Current Topics

Recent Progress in the Research of Insulin Secretion

Glucose-Sensing Receptor T1R3: A New Signaling Receptor Activated by Glucose in Pancreatic β-Cells

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Subunits of the sweet taste receptors T1R2 and T1R3 are expressed in pancreatic β-cells. Compared with T1R3, mRNA expression of T1R2 is considerably lower. At the protein level, expression of T1R2 is undetectable in β-cells. Accordingly, a major component of the sweet taste-sensing receptor in β-cells may be a homodimer of T1R3 rather than a heterodimer of T1R2/T1R3. Inhibition of this receptor by gurmarin or deletion of the T1R3 gene attenuates glucose-induced insulin secretion from β-cells. Hence the T1R3 homodimer functions as a glucose-sensing receptor (GSR) in pancreatic β-cells. When GSR is activated by the T1R3 agonist sucralose, elevation of intracellular ATP concentration ([ATP]i) is observed. Sucralose increases [ATP], even in the absence of ambient glucose, indicating that sucralose increases [ATP], not simply by activating glucokinase, a rate-limiting enzyme in the glycolytic pathway. In addition, sucralose augments elevation of [ATP], induced by methylsuccinate, suggesting that sucralose activates mitochondrial metabolism. Nonmetabolizable 3-O-methylglucose also increases [ATP], and knockdown of T1R3 attenuates elevation of [ATP], induced by high concentration of glucose. Collectively, these results indicate that the T1R3 homodimer functions as a GSR; this receptor is involved in glucose-induced insulin secretion by activating glucose metabolism probably in mitochondria.

Key words insulin secretion; β-cell; glucose; sweet taste receptor; glucose-sensing receptor; calcium

1. SWEET TASTE RECEPTOR IN TASTE CELLS OF THE TONGUE

The sense of sweet taste is quite important for living animals since it provides information that ingested food is nutritious and rich in carbohydrates. Sweet taste is detected in taste cells located in the taste buds of the tongue. Taste buds consist of at least three types of taste cells. Among them, the type II cells express sweet taste receptor subunits and downstream signaling molecules. Type II taste cells transmit the signals to the afferent nerve fiber, which locates close to the type II taste cells. Interestingly, type II cells do not express voltage-dependent calcium channels and typical synaptic formation is not observed.11 On activation, type II taste cells release ATP, which activates P2X receptor expressed in the afferent nerve fibers.21 The key molecule involved in detection of sugars and other sweet substances is the sweet taste receptor located in the tip of the taste cells. The molecular nature of the sweet taste receptor was revealed more than a decade ago by several groups.3–6 It is now established that the sweet taste receptor is a heterodimer of two class-C G protein-coupled receptors (GPCRs), namely T1R2 and T1R3.6,7 In accordance with this notion, deletion of either T1R2 or T1R3 gene results in inhibition of behavioral preference for sweeteners in mice.8,9 It should be noted that sweet sensation remains to some extent in T1R2-knockout and T1R3-knockout mice. Possibly, other types of dimer may function as the sweet taste receptor at least in some occasions.

Both T1R2 and T1R3 are members of the class-C GPCR, which possesses a large N-terminal extracellular domain. This domain is called the “Venus flytrap” domain and is thought to provide ligand-binding sites. The cysteine-rich domain locates between the Venus flytrap domain and the transmembrane segments. Like other members of the class-C GPCR, various ligands including natural sugars and small molecule sweeteners, such as aspartame and neotame, bind to the Venus flytrap domain.30 Indeed, natural sugars including sucrose and glucose bind to the Venus flytrap domain of both T1R2 and T1R3.13 Specifically, Venus flytrap domain of T1R3 binds sucrose with higher affinity than that of T1R2.13 Conversely, Venus flytrap domain of T1R2 binds glucose with higher affinity than that of T1R3.13 Artificial sweeteners aspartame and neotame bind to the Venus flytrap domain of T1R2. In contrast, protein sweeteners such as thaumatin and monellin bind to the cysteine-rich domain.12 In addition, some small molecules including cyclamate and lactisole bind to the transmembrane domain of T1R3.13 In this regard, the sweet taste receptor is quite singular in that various types of sweet molecules bind to multiple sites of the receptor. It is not certain, however, whether ligands binding to different domains of the receptor all activate a common intracellular signaling pathway.

The sweet taste receptor is also expressed in extragustatory organs. Thus the sweet taste receptor and downstream signaling molecules are expressed in the airways.44 For example, T1R3 is expressed in solitary chemosensory cells (SCC).15 These cells resemble taste cells in their morphology but unlike taste cells of the taste buds, SCCs are scattered singly in the

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nasal epithelium, larynx, trachea, and bronchi. In particular, SCCs in upper airway are heavily innervated and formation of typical synapses is observed. In addition to the sweet taste receptor, SCCs express bitter taste receptors T2Rs. Recent studies suggest that SCCs play a significant role in protective airway reflexes. Interestingly, SCCs are activated by bitter substances through activation of T2Rs but are inhibited by sugars and artificial sweeteners through activation of T1R2/3. It is thought that the sweet taste receptor is involved in innate immune responses.

Sweet taste receptors are also expressed in gastrointestinal tract. Thus T1Rs are expressed in enteroendocrine L- and K-cells, which secrete glucagon-like peptide-1 and glucose-dependent insulino-tropic peptide, respectively. The apical portion of these cells is exposed to the gastrointestinal lumen and ingested carbohydrates including glucose activate the sweet taste receptor and thereby stimulate secretion of incretins. Secreted incretins potentiate insulin secretion from pancreatic β-cells. Activation of the sweet taste receptor also upregulates the expression of glucose transporter in intestinal epithelial cells and increases glucose uptake.

Recent studies suggest that the sweet taste receptor is expressed various types of cells regulating carbohydrate metabolism. Thus the sweet taste receptor is expressed in pancreatic β-cells, adipocytes, and glucose-responsive neurons in the hypothalamus.

2. SWEET TASTE RECEPTOR FUNCTIONS AS THE GLUCOSE-SENSING RECEPTOR IN PANCREATIC β-CELLS

Subunits of the sweet taste receptor T1R2 and T1R3 are expressed in pancreatic islets. mRNA for T1R2 and T1R3 as well as α-subunit of gustducin is detected in mouse islets. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that the expression level of T1R2 is ~1% of that of T1R3. Consistent with this observation, immunoreactivity of T1R3 is abundantly found in mouse pancreatic islets while that of T1R2 is not detected. All islet β-cells are positive for immunoreactive T1R3 and some small portion of α-cells are also positive for T1R3. Collectively, T1R3 is the dominant subunit expressed in pancreatic islets and T1R3 is expressed mainly in β-cells. Whether the sweet taste receptor functions as a signaling receptor can be assessed by adding an artificial sweetener such as sucralose, which activates T1R3. Indeed, activation of T1R3 by sucralose upregulates insulin secretion. Hence the sweet taste receptor subunit T1R3 expressed in pancreatic β-cells is functional and activation of the receptor augments insulin secretion. When T1R3 is knocked down by short hairpin RNA (shRNA) in insulin-secreting MIN6 cells, the effect of sucralose is inhibited. In contrast, knockdown of T1R2 did not affect the effect of sucralose. These observations, together with the observation that expression of T1R2 in β-cells is considerably less than that of T1R3, suggest that the majority of functional ‘sweet taste-sensing receptors’ in β-cells comprises a homodimer of T1R3 rather than a heterodimer of T1R2/T1R3. It has been suggested that the T1R3 homodimer is not functional; this notion is based on observations obtained in HEK cells expressing T1R3. Nonetheless, a recent study by Tordoff et al. demonstrates that T1R3 alone expressed ectopically in HEK cells is indeed functional. We have also shown that T1R3 alone expressed in HEK cells functions as a signaling receptor. Collectively, these results suggest that the T1R3 homodimer is able to transmit a signal and activate β-cells. We do not exclude the possibility that a heterodimer of T1R2/T1R3 also functions as a signaling receptor albeit the expression level is quite low. In addition, it is possible that T1R3 forms a heterodimer with other class-C GPCR. In any event, the majority of the ‘sweet taste-sensing receptors’ expressed in pancreatic β-cells seems a homodimer of T1R3.

A crucial question is whether glucose activates the sweet taste-sensing receptor expressed in pancreatic β-cells under physiological conditions. In this regard, we showed that glucose-induced insulin secretion from isolated mouse pancreatic islets is significantly inhibited by gurmarin, an inhibitor of the sweet taste receptor. Geraedts et al. showed that exocytosis of insulin granules induced by glucose is markedly blunted in isolated β-cells obtained from T1R3 knockout mice. Our unpublished observation also suggests that glucose-induced insulin secretion is impaired in isolated islets obtained from T1R3-null mice (Nakagawa and Kojima, unpublished observation). Collectively, glucose action in pancreatic β-cells is dependent on the function of the sweet taste-sensing receptor in β-cells, implying that this receptor is activated by glucose and involved in the action of glucose on insulin secretion. Therefore, we designate this receptor “glucose-sensing receptor” (GSR).

It is well known that glucose-induced insulin secretion by β-cells is dependent on glucose metabolism and the products of glucose metabolism evoke dynamic changes in intracellular signals including Ca2+ and cAMP. For example, elevation of ATP or ADP ratio due to metabolism of glucose closes the ATP-sensitive potassium (KATP) channel, which leads to depolarization of the β-cell plasma membrane. Then, voltage-gated Ca2+ channels are activated, Ca2+ enters the cells, and cytoplasmic Ca2+ concentration ([Ca2+]i) is elevated. The elevation of [Ca2+]i, triggers exocytosis of insulin granules. In addition to these initial events, metabolites of glucose presumably sensitize the exocytotic machinery and augment Ca2+-evoked exocytosis of insulin.

Given that the GSR is involved in the action of glucose in β-cells and the glucose action is dependent on its metabolism, a critical question is how GSR modulates insulin secretion. An interesting possibility is that GSR modulates metabolism of glucose and thereby augments insulin secretion. We addressed this possibility using glucose-responsive MIN6 cells, an insulinoma cell line. We prepared luciferase-expressing MIN6 cells and monitored changes in intracellular concentration of ATP ([ATP]) in living cells. As shown in Fig. 1, high concentration of glucose increases [ATP], in MIN6 cells. The effect of glucose on [ATP], is detected at a concentration of 8.3 mM and observed in a dose-dependent manner. Interestingly, glucose induced a biphasic increase in [ATP]. The initial peak occurs around 1 min, followed by a secondary sustained phase (Fig. 1). We then activated GSR by adding sucralose. To our surprise, sucralose induces a marked elevation of [ATP], (Fig. 2). The effect of sucralose is observed at as low as 0.3 mM, and [ATP] is increased in a dose-dependent manner. Indeed, the effect of 5 mM sucralose is much greater than that of 16.7 mM glucose. Since sucralose is an artificial sweetener, which does not enter β-cells nor is metabolized in β-cells, it
exerts its effect by stimulating GSR. [ATP] is determined by a balance between production and consumption of ATP. It seems rather unlikely that sucralose markedly reduces ATP consumption. The results therefore suggest that activation of GSR leads to an increase in [ATP], probably by stimulating ATP production. Then, a crucial question is whether glucose also activates the receptor and thereby increases [ATP]. As shown in Fig. 3, addition of 3-O-methylglucose (3OMG), a nonhydrolyzable analog of glucose, increases [ATP], 3OMG enters β-cells via the glucose transporter-2 but is not catalyzed by glucokinase. Consequently, 3OMG cannot serve as a substrate for the glycolytic pathway. In contrast, 3OMG tastes sweet and is able to activate T1R3. The results shown in Fig. 3 indicate that a nonmetabolizable glucose is able to promote metabolism and increase [ATP]. Consistent with this notion, knockdown of T1R3 by shRNA results in reduction of glucose-induced elevation of [ATP] (Fig. 4). This result clearly shows that glucose acts on GSR T1R3 and promotes its own metabolism. In other words, glucose is not capable of eliciting its full action in the absence of T1R3. Indeed, knockdown of T1R3 significantly inhibits glucose-induced insulin secretion in MIN6 cells. Based on these results, we postulate a new model for glucose action on insulin secretion (Fig. 5). Glucose first acts on the cell-surface GSR, which primes the metabolic pathway of glucose and induces initial increase in [ATP], (priming phase). Glucose then enters β-cells and is metabolized through already activated metabolic pathway, and produces massive increase in [ATP], which activates well-known ATP-dependent pathway (metabolic phase). Glucose is able to augment insulin secretion even in the absence of the cell-surface GSR; however, initiation of secretion is delayed and the magnitude of the secretory response is blunted.

The full action of glucose is provided by synergic activation of two pathways: receptor-mediated priming of metabolism and ATP-generation through the primed metabolic pathway.
A critical question is how GSR augments glucose metabolism. We only have fragmental information at present. Sucralose, an agonist for T1R3, increases [ATP]i in the presence of 2.7 or 5.5 mM glucose. Interestingly, sucralose is able to increase [ATP]i even in the absence of ambient glucose. As shown in Fig. 6, when luciferase-expressing MIN6 cells are incubated for 60 min in glucose-free medium, basal [ATP]i level is reduced. Nonetheless, sucralose induces a marked elevation of [ATP]i. The magnitude of the response is comparable to that observed in the presence of ambient glucose (Fig. 6). In this condition, intracellular glucose is depleted and glycogen, if any, is also negligible. This implies that sucralose elevates [ATP]i by not simply activating glucokinase, a rate-limiting enzyme in the glycolytic pathway. Instead, sucralose activates a step(s) downstream of glucose-6-phosphate. Since sucralose increases [ATP]i, in the absence of ambient glucose, it perhaps mobilizes substrates from sources other than glucose. As shown in Fig. 7, addition of methylsuccinate leads to an increase in [ATP]i. Methylsuccinate is a membrane-permeable analogue of succinate and serves as a mitochondrial fuel. Interestingly, sucralose acts synergistically with methylsuccinate and markedly increases [ATP]i. Thus activation of GSR promotes metabolism of succinate in mitochondria and augments ATP production from succinate. Sucralose therefore acts on mitochondria and promotes metabolism through the Krebs cycle or activates the reduced nicotinamide adenine dinucleotide (NADH) shuttle. In any case, further study is needed to identify the metabolic step(s) regulated by GSR.

It is also important to understand how activation of GSR leads to facilitation of metabolism in β-cells. Again, we do not have an exact answer for this question at present. This is partly because the intracellular signaling system activated by GSR is very complicated. When we stimulate a GPCR by multiple agonists, either natural or synthetic agonists, they usually induce a uniform set of cellular responses. For example, if we stimulate α1-adrenergic receptor by adding norepinephrine, epinephrine, and phenylephrine, these agonists all induce hydrolysis of polyphosphoinositide and increase [Ca2+]c. Many GPCRs are known to behave in this way. However, there are some exceptions. For example, if we stimulate metabotropic glutamate receptor-1a by either glutamate or Gd3+, these two agonists induce different patterns of changes in intracellular messengers, Ca2+ and cAMP. The situation is much more complicated in the case of the GSR. When we stimulate GSR in β-cells by sucralose, this artificial sweetener increases both [Ca2+]c and [cAMP]. Sucralose increases [Ca2+]c by mobilizing Ca2+ from intracellular pools and also by promoting Ca2+ entry, which is dependent on extracellular Na+. Elevation of
cAMP is due to activation of Gs. The action of sucralose is by itself unique in the sense that it activates both the Ca2+ and cAMP messenger systems. Intriguingly, other GSR agonists elicit different patterns of intracellular responses. When we stimulate GSR by adding saccharin, which probably binds to a different portion of the GSR, this sweetener increases [cAMP], but [Ca2+]i is not changed. Furthermore, when we stimulate GSR by adding glycyrrhizin, a natural sweetener structurally different from sugars, this compound increases [Ca2+]i but cAMP is not changed. Although the precise mechanism is still unclear, GSR agonists induce multiple patterns of changes in intracellular messengers depending on the types of agonists. Perhaps, these agonists bind to different portions of the receptor, and induce different patterns of activation of downstream transducers and effectors. Hence GSR agonists act as biased agonists and induce multiple patterns of changes in intracellular messengers. In this regard, we need to identify which types of intracellular responses are induced by glucose through activation of GSR expressed in β-cells. It is likely that the effects of glucose may be different from those elicited by artificial sweeteners.

It is well known that glucose induces changes in intracellular [Ca2+]i and [cAMP]. Glucose first reduces [Ca2+]i, which is followed by oscillatory elevation of [Ca2+]i. Glucose-induced reduction of [Ca2+]i is due to sequestration of Ca2+ into intracellular pools, likely in endoplasmic reticulum. However, the mechanism by which glucose stimulates sequestration of [Ca2+]i has not been identified. Glucose-induced oscillation of [Ca2+]i is dependent on glucose metabolism. Given that glucose metabolism is modulated by GSR, it is possible that oscillation of [Ca2+]i is also dependent in part on activation of GSR. In any case, the mechanism by which glucose induces changes in ion fluxes in β-cells should be reevaluated. With regard to cAMP, glucose-mediated elevation of cAMP is thought due to elevation of [Ca2+]i, according to the current understanding. However, we have shown that many GSR agonists activate Gs and thereby increase [cAMP]. It is thus possible that glucose also activates Gs by acting on GSR. Again, the mechanism by which glucose elevates [cAMP] should be reevaluated. Elucidation of the signaling systems activated by glucose through stimulation of GSR will help to identify how glucose induces glucose metabolism in pancreatic β-cells.

In summary, T1R3, a subunit of the sweet taste receptor, is expressed in pancreatic β-cells. A homodimer of T1R3 functions as the GSR and is involved in the action of glucose. Elucidation of the function and significance of GSR will provide new insights into our understanding of the action of glucose in pancreatic β-cells.

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Conflict of Interest The authors declare no conflict of interest.

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