be involved in β-cell dysfunction and the early phase of type 2 diabetes. Third, basal insulin secretion showed no statistically significant correlation with the effects of α-gustducin knockdown and sucralose treatment on insulin secretion (P = 0.056). These results suggest that α-gustducin is not involved in the sucralose–taste receptor signaling axis in pancreatic β-cells.

Previously, Nakagawa et al. reported sucralose-stimulated insulin secretion in the mouse pancreatic β-cell line, MIN6, due to higher cAMP and Ca^{2+} levels. They concluded that these effects of sucralose were, at least partially, mediated by the heterodimer of T1r2 and T1r3, the sweet receptor found in taste cells. Furthermore, Nakagawa et al. showed that expression levels of α-gustducin were extremely low in MIN6 cells, and that α-gustducin knockdown did not affect [Ca^{2+}]i responses to various artificial sweeteners. Some differences of their results and the present results could be explained either by species difference (between mice and rats) or by the difference between tumor cell lines. We confirmed high expression of α-gustducin, Tas1r1, Tas1r2 and Tas1r3 mRNA in rat islets as positive controls, but exact reasons for apparent discrepancies between the findings of Nakagawa et al. and the present study regarding the roles of α-gustducin in insulin secretion remain unknown. Interestingly, sucralose increased basal insulin secretion even in cellular states with abnormal glucose responsiveness after HFD challenge. In order to determine how this affects damaged β-cells, further studies are necessary.

The previous studies investigated different β-cell lines; the expression of T1r2 mRNA was not detected in the INS-1 cells used by us, and the classical sweet receptor α-gustducin pathway (mediated by T1r2 + T1r3) involved in insulin secretion might be absent from INS-1 cells. Therefore, taken together, α-gustducin might couple with other G protein-coupled receptors, in addition to the classical taste receptor, T1r3. Currently, we do not know which receptors couple with α-gustducin and mediate the effect of insulin secretion in INS-1 cells. Nakagawa et al. suggested that T1r3 acts as a homodimer; however, its association with α-gustducin is not yet clear. We were not able to verify the association between α-gustducin and T1r3 in INS-1 cells, because we could not silence the T1r3 gene in INS-1 cells. We observed that certain subtypes of T1rs (Figure 1a) and T2rs (data not shown) were expressed in INS-1 cells, in which T1r2 was absent. The types of extracellular factors that couple with α-gustducin in β-cells are yet to be explored. In taste cells, sweet compounds bind to and activate specific G protein-coupled receptors that interact with α-gustducin, Gβ3, and Gγ13.43,30. The present data showed that during sucralose-stimulated insulin secretion, sucralose is not absorbed from the intestine, and does not affect β-cells directly in vivo. Wong et al. reported that α-gustducin knockout mice showed reduced response to bitter, sweet and umami stimuli. We examined whether α-gustducin participates in insulin secretion stimulated by possible taste receptor ligands other than glucose and sweeteners. Although various substances, such as fructose, denatonium, leucine and monosodium glutamate, and the hormones, GLP-1 and glucagon, induced insulin secretion in INS-1 cells, the effects of α-gustducin knockdown on insulin secretion were not significant (data not shown). However, Kyriazis et al. showed that ablation of sweet taste receptor protein, T1r2, obliterates fructose-induced calcium responses and insulin release in the mouse pancreatic β-cell line, MIN6, and mouse islets. In addition, taste receptor complexes of T1r1 and T1r3 regulate the amino acid-induced insulin secretion in the mouse pancreatic β-cell line, MIN6. Li et al. showed that α-gustducin coupled with the fatty acid receptor in the colon. Taken together, α-gustducin is speculated to be associated with amino acid or fatty acid signaling in pancreatic β-cells. Further studies are required to identify the ligands and/or receptors that activate α-gustducin in vivo.

At the basal state, α-gustducin maintains tonically low cAMP level and insulin secretion in INS-1 cells. It has been reported that cAMP exerts pleiotropic effects and is important not only
for insulin secretion, but also for β-cell proliferation or survival. It would be interesting to explore the possible role of α-gustducin in this respect.

In conclusion, α-gustducin is expressed in rat and mouse islets, and in INS-1 cells and MIN6 cells, and negatively regulates [Ca²⁺]i and cAMP levels, and insulin secretion in INS-1 cells. The present results show a potential role for α-gustducin and a novel mechanism regulating insulin secretion in pancreatic β-cells. Furthermore, the change in α-gustducin expression indicates its role in the development of type 2 diabetes, because expression of α-gustducin is reduced in pancreatic islets of HFD-fed mice and diabetic db/db mice. Further in vivo studies could elucidate the significance of these findings in normal physiology, as well as in pathological states, such as diabetes mellitus.

ACKNOWLEDGMENTS

We thank Dr CB Wollheim and Dr N Sekine for the INS-1 cell line; Dr Miyazaki for the MIN6 cell line; Dr Y Kaburagi for helpful discussion; M Nakano for Ca²⁺ analysis; and D Suzuki and K Nagase for technical assistance. This study was supported by a grant from the National Center for Global Health and Medicine (KY and HU), grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (KY and HU), and a grant from Research on Publicly Essential Drugs and Medical Devices, Japan Health Sciences Foundation (KY).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Li X, Staszewski L, Xu H, et al. Human receptors for sweet and umami taste. Proc Natl Acad USA 2002; 99: 4692–4696.
2. Nelson G, Hoon MA, Chandrashekar J, et al. Mammalian sweet taste receptors. Cell 2001; 106: 381–390.
3. Nelson G, Chandrashekar J, Hoon MA, et al. An amino-acid taste receptor. Nature 2002; 416: 199–202.
4. McLaughkin SK, McKinnon PJ, Margolskee RF. Gustducin is a taste-cell-specific G protein closely related to the transducins. Nature 1992; 357: 563–569.
5. Hofer D, Asan E, Dreneckhahn D. Chemosensory perception in the gut. News Physiol Sci 1999; 14: 18–23.
6. Hofer D, Dreneckhahn D. Identification of the taste cell G-protein, α-gustducin, in human cells of the rat pancreatic duct system. Histochern Cell Biol 1998; 110: 303–309.
7. Hofer D, Puschel B, Dreneckhahn D. Taste receptor-like cells in the rat gut identified by expression of α-gustducin. Proc Natl Acad Sci USA 1996; 93: 6631–6634.
8. Jang HJ, Kokrashvili Z, Theodorakis MJ, et al. Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. Proc Natl Acad Sci USA 2007; 104: 15069–15074.
9. Kokrashvili Z, Mosinger B, Margolskee RF. T1r3 and alpha-gustducin in gut regulate secretion of glucagon-like peptide-1. Ann N Y Acad Sci 2009; 1170: 91–94.
10. Mokadem M, Zechner JF, Margolskee RF, et al. Effects of Roux-en-Y gastric bypass on energy and glucose homeostasis are preserved in two mouse models of functional glucagon-like peptide-1 deficiency. Mol Metab 2013; 3: 191–201.
11. Nakagawa Y, Nagasawa M, Yamada S, et al. Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. PLoS ONE 2009; 4: e5106.
12. Nakagawa Y, Nagasawa M, Mogami H, et al. Multimodal function of the sweet taste receptor expressed in pancreatic β-cells: generation of diverse patterns of intracellular signals by sweet agonists. Endocr J 2013; 60: 1191–1206.
13. Oya M, Suzuki H, Watanabe Y, et al. Amino acid taste receptor regulates insulin secretion in pancreatic β-cell line MIN6 cells. Genes Cells 2011; 16: 608–616.
14. Kyriazis GA, Soundarapandian MM, Tyberg B. Sweet taste receptor signaling in beta cells mediates fructose-induced potentiation of glucose-stimulated insulin secretion. Proc Natl Acad Sci USA 2012; 109: 524–532.
15. Asfari M, Janjic D, Meda P, et al. Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 1992; 130: 167–178.
16. Wollheim CB, Meda P, Halban PA. Isolation of pancreatic islets and primary culture of the intact microorgans or of dispersed islet cells. Methods Enzymol 1990; 192: 188–223.
17. Nagamatsu S, Nakamichi Y, Yamamura C, et al. Decreased expression of t-SNARE, syntaxin 1, and SNAP-25 in pancreatic beta-cells is involved in impaired insulin secretion from diabetic GK rat islets: restoration of decreased t-SNARE proteins improves impaired insulin secretion. Diabetes 1999; 48: 2367–2373.
18. Kawaguchi M, Minami K, Nagashima K, et al. Essential role of ubiquitin-proteasome system in normal regulation of insulin secretion. J Biol Chem 2006; 281: 13015–13020.
19. Nishimura W, Yao I, Iida J, et al. Interaction of synaptic scaffolding molecule and Beta-catenin. J Neurosci 2002; 22: 757–765.
20. Nishimura W, Kondo T, Salameh T, et al. A switch from MaB to MafA expression accompanies differentiation to pancreatic beta-cells. Dev Biol 2006; 293: 526–539.
21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 2001; 25: 402–408.
22. Feldman JM, Lebovitz HE. Mechanism of epinephrine and serotonin inhibition of insulin release in the golden hamster in vitro. Diabetes 1970; 19: 480–486.
23. Peterhoff M, Sieg A, Brede M, et al. Inhibition of insulin secretion via distinct signaling pathways in alpha2-adrenergic receptor knockout mice. Eur J Endocrinol 2003; 149: 343–350.
24. Clapp TR, Trubey KR, Vandenbeuch A, et al. Tonic activity of Gaα-gustducin regulates taste cell responsivity. FEBS Lett 2008; 582: 3783–3787.
25. Attané C, Peyot ML, Lussier R, et al. Differential insulin secretion of high-fat diet-fed C57BL/6NN and C57BL/6NJ mice: implications of mixed genetic background in metabolic studies. PLoS ONE 2016; 11: e0159165.
26. Meloni AR, DeYoung MB, Lowe C, et al. GLP-1 receptor activated insulin secretion from pancreatic β-cells: mechanism and glucose dependence. Diabetes Obes Metab 2013; 15: 15–27.
27. Remaury A, Larrouy D, Daviaud D, et al. Coupling of the alpha 2-adrenergic receptor to the inhibitory G-protein Gi and adenylate cyclase in HT29 cells. Biochem J 1993; 15: 283–288.
28. Sallam N, Fisher A, Golbidi A, et al. Weight and inflammation are the major determinants of vascular dysfunction in the aortae of db/db mice. Naunyn-Schmied Arch Pharmacol 2011; 383: 483–492.
29. Nakagawa Y, Ohtsu Y, Nagasawa M, et al. Glucose promotes its own metabolism by acting on the cell-surface glucose-sensing receptor T1R3. Endocr J 2013; 60: 1191–1206.
30. Huang L, Shanker YG, Dubauskaite J, et al. Gamma13 colocalizes with gustducin in taste receptor cells and mediates IP3 responses to bitter denatonium. Nat Neurosci 1999; 2: 1055–1062.
31. Reports ADA. Position of the American Dietetic Association: Use of nutritive and nonnutritive sweeteners. J Am Diet Assoc 2004; 104: 255–275.
32. Wong GT, Gannon KS, Margolskee RF. Transduction of bitter and sweet taste by gustducin. Nature 1996; 381: 796–800.
33. Li Y, Kokrashvili Z, Mosinger B, et al. Gustducin couples fatty acid receptors to GLP-1 release in colon. Am J Physiol Endocrinol Metab 2013; 67: 651–660.