

Return of the glucoreceptor: Glucose activates the glucose-sensing receptor T1R3 and facilitates metabolism in pancreatic β -cells

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ABSTRACT

Subunits of the sweet taste receptor, namely T1R2 and T1R3, are expressed in mouse pancreatic islets. Quantitatively, the expression of messenger ribonucleic acid for T1R2 is much lower than that of T1R3, and immunoreactive T1R2 is in fact undetectable. Presumably, a homodimer of T1R3 could function as a signaling receptor. Activation of this receptor by adding an artificial sweetener, sucralose, leads to an increase in intracellular adenosine triphosphate ([ATP]_c). This increase in [ATP]_c is observed in the absence of ambient glucose. Sucralose also augments elevation of [ATP]_c induced by methylsuccinate, a substrate for mitochondria. Consequently, activation of T1R3 promotes metabolism in mitochondria and increases [ATP]_c. 3-O-Methylglucose, a non-metabolizable analog of glucose, also increases [ATP]_c. Conversely, knockdown of T1R3 attenuates elevation of [ATP]_c induced by glucose. Hence, glucose promotes its own metabolism by activating T1R3 and augmenting ATP production. Collectively, a homodimer of T1R3 functions as a cell surface glucose-sensing receptor and participates in the action of glucose on insulin secretion. The glucose-sensing receptor T1R3 might be the putative glucoreceptor proposed decades ago by Niki *et al.* The glucose-sensing receptor is involved in the action of glucose and modulates glucose metabolism in pancreatic β -cells.

INTRODUCTION

Fuel metabolism is tightly regulated in the body to maintain energy homeostasis. Dysregulation of the fuel metabolism leads to a variety of metabolic disorders including diabetes mellitus, dyslipidemia, metabolic syndrome, atherosclerosis, stroke, chronic kidney disease and cardiovascular diseases. The most important regulator of fuel metabolism is insulin, a polypeptide hormone secreted from pancreatic islet β -cells¹. Thus, insulin controls carbohydrate metabolism, and also regulates metabolism of other nutrients including amino acids, proteins and lipids. With regard to carbohydrate metabolism, production and secretion of insulin are strictly controlled by glucose, a major fuel in the body, and elevation of the plasma glucose concentration augments insulin secretion. In contrast, insulin maintains the plasma glucose concentration in a relatively narrow range by stimulating glycogen synthesis in the liver, and promoting glucose uptake in skeletal muscle and adipose tissues.

Pancreatic β -cells thus function as fuel sensors and detect changes in the plasma concentrations of not only glucose, but also amino acids and lipids including fatty acids. In this regard, it is well known that pancreatic β -cells express cell surface G protein-coupled receptors (GPCRs) detecting long-chain fatty acids^{2–4} and various types of amino acids^{5–7}. These GPCRs function as cell surface nutrient sensors and thereby modulate secretion of insulin. Given that β -cells express a variety of GPCR sensors for lipids and amino acids, it seems quite possible that β -cells express GPCR that functions as a sugar sensor. These considerations led us to hypothesize that the sugar-sensing receptor would be expressed on the cell surface of pancreatic β -cells.

SWEET TASTE RECEPTOR IN PANCREATIC β -CELLS

To determine whether or not the sugar-sensing receptor is expressed in pancreatic β -cells, we investigated the expression of the sweet taste receptor in pancreatic β -cells. The sweet taste receptor is expressed in the taste cells of taste buds in the

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tongue, and detects sweet substances in the oral cavity^{8,9}. The sweet taste receptor senses sweet molecules, such as sugars including sucrose, glucose and fructose. It also senses sweet amino acids, sweet proteins and various artificial sweeteners with diverse chemical structures. Structurally, the sweet taste receptor is thought to be a heterodimer of T1R2 and T1R3, both of which belong to the class C GPCR, which contains a large extracellular domain^{10–12}. When the messenger ribonucleic acid (mRNA) expression of the sweet taste receptor subunits was measured in mouse pancreatic islets and in insulin-secreting MIN6 cells, mRNA for both T1R2 and T1R3 was detected¹³. Immunohistochemically, immunoreactivity of T1R3 was observed mainly in β -cells in mouse islets, and insulin-producing MIN6 cells were also positive for immunoreactive T1R3¹³. It is thought that pancreatic β -cells express the sweet taste receptor. With regard to its function, stimulation of the receptor by adding an artificial sweetener sucralose resulted in an increase in insulin secretion in both mouse pancreatic islets and in MIN6 cells, showing that the sweet taste receptor in β -cells was functionally active. It should be noted that relatively high concentrations of sucralose were required to stimulate insulin secretion. In any event, the sugar-sensing sweet taste receptor is expressed in pancreatic β -cells, and an activation of the receptor augments insulin secretion¹³. The signal transduction mechanism of the sweet taste receptor in β -cells was investigated in MIN6 cells by monitoring changes in intracellular concentrations of Ca^{2+} and cyclic adenosine monophosphate (cAMP), and translocation of a protein kinase C myristoylated alanine-rich C-kinase substrate. Interestingly, activation of the sweet taste receptor led to increases in both Ca^{2+} and cAMP. Simultaneously, translocation of myristoylated alanine-rich C-kinase substrate was also induced by sucralose. These results show that the sweet taste receptor expressed in β -cells is coupled to both Ca^{2+} and cAMP messenger systems¹³.

Subsequently, we examined the expression of subunits of the sweet taste receptor expressed in β -cells more precisely¹⁴. When the expression of mRNA for T1R2 and T1R3 in pancreatic islets was measured by quantitative reverse transcription polymerase chain reaction, the expression of T1R2 was <1% of that of T1R3¹⁴. Consistent with this observation, immunoreactive T1R2 was undetectable in β -cells, whereas immunoreactive T1R3 was abundantly found in β -cells¹⁴. This raises the possibility that, unlike in taste cells of the tongue, a major component of the 'sweet taste receptor' expressed in pancreatic β -cells might be a homodimer of T1R3 rather than a heterodimer. In accordance with this notion, knockdown of T1R3 by using short hairpin RNA (shRNA) abolished the effect of artificial sweeteners in MIN6 cells, whereas knockdown of T1R2 had little effect¹⁵. These results suggest that the sweet taste receptor in β -cells is a homodimer of T1R3. It was proposed originally that a homodimer of T1R3 was not functional¹⁰. However, a recent study showed that a homodimer of T1R3 was indeed functional¹⁶. We also examined whether or not a homodimer of T1R3 functions as a signaling receptor by transfecting T1R3

alone in HEK cells¹⁷. The results clearly showed that the homodimer of T1R3 was able to transmit the signal of sweet molecules¹⁷. At present, we feel it reasonable that a homodimer of T1R3 functions as a 'sweet taste-sensing receptor' in pancreatic β -cells. In a strict sense, we cannot totally rule out the possibility the T1R3 forms a heterodimer with another type of class C GPCR; for example, the metabotropic glutamate receptor or Ca^{2+} -sensing receptor.

An important question as to the function of the sweet taste-sensing receptor, a homodimer of T1R3, in pancreatic β -cells is whether or not glucose at physiological concentrations activates the receptor. In this regard, the sweet taste receptor expressed in taste cells of the tongue is activated by sugars, but the glucose-sensitivity of the receptor is not remarkable^{8,9,18}. Accordingly, if the sweet taste-sensing receptor in β -cells has the same property as the sweet taste receptor expressed in taste cells, the receptor might not be activated by glucose at physiological concentrations¹⁸. Nevertheless, as the sweet taste-sensing receptor expressed in β -cells is a homodimer of T1R3, and is slightly different from that expressed in taste cells of the tongue, it is an open question whether or not physiological concentration of glucose activates the receptor. This issue should be examined experimentally. When the effect of glucose on insulin secretion was examined in MIN6 cells, knockdown of T1R3 significantly reduced glucose-induced insulin secretion¹⁹ (Figure 1). Furthermore, glucose-induced insulin secretion from mouse pancreatic islets was inhibited significantly by an addition of gurmardin¹⁴, an inhibitor of the sweet taste receptor²⁰. Involvement of T1R3 in glucose-evoked insulin secretion was also shown in islets

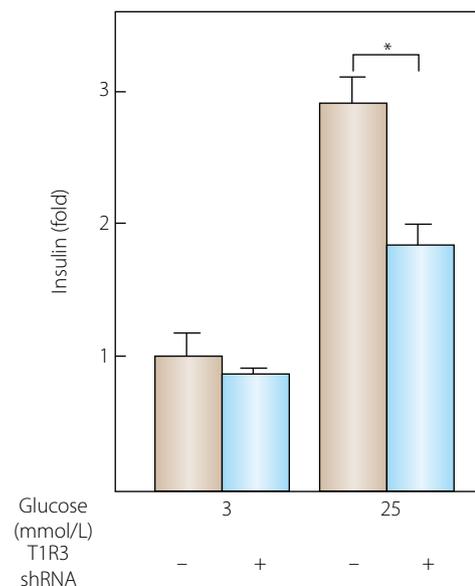


Figure 1 | Effect of knockdown of T1R3 on glucose-induced insulin secretion in MIN6 cells (□)¹⁹. Insulin secretion induced by 25 mmol/L glucose was measured in MIN6 cells. T1R3 (□) was knocked down by using short hairpin ribonucleic acid (shRNA). * $P < 0.05$.

obtained from T1R3-null mouse²¹. Geraedts *et al.*²¹ measured exocytosis of insulin granules in T1R3-null β -cells and found that exocytosis of insulin granules induced by glucose was markedly delayed in T1R3-null β -cells. Collectively, it is clear that T1R3 is involved in the action of glucose in pancreatic β -cells. This shows that T1R3 is activated by glucose at physiological concentrations. We therefore designated a homodimer of T1R3 as the 'glucose-sensing receptor'¹⁹.

REGULATION OF GLUCOSE METABOLISM BY THE GLUCOSE-SENSING RECEPTOR

When T1R3 was inhibited by gurmarin, insulin secretion induced by 16.7 mmol/L glucose was reduced by approximately 65%¹⁴. It is well known that glucose induces biphasic insulin secretion^{22,23}. When glucose-induced insulin secretion was measured in a perfusion system, T1R3 inhibitor gurmarin inhibited both the first and second phases of insulin secretion (Nakagawa Y and Kojima I, unpublished observation, 2013). Hence, T1R3 might be involved in rapid and sustained actions of glucose in β -cells. As glucose-induced insulin secretion is totally dependent on glucose metabolism^{24,25}, we made a hypothesis that the glucose-sensing receptor T1R3 would modify glucose metabolism in pancreatic β -cells. We examined this possibility by monitoring the concentration of intracellular ATP ($[ATP]_c$) in glucose-responsive MIN6 cells expressing firefly luciferase¹⁹. We first administered a T1R3 agonist, sucralose. Sucralose is an artificial sweetener and is not metabolized in the cells²⁶. Indeed, sucralose induced a rapid and marked elevation of $[ATP]_c$ in the presence of 5.5 mmol/L glucose (Figure 2). The effect of sucralose was observed at a concentration of 0.3 mmol/L, and $[ATP]_c$ response induced by 5 mmol/L sucralose was greater than that induced by 25 mmol/L glucose. Given that sucralose activates the glucose-sensing receptor T1R3¹³, but is not metabolized in β -cells, activation of the glucose-sensing receptor T1R3 might have facilitated glucose-metabolism and thereby increased $[ATP]_c$. Consistent with this notion, knockdown of T1R3 attenuated elevation of $[ATP]_c$ induced by sucralose¹⁹.

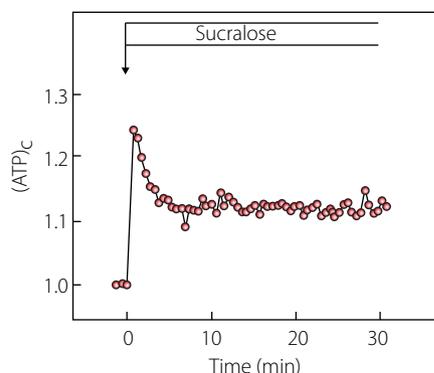


Figure 2 | Effect of sucralose on intracellular adenosine triphosphate ($[ATP]_c$) in MIN6 cells¹⁹. MIN6 cells expressing luciferase were stimulated by 5 mmol/L sucralose and changes in $[ATP]_c$ were monitored.

To address the site of sucralose action, we removed ambient glucose and observed the effect of sucralose on $[ATP]_c$. When sucralose was added in the absence of ambient glucose, sucralose was still able to increase $[ATP]_c$. This shows that sucralose increases $[ATP]_c$ not simply by activating glucokinase, a rate-limiting step in the glycolytic pathway. To our surprise, the sucralose-induced elevation of $[ATP]_c$ was similarly observed when ambient glucose was removed 1 h before the stimulation. In this condition, availability of glucose-6-phosphate either from glucose or glycogen, if any, might be negligible. This result suggests that sucralose increases $[ATP]_c$ by not simply increasing the availability of glucose-6-phosphate. The site of action of sucralose might be located downstream of glucose-6-phosphate. To address the involvement of mitochondria, we added methylsuccinate, a membrane-permeable analog of succinate. As shown in Figure 3, methylsuccinate induced a sustained elevation of $[ATP]_c$. When sucralose was added together with methylsuccinate, the resultant increase in $[ATP]_c$ was marked. In fact, the effect of a combination of sucralose and methylsuccinate was more than the additive of $[ATP]_c$ responses induced by respective agents. Hence, a signal derived from the glucose-sensing receptor greatly facilitates the metabolism of succinate, and augments ATP production. In this regard, the glucose-sensing receptor signal could activate the metabolism in mitochondria and increase the ATP production. A critical question is whether or not glucose actually activates the glucose-sensing receptor and thereby facilitates the metabolism. To address this issue, we administered 3-*O*-methylglucose, a non-metabolizable analog of glucose, and monitored changes in $[ATP]_c$. As shown in Figure 4, although 3-*O*-methylglucose is not catalyzed by glucokinase, it significantly increased ATP. This result clearly shows that glucose in fact activates the glucose-sensing receptor and facilitates its own metabolism. When glucose-induced elevation of $[ATP]_c$ is observed, it means that the two events take place simultaneously: one is a rapid activation of the glucose-

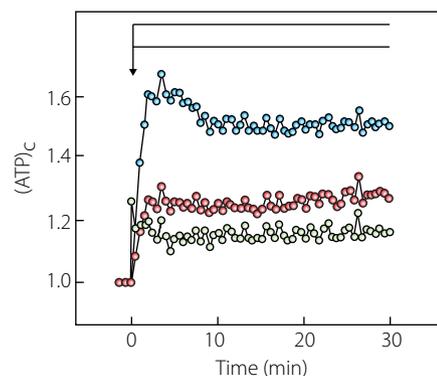


Figure 3 | Effect of methylsuccinate and sucralose on intracellular adenosine triphosphate ($[ATP]_c$) in MIN6 cells¹⁹. MIN6 cells expressing luciferase were stimulated by 10 mmol/L methylsuccinate (●), 3 mmol/L sucralose (○), and a combination of methylsuccinate and sucralose (◐). Changes in $[ATP]_c$ were monitored.

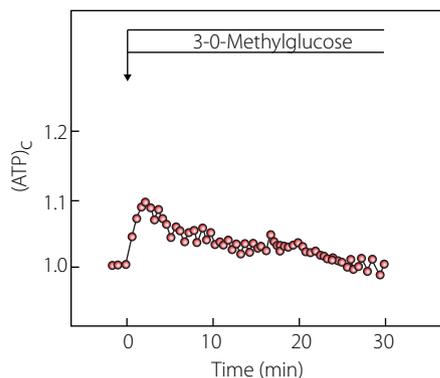


Figure 4 | Effect of 3-O-Methylglucose on intracellular adenosine triphosphate ([ATP]_c) in MIN6 cells¹⁹. MIN6 cells expressing luciferase were stimulated by 25 mmol/L 3-O-methylglucose, and changes in [ATP]_c were monitored.

sensing receptor and subsequent facilitation of the glucose metabolism; and the second is metabolism of glucose through an already activated metabolic pathway. Synergic interaction of two pathways could be a basis for glucose-induced insulin secretion. Consistent with this notion, knockdown of the glucose-sensing receptor, T1R3, significantly reduced elevation of [ATP]_c induced by glucose (Figure 5). Based on these observations, we made a new model for the action of glucose in pancreatic β -cells (Figure 6). Glucose first acts on the cell surface glucose-sensing receptor, T1R3, and facilitates the metabolism presumably by activating the metabolism in mitochondria. Then glucose enters β -cells through glucose transporters, and is then metabolized through the already facilitated metabolic pathway¹⁹.

An important point in our new model is that glucose exerts its effect by acting on two pathways, and both pathways act synergistically to stimulate insulin secretion. The new model includes the currently established model, which states that the effect of glucose is dependent on its metabolism. It is also consistent with many previous observations showing that inhibition of glucose metabolism blocks glucose-induced insulin secretion^{24,25}. The new model extends in that the full activity of glucose also depends on the glucose-sensing receptor signals. The model explains why exocytosis of insulin induced by glucose is delayed and blunted in β -cells obtained from T1R3-null mice²¹. In addition, the model explains why stimulation of the glucose-sensing receptor by sucralose augments glucose-evoked insulin secretion¹³. Based on this model, it is expected that any increase in the expression levels of T1R3 would augment glucose-induced insulin secretion. Studies on the expression levels of T1R3 in pancreatic β -cells showed that the expression levels of T1R3 change significantly depending on nutritional states. For example, the expression of T1R3 in β -cells is high in fasting mice and decreases rather quickly after the intake of food¹⁴. In other words, the expression of T1R3 is high when intake of carbohydrates is required. Consistent with this change, the

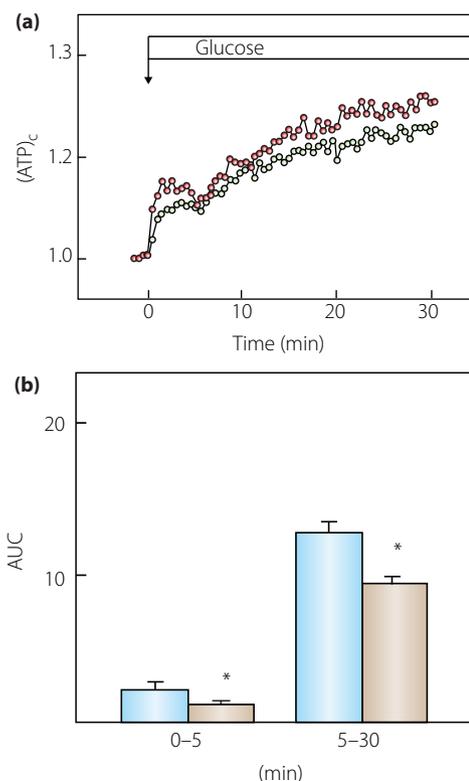


Figure 5 | Effect of knockdown of T1R3 on glucose-induced elevation of intracellular adenosine triphosphate ([ATP]_c)¹⁹. (a) [ATP]_c response to 25 mmol/L glucose was measured in MIN6 cells (●) or T1R3-knocked down MIN6 cells (○). (b) Experiments were carried out as shown in (a), and area under the curve (AUC) from 0 to 5 min and from 5 to 30 min were calculated. (■), MIN6 cells; (□), T1R3-knocked down MIN6 cells. **P* < 0.05 vs control.

amount of insulin secreted in response to glucose is higher in the fasting state compared with the fed state¹⁴, and the effect of inhibitor of T1R3 on insulin secretion is greater compared with that in fed mice. Glucose-sensing receptor expressed in β -cells might show diurnal changes depending on the timing of feeding. From a physiological point of view, this is a reasonable regulation, as a larger amount of insulin is secreted when the demand of carbohydrate intake is higher. It should be noted that these rapid changes in the expression of T1R3 are not accompanied by changes in the mRNA levels. Presumably, short-term downregulation of T1R3 after feeding might be a result of post-transcriptional events. In addition to the short-term regulation, the expression levels of T1R3 were reduced in β -cells obtained from animals with type 2 diabetes including Goto-Kakizaki rats¹⁴. In these animals, the expression of T1R3 was reduced in both mRNA and protein levels. Indeed, reduced expression of T1R3 was recovered by treatment of these diabetic animals with insulin. These results suggest that chronic exposure of β -cells to hyperglycemia downregulates T1R3 by a transcriptional mechanism. In any event, the expression of the

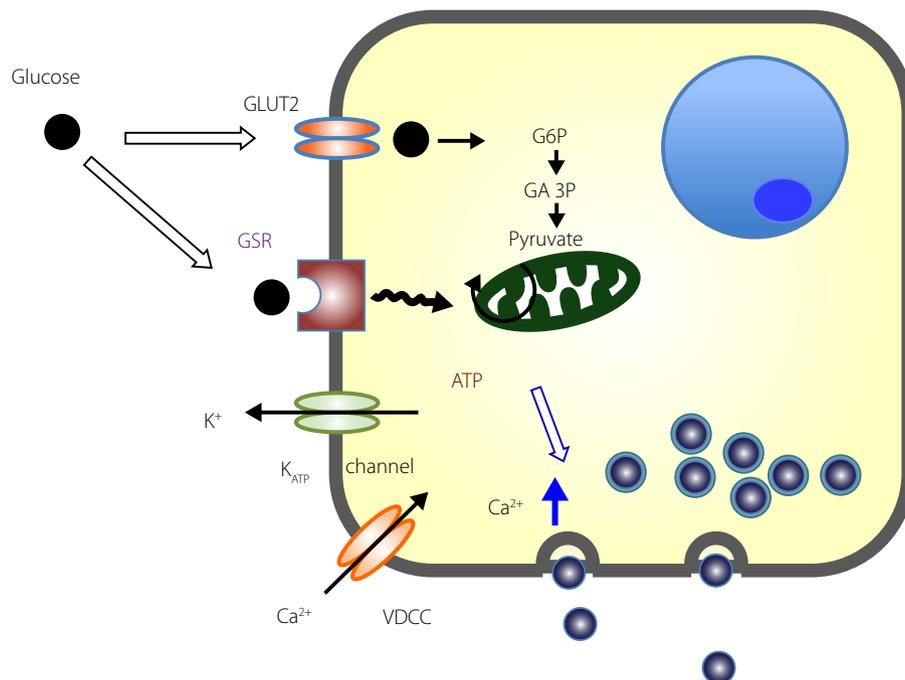


Figure 6 | Schematic presentation of the action of glucose¹⁹. Glucose first acts on the cell surface glucose-sensing receptor (GSR), which generates a signal(s) to facilitate metabolism in mitochondria. Glucose then enters the cell through the glucose transporter, and is then metabolized through an already primed metabolic pathway. An increase in the amount of substrate together with the signal through the GSR leads to production of adenosine triphosphate (ATP). GLUT2, glucose transporter 2; K_{ATP} channel, adenosine triphosphate-sensitive K⁺ channel; VDCC, voltage-dependent Ca²⁺ channel.

glucose-sensing receptor, T1R3, is affected by various nutritional and metabolic states.

T1R3 FUNCTIONS AS THE 'GLUCORECEPTOR'

Elucidation of the function of T1R3 as a glucose-sensing receptor reminds us of the putative cell surface 'glucoreceptor' proposed some decades ago. The glucoreceptor represents a molecule(s) in β -cells that binds to and senses glucose. The glucoreceptor then transmits some signals into the cells, and eventually induces exocytosis of insulin presumably by changing the ion fluxes of β -cells. There were two major models as to the glucoreceptor. One is the regulator site model and another is the substrate site model¹. According to the regulator site model, it was thought that glucose binds to the cell surface glucoreceptor and activates the receptor molecule. The molecular nature and function of the cell surface glucoreceptor were, however, not determined in those days because of the technical limitation. In this regard, Niki *et al.*²⁷ showed that β -cells discriminated α - and β -anomers of D-glucose. Thus, they showed that α -anomer is a more efficient stimulator of insulin secretion compared with β -anomer. They also showed that β -cells recognize α - and β -anomers of mannose²⁸. Their findings are consistent with the notion that β -cells express a molecule that recognizes the fine structure of hexose anomers. Subsequently, Matchinsky²⁴ and others²⁹ reported that enzymes involved in the glycolytic pathway, such as phosphoglu-

cose isomerase²⁹ and glucokinase²⁴, are able to discriminate anomers of glucose. They postulated that the nature of the glucoreceptor is an enzyme catalyzing glucose (substrate site model)¹. Given that, however, intracellular conversion of α -anomer to β -anomer is quite rapid, it seems rather difficult to assume that glycolytic enzymes could be responsible for the preferential effect of α -anomer over β -anomer in glucose-induced insulin secretion. Although the true molecular nature of the glucoreceptor was not elucidated in those days, Niki and Niki³⁰ realized an important issue; that is, the similarity of β -cells and taste cells of the tongue in terms of recognition of glucose. In taste cells of the tongue, sweet molecules, such as sugars, are recognized by a cell surface receptor molecule, namely the sweet taste receptor⁸. This receptor is capable of discriminating α - and β -anomers of hexose³¹. Thus, α -D-glucose is sweeter than β -D-glucose. Niki *et al.*^{32,33} then investigated whether or not inhibitors of the sweet taste receptor also modified the action of glucose on insulin secretion in β -cells. Indeed, they found that α -anomers of *p*-nitrophenyl-D-glucopyranoside (PNP-Glu)³² and *p*-nitrophenyl-D-mannopyranoside (PNP-Man)³³ dose-dependently inhibited glucose-induced insulin secretion in pancreatic β -cells without affecting glucose oxidation. They also showed that β -anomers of PNP-Glu and PNP-Man were ineffective. They further showed that other competitive inhibitors of sweet taste sensation, namely methyl-4, 6-dichloro-4, 6-dideoxyglucopyranoside

and methyl-4, 6-dichloro-4, 6-dideoxy-D-garactopyranoside, also inhibited glucose-induced insulin secretion³³. These compounds do not enter β -cells because of the molecular structure. These results strongly suggest that β -cells express a cell surface molecule that recognizes glucose and other sugars in a manner similar to that of the sweet taste receptor in taste cells. Niki *et al.*³⁴ also pointed out an important issue, namely that recognition of the anomeric structure of glucose is impaired in diabetes mellitus. They further showed that impaired recognition of glucose anomers was recovered to some extent when hyperglycemia was corrected in Goto–Kakizaki rats, a rat model of type 2 diabetes³⁵.

When we characterize the glucose-sensing receptor, T1R3, expressed in pancreatic β -cells, there are lines of similarities between the glucose-sensing receptor T1R3 and the glucoreceptor proposed by Niki *et al.* First, as T1R3 is a component of the sweet taste receptor, it is not surprising that it is able to discriminate α - and β -anomers of glucose. Second, the glucose-sensing receptor, T1R3, recognizes glucose and mannose, as does the glucoreceptor. Third, inhibitors of the sweet taste receptor used by Niki *et al.*^{32,33} do inhibit the activity of the glucose-sensing receptor, T1R3, and attenuate the action of glucose in β -cells (Nakagawa Y and Kojima I, unpublished observation, 2013). Fourth, expression of the glucose-sensing receptor is reduced in animal models of type 2 diabetes, including Goto–Kakizaki rats, and correction of hyperglycemia recovers the expression of the glucose-sensing receptor¹⁴. Based on these considerations, it seems reasonable to conclude that the glucose-sensing receptor, T1R3, represents the glucoreceptor described by Niki *et al.*^{27–30,32–35}. The glucoreceptor modifies metabolism of glucose and augments ATP production. Furthermore, as activation of the glucoreceptor induces changes in Ca^{2+} fluxes, increases cAMP and activates C-kinase, it is also possible that those signals directly modify exocytosis of insulin granules.

INTRACELLULAR SIGNALING SYSTEM ACTIVATED BY THE GLUCOSE-SENSING RECEPTOR

As aforementioned, activation of the glucose-sensing receptor leads to increases in $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ ¹³. Elevation of $[\text{Ca}^{2+}]_c$ is largely dependent on Ca^{2+} entry through voltage-dependent Ca^{2+} channels (VDCC). Also, Ca^{2+} entry is dependent on extracellular Na^+ . Presumably, activation of the sweet taste receptor somehow stimulates Na^+ entry by activating a Na^+ -permeable channel. Resultant depolarization causes activation of the nifedipine-sensitive VDCC¹³. The glucose-sensing receptor also increases $(\text{cAMP})_c$, and this is presumably mediated by activation of G_s .

It is known that the sweet taste receptor is activated by a variety of sweet molecules with different chemical structures⁹. An interesting question is whether or not these varieties of sweet molecules induce a common set of changes in intracellular messengers. In other words, it is an interesting question whether or not all of the sweet molecules induce uniform signals inside the cells. However, this question has never been

answered in taste cells. We addressed this question in β -cells by stimulating the glucose-sensing receptor, T1R3, with different sweet molecules¹⁵. We stimulated MIN6 cells expressing T1R3 by four sweet agonists with different chemical structures, namely sucralose, acesulfame-K, saccharin and glycyrrhizin, and measured changes in $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ ¹⁵. To our surprise, four sweet molecules activated the glucose-sensing receptor in different ways and induced different patterns of changes in $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$. For example, both sucralose and acesulfame-K elevated $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$. In contrast, saccharin increased $[\text{cAMP}]_c$ but not $[\text{Ca}^{2+}]_c$, whereas glycyrrhizin increased $[\text{Ca}^{2+}]_c$ without affecting $[\text{cAMP}]_c$. Furthermore, although sucralose and acesulfame-K elevated both $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$, the mode of actions of these two agonists on $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ was different. Collectively, the glucose-sensing receptor in MIN6 responds to four sweet agonists in different manners, and evokes distinct patterns of intracellular signals. Sweet agonists act as biased agonists, and induce complex changes in the intracellular signaling pathways. This is a unique property of T1R3, and provides new information as to the function of class C GPCR. In this regard, it is possible that the sweet taste receptor in taste cells of the tongue behaves similarly. This notion should be examined experimentally. Our recent study showed that the glucose-sensing receptor, T1R3, has an additional property. We examined the effect of the same four sweeteners on $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ in T1R3-expressing Hutu-80 cells, which secrete glucagon-like peptide-1 (GLP-1)³⁶. As in MIN6 cells, Hutu-80 cells predominantly expressed T1R3 and secrete GLP-1 in response to four sweet molecules³⁶. When the changes in $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ were measured, the four sweet molecules indeed all showed distinct patterns of $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ as in MIN6 cells. In addition, when compared with their effects in MIN6 cells, the patterns of changes in $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ observed in Hutu-80 cells were different. For example, saccharin increased both $[\text{Ca}^{2+}]_c$ and $[\text{cAMP}]_c$ in Hutu-80 cells³⁶. Hence, signals generated by the same agonists are different in two types of cells. Taken together, intracellular signals generated by the glucose-sensing receptor are quite diverse, and they are dependent on the types of agonists and types of cells expressing the receptor. In other words, coupling of the glucose-sensing receptor to transducers, for example G proteins, is dependent on the types of agonists and types of cells expressing the cells. It is now known that the glucose-sensing receptor, T1R3, is expressed in many types of cells regulating energy metabolism^{13,17,36,37}, and intracellular signals produced by sweet molecules should be evaluated precisely. In this regard, we still have few data as to the intracellular signals generated by the glucose-sensing receptor stimulated by glucose. Intracellular signals evoked by glucose in β -cells should be re-evaluated extensively.

PERSPECTIVES

Many issues still remain unsolved. First, the molecular nature of the glucose-sensing receptor is not totally identified.

Although we have shown that the major component of the glucose-sensing receptor is a homodimer of T1R3, it remains possible that other members of the class C GPCR form a heterodimer with T1R3, and also function as the glucose-sensing receptor. Possible candidates for heterodimerization are the Ca²⁺-sensing receptor, metabotropic glutamate receptor 5 and T1R2. These GPCRs are expressed in pancreatic β -cells^{13,38,39}, and could possibly form a heterodimer with T1R3. In this regard, Kyriazis *et al.*³⁹ suggested that a heterodimer of T1R2 and T1R3 functions as the sweet taste receptor in β -cells, and is involved in fructose-mediated potentiation of insulin secretion. Specifically, they showed that fructose action was impaired in T1R2-knockout mice. This observation is apparently contradictory to our proposal that a homodimer of T1R3 functions as the glucose-sensing receptor. It is possible that a heterodimer of T1R2 and T1R3 is actually formed in β -cells, although the amount of heterodimer might be much lower compared with a homodimer of T1R3. It is therefore possible that the heterodimer of T1R2 and T1R3 is activated by fructose. In a strict sense, however, we have not examined whether or not either a T1R3 homodimer or a T1R2-T1R3 heterodimer is activated by fructose. This should be examined experimentally. Kyriazis *et al.*⁴⁰ recently showed that in T1R2 knockout mice, basal insulin secretion was increased. They postulated that the 'sweet taste receptor' is involved in the negative regulation of basal secretion of insulin. Their results are apparently contradictory to their previous work stating that activation of the T1R2-T1R3 heterodimer augments insulin secretion³⁹. In any case, the expression of T1R2 in mouse islets is much lower than that of T1R3, and deletion of T1R2 would increase the T1R3 homodimer. Therefore, interpretation of the results obtained in T1R2 knockout mice should be done with caution. Additional studies are required to understand the underlying reasons for apparent contradictory results. Second, our previous study showed that the glucose-sensing receptor activates complex signaling pathways in pancreatic β -cells¹⁵. Depending on types of agonists or possibly their binding sites in the receptor, various types of signaling molecules were activated. At present, however, little is known about the mechanism by which the glucose-sensing receptor activates different signaling cascades. Presumably, different agonists activate different sets of G-proteins, and activate downstream effectors depending on the conformational changes induced by the binding of agonists. We need to identify the switching mechanism. Also, the site in T1R3 to which various agonists, including glucose, bind should be identified in order to elucidate the molecular mechanism for differential activation of G proteins. In this regard, we still do not know which of the intracellular signals evoked by glucose is in fact mediated by the glucose-sensing receptor.

Third, we still do not know how the glucose-sensing receptor augments glucose metabolism. We already know that the glucose-sensing receptor facilitates metabolism of succinate in mitochondria¹⁶. Also, the receptor activates a metabolic pathway downstream of glucose-6-phosphate. We need to identify

the metabolic steps regulated by the glucose-sensing receptor. In this regard, sucralose is able to increase [ATP]_c in the absence of ambient glucose¹⁶. This suggests that sucralose delivers some substrates from (a) compound(s) outside the glycolytic pathway or the tricarboxylic acid cycle. We need to identify the source(s) of substrate for ATP production. In addition, we have to understand how the glucose-sensing receptor activates the metabolism. We need to identify which type(s) of intracellular signal(s) is/are responsible for the activation of the metabolism of glucose and related compounds.

Fourth, recent studies including ours showed that the expression of the glucose-sensing receptor is altered in various nutritional and metabolic conditions^{14,40}. As changes in the expression of the glucose-sensing receptor would alter the secretory responses of insulin, molecular mechanism by which metabolic changes affect the expression should be clarified. Obviously, further studies are required to elucidate the role of this receptor in physiological regulation of insulin secretion and impaired secretion in various metabolic disorders.

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