Lactisole inhibits the glucose-sensing receptor T1R3 expressed in mouse pancreatic β-cells

Kunihisa Hamano1,2, Yuko Nakagawa1, Yoshiaki Ohtsu1, Longfei Li1, Johan Medina1, Yuji Tanaka2, Katsuyoshi Masuda3, Mitsuhisa Komatsu4 and Itaru Kojima1

1Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-8512, Japan
2Department of General Medicine, National Defense Medical College, Tokorozawa, Japan
3Suntory Institute for Bioorganic Research, Osaka, Japan
4Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

Abstract

Glucose activates the glucose-sensing receptor T1R3 and facilitates its own metabolism in pancreatic β-cells. An inhibitor of this receptor would be helpful in elucidating the physiological function of the glucose-sensing receptor. The present study was conducted to examine whether or not lactisole can be used as an inhibitor of the glucose-sensing receptor. In MIN6 cells, in a dose-dependent manner, lactisole inhibited insulin secretion induced by sweeteners, acesulfame-K, sucralose and glycyrrhizin. The IC50 was ~4 mmol/l. Lactisole attenuated the elevation of cytoplasmic Ca2+ concentration ([Ca2+]c) evoked by sucralose and acesulfame-K but did not affect the elevation of intracellular cAMP concentration ([cAMP]c) induced by these sweeteners. Lactisole also inhibited the action of glucose in MIN6 cells. Thus, lactisole significantly reduced elevations of intracellular [NADH] and intracellular [ATP] induced by glucose, and also inhibited glucose-induced insulin secretion. To further examine the effect of lactisole on T1R3, we prepared HEK293 cells stably expressing mouse T1R3. In these cells, sucralose elevated both [Ca2+]c and [cAMP]c. Lactisole attenuated the sucralose-induced increase in [Ca2+]c but did not affect the elevation of [cAMP]c. Finally, lactisole inhibited insulin secretion induced by a high concentration of glucose in mouse islets. These results indicate that the mouse glucose-sensing receptor was inhibited by lactisole. Lactisole may be useful in assessing the role of the glucose-sensing receptor in mouse pancreatic β-cells.

Key Words

- β-cell
- insulin secretion
- sweet taste receptor
- glucose

Introduction

Glucose is a primary stimulator of insulin secretion in pancreatic β-cells and modulates the effects of incretins and acetylcholine (Ashcroft & Rorsman 1989, Rasmussen et al. 1990, Rorsman 1997, Newsholme et al. 2014). We have shown recently that T1R3, a subunit of the sweet taste receptor (Nelson et al. 2001, Temussi 2007), functions as a glucose-sensing receptor in β-cells (Malaisse 2014, Nakagawa et al. 2014, Kojima et al. 2015). This receptor is activated by glucose, and promotes metabolism of fuels in β-cells leading to an increase in ATP (Nakagawa et al. 2014). Since knockdown of T1R3 attenuates glucose-induced elevation of intracellular ATP ([ATP]c), the glucose-sensing receptor T1R3 is involved in the action of glucose (Kojima et al. 2015). At present, the
physiological role of this receptor in β-cells is not completely elucidated. A specific and useful inhibitor of T1R3 would be helpful in assessing the physiological functions of the glucose-sensing receptor in β-cells.

T1R3 belongs to the class C G protein-coupled receptor and forms a heterodimer with T1R2 or T1R1. The T1R2/T1R3 heterodimer functions as the sweet taste receptor while T1R3/T1R1 functions as the umami receptor (Nelson et al. 2001, Roper 2007, 2013, Temussi 2007). T1R3 is a key molecule in sweet taste perception and many activators and inhibitors were identified (Temussi 2007). Among these inhibitors, lactisole, which is 2-(4-methoxyphenol) propionic acid, inhibits perception of sweet taste by acting on T1R3 (Johnson et al. 1994, Sclafani & Perez 1997, Xu et al. 2004). Jiang et al. (2005) showed that lactisole acts on the transmembrane domain (TMD) of T1R3. They further narrowed down the specific region in TMD that interacts with lactisole and identified critical residues in the TMD of T1R3 (Jiang et al. 2005).

Subunits of the sweet taste receptor are also expressed in extragustatory organs including enteroendocrine cells (Bezencon et al. 2007, Margolskee et al. 2007, Jang et al. 2010), pancreatic β-cells (Nakagawa et al. 2009, Geraedts et al. 2012, Kyriazis et al. 2012) and adipocytes (Masubuchi et al. 2013). Unlike taste cells of the tongue, the expression level of T1R2 is much lower compared to that of T1R3 in these cells (Masubuchi et al. 2013, Nakagawa et al. 2013, Medina et al. 2014, Ohtsu et al. 2014), and a homodimer of T1R3 may function as the sweet taste-sensing receptor (Masubuchi et al. 2013, Nakagawa et al. 2013, Medina et al. 2014, Ohtsu et al. 2014). Specifically in β-cells, the expression of T1R2 is far less than that of T1R3 (Medina et al. 2014). Furthermore, knockdown of T1R3 attenuated the effects of sweeteners whereas knockdown of T1R2 was ineffective (Nakagawa et al. 2013). It is quite likely that a major component of the sweet taste-sensing receptor in β-cells may be a homodimer of T1R3 (Nakagawa et al. 2013, Kojima et al. 2015). The physiological function and its role in the regulation of insulin secretion are largely unknown. In this regard, the inhibitor of the glucose-sensing receptor, if any, is useful to assess the role of the receptor in the regulation of insulin secretion.

To block the glucose-sensing receptor in β-cells, the putative compound should inhibit the homodimer of T1R3. Among various inhibitors of sweet taste perception, lactisole is the most interesting candidate since it acts on the specific region of T1R3 (Xu et al. 2004, Jiang et al. 2005). Unfortunately, it was reported that the effect of lactisole was dependent on species. Thus, it inhibits sweet taste sensation in humans (Johnson et al. 1994) whereas it is ineffective in rodents (Johnson et al. 1994, Jiang et al. 2005). These reports negate the function of lactisole as an inhibitor of the glucose-sensing receptor in rodents. In this regard, Oya et al. (2011) reported recently that lactisole inhibits the umami receptor function in MIN6 cells. Their results suggest that mouse T1R3 (mT1R3) is not totally insensitive to lactisole. Nevertheless, since their results are apparently contradictory to previous reports (Johnson et al. 1994, Jiang et al. 2005), investigators in the field of taste research cast some doubts on their results. This is an important issue since, if it is true, lactisole can be used as an inhibitor of the glucose-sensing receptor in humans and rodents. Given that properties of the glucose-sensing receptor in β-cells may be slightly different from those of the canonical sweet taste receptor in the taste buds, it is indeed worth testing whether the glucose-sensing receptor expressed in mouse β-cells is inhibited by lactisole. In the present study, we examined this possibility. The results clearly show that lactisole inhibits the mouse glucose-sensing receptor mT1R3. Lactisole may provide a useful pharmacological tool to assess the function of the glucose-sensing receptor in pancreatic β-cells.

Materials and methods

Materials

Lactisole and sucralose were obtained from Sigma–Aldrich. Acesulfame-K and glycyrrhizin were from Wako Pure Chemical Industry (Osaka, Japan). These chemicals were dissolved in Hank’s balanced salt solution (HBSS) containing 138 mmol/l NaCl, 5.4 mmol/l KCl, 1.3 mmol/l CaCl₂, 0.44 mmol/l KH₂PO₄, 0.5 mmol/l MgCl₂, 0.38 mmol/l MgSO₄, 0.34 mmol/l Na₃HPO₄, 5.5 mmol/l tris-glucose and 10 mmol/l HEPES/NaOH (pH 7.4). To prepare lactisole-containing buffer, we dissolved the compound in HBSS or Krebs–Ringer–HEPES (KRH) buffer and pH was adjusted to 7.4 by adding NaOH.

Cell culture

MIN6 cells (Miyazaki et al. 1990) were grown in DMEM containing a high concentration of glucose (Invitrogen) and 10% fetal bovine serum (FBS) (Sigma–Aldrich) and cultured in a humidified incubator with 95% air and 5% CO₂ at 37 °C. HEK293 cells were cultured in DMEM containing 10% FBS.

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Measurement of insulin secretion
MIN6 cells were cultured in a 24-well plate and incubated for 48 h. Cells were then incubated for 1 h in KRH buffer containing 136 mmol/l NaCl, 4.8 mmol/l KCl, 1.3 mmol/l CaCl$_2$, 1.2 mmol/l MgSO$_4$, 1.2 mmol/l KH$_2$PO$_4$, 5.0 mmol/l NaHCO$_3$, 10 mmol/l HEPES/NaOH (pH 7.4) and 0.1% BSA. Cells were then incubated for 1 h in the same buffer containing 5.5 mmol/l glucose in the presence and absence of various agents. The supernatant was collected and centrifuged at 300 g for 10 min to remove cell debris. The insulin concentration in the supernatant was measured by RIA (Eiken Chemical, Tokyo, Japan) according to the manufacturer’s protocol (Nakagawa et al. 2009). Insulin content was measured as described previously (Nakagawa et al. 2009) and secreted insulin was expressed as a percent of the insulin content. Statistical analysis was performed by using Student’s t-test.

Measurement of cytoplasmic Ca$^{2+}$ concentration and NADH in MIN6 cells
HBSS was used for measurement of cytoplasmic Ca$^{2+}$ concentration ([Ca$^{2+}$]) and intracellular NADH concentration [NADH].

[Ca$^{2+}$], was monitored by using a fluorescent Ca$^{2+}$ indicator fluo-8. MIN6 cells were loaded with fluo-8 by incubating in HBSS containing 4 μmol/l fluo-8/AM for 20 min at room temperature. Cells were placed on a 35 mm glass bottom culture dish. Before the measurement, cells were incubated in HBSS and were visualized with a 40 UApo/340 objective lens (Olympus). To detect the fluorescence images, we used the AQUACOSMOS/ASHURA imaging system (Hamamatsu Photonics, Hamamatsu, Japan; Nakagawa et al. 2009). The values (F) were normalized to each initial value (F$_0$) and the relative fluorescence change was referred to as F/F$_0$ (Nakagawa et al. 2009). [NADH] was monitored by measuring auto-fluorescence using a 380 nm emission filter. In each experiment, recordings were obtained in more than ten cells.

Results are expressed as mean ± S.E.M. of accumulated data obtained in multiple experiments. For determination of dose–response relationship and analyses of the effects of inhibitors, the area under the curve (AUC) was calculated. Statistical analysis was performed using Student’s t-test.

Measurement of cytoplasmic cAMP concentration in MIN6 cells
Changes in cytoplasmic cAMP concentration ([cAMP]$_c$) were monitored by using Epac1-camps (Nikolaev et al. 2004) kindly provided by Dr Martin Lohse (University of Würzburg, Germany). MIN6 cells were transiently transfected with 5.4 μg of plasmid encoding Epac1-camps using Lipofectamine 2000 transfection reagent (Invitrogen; Nakagawa et al. 2009). Transfected cells were incubated in HBSS. For imaging, cells were visualized with a 40 UApo/340 objective lens (Olympus). The Epac1-camps excitation wavelength was 440 nm and attenuated by 6% using neutral density filters. For dual emission ratio imaging for enhanced cyan fluorescent protein and enhanced yellow fluorescent protein, we used AQUACOSMOS/ASHURA, a 3CCD based fluorescence energy transfer imaging system (Hamamatsu Photonics).

Measurement of cytoplasmic ATP in MIN6 cells
MIN6 cells expressing luciferase (Nakagawa et al. 2014) were seeded into a culture plate-96 and incubated for 2–3 days before the measurement of ATP. Cells were then incubated for 20 min in HBSS containing 50 μmol/l D-luciferin potassium salt (Wako Pure Chemical Industry) in a 37 °C incubator. To measure the basal ATP concentration ($L_0$), we counted the emitted photons with an EnSpire (Perkin Elmer, Waltham, MA, USA) for 20 min. The cells were then incubated in the presence or absence of stimulatory agents. We counted the emitted photon for 1 h. These values ($L$) were normalized to each basal value ($L_0$) so that the relative luminescence changes were referred to as $L/L_0$ (Nakagawa et al. 2014).

Establishment of HEK293 cell lines expressing mT1R3
HEK293 cells cultured in a 100 mm culture dish were dispersed with PBS containing 0.05% trypsin–EDTA (Life Technologies). After washing with growth medium, cells were resuspended in Electroporation Buffer (Bio-Rad). Then 0.55 ml of cell aliquot was mixed with 0.2 ml of mT1R3 pcDNA3.1 + neo (a gift from Yutaka Maruyama, Ajinomoto Co., Inc., Kawasaki, Japan; 30 μg) in a 0.4 cm-gap cuvette.
of various concentrations of lactisole, and insulin in the medium was measured. Effects of lactisole in the absence of stimulator were also examined (E). Values are the mean ± S.E.M. for four experiments.

Figure 1
Effect of lactisole on insulin secretion in MIN6 cells. MIN6 cells were incubated with 40 mmol/l sucralose (A), 40 mmol/l acesulfame-K (B), 3 mmol/l glycyrrhizin (C) and 40 mmol/l KCl (D) in the presence and absence of lactisole F. In Fig. 1E, lactisole did not affect the basal secretion.

We then examined the effect of lactisole on sucralose-induced elevation of [Ca^{2+}]_c. As shown in Fig. 2A, sucralose induced an immediate increase in [Ca^{2+}]_c, which was followed by sustained elevation of [Ca^{2+}]_c. The effect of sucralose was markedly inhibited by 5 mmol/l lactisole. Figure 2B depicts the effect of lactisole on sucralose-induced elevation of [Ca^{2+}]_c, assessed by measuring AUC. Likewise, elevation of [Ca^{2+}]_c induced by acesulfame-K was significantly inhibited by lactisole (Fig. 2C and D). In contrast, lactisole did not affect elevation of [Ca^{2+}]_c induced by a high concentration of potassium (Fig. 2E and F). In addition to [Ca^{2+}]_c, sucralose also induced elevation of [cAMP]_c (Fig. 3A). In sharp contrast to the effects on [Ca^{2+}]_c, lactisole did not affect sucralose-induced elevation of [cAMP]_c (Fig. 3A and B). Similarly, lactisole did not affect the elevation of [cAMP]_c induced by acesulfame-K (Fig. 3C and D).

The preceding results suggest that lactisole inhibits [Ca^{2+}]_c response mediated by mT1R3. To further confirm this, we permanently expressed mT1R3 in HEK293 cells and examined the effect of lactisole. In naïve HEK293 cells or mock-transfected HEK293 cells, sucralose did not cause any change in [Ca^{2+}]_c (data not shown). In HEK293 cells expressing mT1R3, sucralose induced a transient elevation of [Ca^{2+}]_c (Fig. 4A). It is of note that [Ca^{2+}]_c response to sucralose was obtained by transfection of mT1R3 alone and transfection of gustducin or a chimeric G protein (Nelson et al. 2001) was not required. Elevation of [Ca^{2+}]_c induced by sucralose was markedly inhibited by lactisole (Fig. 4B). In contrast, lactisole did not affect elevation of [cAMP]_c induced by sucralose (Fig. 4B and C). Likewise, acesulfame-K induced biphasic increase in [Ca^{2+}]_c, which was inhibited by lactisole (Fig. 4E and F).

We reported previously that activation of the glucose-sensing receptor led to an increase in [ATP]_c by facilitating

before a single pulse of electroporation was administered using Gene Pulser Xcell (Bio-Rad) set at voltage 200 V and time constant 28 ms. Cells were selected for antibiotic resistance to G-418 sulfate (1 g/l, Wako Pure Chemical Industry) for 10 days. One hundred and twenty resistant colonies were expanded, and their Ca^{2+} responses to the sweet taste receptor agonist were evaluated by using fluorescence microscopy. Six responding colonies were isolated, and the strongest responding cell line was used for all analyses.

Results
We first examined the effect of various concentrations of lactisole on insulin secretion induced by T1R3 agonists in MIN6 cells. In these cells, an artificial sweetener sucralose, which activates the glucose-sensing receptor T1R3 (Nakagawa et al. 2009, 2013, 2014), induced an approximately threefold increase in insulin secretion in the presence of 5.5 mmol/l glucose. This increase in insulin secretion induced by sucralose was inhibited by an addition of lactisole (Fig. 1A). In the presence of 3 mmol/l lactisole, sucralose-induced secretion was ~60%. At 10 mmol/l, lactisole nearly completely blocked the effect of sucralose. The IC_{50} of the effect of lactisole was ~4.0 mmol/l. Similarly, acesulfame-K induced a fourfold increase in insulin secretion and lactisole inhibited acesulfame-K-induced insulin secretion in a dose-dependent manner (Fig. 1B). In addition, insulin secretion induced by glycyrrhizin was inhibited by lactisole (Fig. 1C). In sharp contrast, lactisole did not affect insulin secretion induced by a depolarizing concentration of KCl (Fig. 1D). Lactisole did not affect the viability of the cells as assessed by using Trypan blue (data not shown). Kyriazis et al. (2014) recently reported that lactisole increased basal insulin secretin in human β-cells. We examined the effect of lactisole on basal secretin in MIN6 cells. As shown in Fig. 1E, lactisole did not affect the basal secretion.

We reported previously that activation of the glucose-sensing receptor led to an increase in [ATP]_c by facilitating

The effect of lactisole on elevation of [NADH] and [ATP]. We next examined whether lactisole modulates the effect of glucose in MIN6 cells. As shown in Fig. 6A, 25 mmol/l glucose induced an increase in [NADH]. Glucose-induced elevation of [NADH] was significantly reduced by lactisole (Fig. 6A and B). High concentration of glucose also evoked a biphasic increase in [ATP]c (Fig. 6C). Lactisole markedly reduced the elevation of [ATP]c induced by glucose (Fig. 6C and D). It is well known that glucose elevates [ATP]c and thereby inhibits the ATP-sensitive potassium (KATP) channel (Ashcroft & Rorsman 1989, Rorsman 1997). Resultant depolarization of the plasma membrane induces Ca2+ entry via the voltage-dependent Ca2+ channel (Ashcroft & Rorsman 1989, Rorsman 1997). As shown in Fig. 6E, 25 mmol/l glucose induced elevation of [Ca2+]c after a certain lag period. The glucose-induced elevation of [Ca2+]c was delayed and significantly blunted by the addition of lactisole (Fig. 6E and F). In agreement with these findings, lactisole strongly reduced the elevation of [cAMP]c induced by glucose (Fig. 6C and D).

Likewise, addition of sucralose induced an immediate increase in [ATP]c, which was markedly inhibited by lactisole (Fig. 5C and D).

We next examined whether lactisole modulates the effect of glucose in MIN6 cells. As shown in Fig. 6A, 25 mmol/l glucose induced an increase in [NADH]. Glucose-induced elevation of [NADH] was significantly reduced by lactisole (Fig. 6A and B). High concentration of glucose also evoked a biphasic increase in [ATP]c (Fig. 6C). Lactisole markedly reduced the elevation of [ATP]c induced by glucose (Fig. 6C and D). It is well known that glucose elevates [ATP]c and thereby inhibits the ATP-sensitive potassium (KATP) channel (Ashcroft & Rorsman 1989, Rorsman 1997). Resultant depolarization of the plasma membrane induces Ca2+ entry via the voltage-dependent Ca2+ channel (Ashcroft & Rorsman 1989, Rorsman 1997). As shown in Fig. 6E, 25 mmol/l glucose induced elevation of [Ca2+]c after a certain lag period. The glucose-induced elevation of [Ca2+]c was delayed and significantly blunted by the addition of lactisole (Fig. 6E and F). In agreement with these findings, lactisole strongly reduced the elevation of [cAMP]c induced by glucose (Fig. 6C and D).

Effect of lactisole on elevation of [Ca2+]c, induced by sucralose and acesulfame-K in MIN6 cells. (A) MIN6 cells were stimulated by 40 mmol/l sucralose in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole and changes in [Ca2+]c were monitored. Values are the mean of ten determinations. Note that 40 mmol/l mannitol did not affect [Ca2+]c. (B) Cells were stimulated as shown in A and quantitative analysis was done by measuring AUC. Values are the mean ± S.E.M. for three experiments. *P<0.01 vs without lactisole. (C) MIN6 cells were stimulated with 30 mmol/l acesulfame-K in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole and changes in [Ca2+]c were monitored. Values are the mean of ten determinations. (D) Cells were stimulated as shown in C and AUC was calculated. Values are the mean ± S.E.M. for three experiments. *P<0.01 vs without lactisole. (E) MIN6 cells were stimulated by 40 mmol/l KCl in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole. Values are the mean of responses of 20 determinations. (F) Experiments were performed as shown in E and AUC was calculated. Values are the mean ± S.E.M. for 20 determinations.

Effect of lactisole on elevation of [cAMP]c, in MIN6 cells. (A) Epac1-camps-expressing MIN6 cells were stimulated by 40 mmol/l sucralose in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole. Changes in [cAMP]c were monitored. Values are the mean of ten determinations. (B) Experiments were performed as mentioned in A and AUC was calculated. Values are the mean ± S.E.M. for ten determinations and representative of two experiments. (C) Cells were stimulated by 40 mmol/l acesulfame-K in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole. Values are the mean of ten determinations. (D) Experiments were performed as in C and AUC was calculated. Values are the mean ± S.E.M. of ten determinations and the representative of two experiments.
with this observation, lactisole significantly reduced insulin secretion induced by glucose (Fig. 7A). It is known that glucose action in β-cells is exerted through two different pathways: a triggering pathway or K_{ATP} channel-dependent pathway, and an amplifying pathway or K_{ATP} channel-independent pathway (Gembal et al. 1992, Sato et al. 1992, Henquin 2009). It was reported that the K_{ATP} channel independent pathway was inhibited by an inhibitor of glucose recognition site in the plasma membrane (Aizawa et al. 1994). To confirm this, we examined whether or not lactisole inhibits insulin secretion induced by glucose in the presence of diazoxide and depolarizing concentration of KCl. As shown in Fig. 7B, lactisole significantly reduced insulin secretion in the presence of glucose, diazoxide and high concentration of KCl.

Finally, we examined the effect of lactisole in mouse pancreatic islets. Lactisole did not alter the basal secretion of insulin (Fig. 8A). Glucose induced an approximately fivefold increase in insulin secretion in mouse islets. Lactisole attenuated insulin secretion induced by glucose (Fig. 8A). In contrast, lactisole did not affect insulin secretion induced by a depolarizing concentration of potassium (Fig. 8B). We also examined the effect of lactisole in islets obtained from T1R3 knockout mice. As shown in Fig. 8C, insulin secretion induced by glucose was reduced in T1R3-null islets. In these islets, lactisole did not affect glucose-induced insulin secretion.

**Discussion**

Results obtained in the present study demonstrated that lactisole inhibited insulin secretion induced by sweeteners
KCl. Likewise, lactisole inhibited \( \text{Ca}^{2+} \) secretion induced by the depolarizing concentration of \([\text{ATP}]_c\) induced by the sweetener. These results clearly showed that \([\text{ATP}]_c\) response to sucralose without affecting \([\text{ATP}]_c\) without lactisole. (C) MIN6 cells were stimulated by 25 mmol/l glucose in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole and changes in \([\text{ATP}]_c\) were monitored. Values are the mean of ten determinations and representative of three experiments. *P < 0.01 vs without lactisole. (D) MIN6 cells were stimulated by 25 mmol/l glucose in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole and changes in \([\text{ATP}]_c\) were monitored. Values are the mean of ten determinations and representative of two experiments. *(B) MIN6 cells were preincubated for 30 min in KRH buffer containing 5.5 mM glucose and 100 \(\mu\)M diazoxide to block the \(K_{\text{ATP}}\) channel. Cells were then incubated for 30 min in KRH buffer containing 5.5 mM lactisole and either 5.5 or 25 mmol/l glucose in the presence and absence of 5 mmol/l lactisole. Diazoxide (100 \(\mu\)M) was included in the buffer. Values are the mean ± S.E.M. for four experiments. *P < 0.01 vs without lactisole.

Figure 6
Effects of lactisole on the effects of glucose. (A) MIN6 cells were stimulated by 25 mmol/l glucose in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole and changes in \([\text{NADH}]_c\) were monitored. Values are the mean of responses of ten determinations. (B) Experiments were carried out as shown in A and AUC was calculated. Values are the mean ± S.E.M. for ten determinations and representative of three experiments. *P < 0.01 vs without lactisole. (C) MIN6 cells were stimulated by 25 mmol/l glucose in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole and changes in \([\text{ATP}]_c\) were monitored. Values are the mean of ten determinations and representative of three experiments. *P < 0.01 vs without lactisole. (D) Experiments are carried out as shown in C and AUC was calculated. Values are the mean ± S.E.M. for four experiments. *(E) MIN6 cells were stimulated by 25 mmol/l glucose in the presence (filled circle) and absence (open circle) of 5 mmol/l lactisole and changes in \([\text{Ca}^{2+}]_c\) were monitored. Values are the mean of ten determinations. Note that glucose concentration in medium before stimulation was 5.5 mmol/l. *(F) Experiments were carried out as shown in E and AUC was calculated. Values are the mean ± S.E.M. for ten determinations and representative of two experiments. *P < 0.01 vs without lactisole.

Figure 7
Effect of lactisole on insulin secretion in MIN6 cells. (A) MIN6 cells were incubated for 60 min with either 5.5 or 25 mmol/l glucose in the presence and absence of 5 mmol/l lactisole, and insulin in the medium was measured. Values are the mean ± S.E.M. for four experiments. *P < 0.05 vs without lactisole. (B) MIN6 cells were preincubated for 30 min in KRH buffer containing 5.5 mM glucose and 100 \(\mu\)M diazoxide to block the \(K_{\text{ATP}}\) channel. Cells were then incubated for 30 min in KRH buffer containing 30 mmol/l KCl and either 5.5 or 25 mmol/l glucose in the presence and absence of 5 mmol/l lactisole. Diaxoxide (100 \(\mu\)M) was included in the buffer. Values are the mean ± S.E.M. for four experiments. *P < 0.01 vs without lactisole.

in dose-dependent manners (Fig. 1). The IC_{50} was ~4 mmol/l. In contrast, lactisole had no effect on insulin secretion induced by the depolarizing concentration of KCl. Likewise, lactisole inhibited \([\text{Ca}^{2+}]_c\) response to sweeteners without affecting the \([\text{Ca}^{2+}]_c\) response to KCl. Furthermore, lactisole blocked the increase in \([\text{ATP}]_c\) induced by the sweetener. These results clearly show that the glucose-sensing receptor in mouse β-cells is inhibited by lactisole. Further support came from the experiments performed in HEK293 cells. In HEK293 cells stably expressing mT1R3, sucralose evoked an elevation of \([\text{Ca}^{2+}]_c\), which was inhibited by lactisole. It should be noted that doses of lactisole that inhibited mT1R3 were slightly higher than that which inhibited human T1R3 (hT1R3; Johnson et al. 1994, Xu et al. 2004, Jiang et al. 2005). Thus, hT1R3 was blocked by 1 mmol/l lactisole (Jiang et al. 2005) whereas 1 mmol/l lactisole showed only a small inhibitory effect on mT1R3 (Fig. 1). mT1R3 was less sensitive to lactisole as compared to hT1R3. In any event, lactisole was able to inhibit mT1R3, although slightly higher concentrations were needed. This notion is apparently different from what has been widely thought (Johnson et al. 1994, Sclafani & Perez 1997, Xu et al. 2004).

In this regard, Jiang et al. (2005) used a fixed dose of lactisole, i.e. 1 mmol/l, and showed that mT1R3 was resistant to lactisole. In fact, they prepared series of chimeric receptors replacing hT1R3 sequences with the mouse ones, and identified a region responsible for inhibition by lactisole. However, even their insensitive chimeras were inhibited when higher concentrations of lactisole were administered (Jiang et al. 2005). Taken
In the present study, we stably transfected mT1R3 in HEK293 cells and observed that mT1R3 responded to the sweeteners. Again, the inhibitory actions of lactisole are biased in some sense. Our previous study (Nakagawa et al. 2013) showed that lactisole increased [cAMP]c by a Gs-dependent mechanism whereas acesulfame-K-induced elevation of cAMP was also dependent on Ca2+. Presumably, lactisole is ineffective to inhibit the Gs-dependent pathway.

Kyriazis et al. (2014) recently postulated that the sweet taste receptor expressed in β-cells inhibits basal secretion of insulin. Their proposal is based on the observation obtained in human islets using lactisole. They showed that lactisole increased insulin secretion in the presence of 3.0 or 5.5 mmol/l glucose in human islets. In that study, they also assessed basal insulin secretion in T1R2 knockout islets. They showed that insulin secretion in the presence of 3.0 or 5.5 mmol/l glucose was not altered in T1R2-null islets (Kyriazis et al. 2012, 2014). These results are apparently inconsistent with their proposal. Because our present results also show that lactisole did not affect basal secretion in mouse β-cells, results obtained in human islets using lactisole may have provided inconsistency. At present, the reason why they observed stimulation of insulin secretion by lactisole is not clear. As mentioned in the ‘Materials and methods’ section, lactisole solution is acidic. If they did not adjust pH of the KRH buffer containing lactisole, pH of the buffer may have been lower than should be. Because β-cells express the proton receptor (Nakakura et al. 2012), low pH might have stimulated insulin secretion independent of the action of lactisole. Further studies are necessary to address these points.

In the present study, we stably transfected mT1R3 in HEK293 cells and observed that mT1R3 responded to...
sucralose. The results clearly indicate that mT1R3 alone functions as the sweet taste-sensing receptor. Presumably, a homodimer of mT1R3 functions as a signaling receptor. It should be mentioned that we did not transfect gustducin or a chimeric G protein simultaneously. This suggests that a homodimer of mT1R3 may couple to endogenous G proteins expressed in HEK cells, presumably G proteins of the Gq family. The present results differ from those that previously reported that a chimeric G protein was required to observe the effect of ectopically expressed T1Rs (Nelson et al. 2001). An exact reason for this discrepancy is not clear at present. The efficiency of transfection or sensitivity of the detection of changes in \([\text{Ca}^{2+}]_c\) may be possible reasons.

We showed recently that sucralose increased \([\text{ATP}]_c\) in MIN6 cells (Nakagawa et al. 2014). The effect of sucralose is observed even in the absence of ambient glucose. Sucralose is an artificial sweetener and is not metabolized in β-cells. We interpret these results that sucralose, by activating the glucose-sensing receptor T1R3, facilitates metabolism in β-cells and increased \([\text{ATP}]_c\). Since sucralose is capable of elevating \([\text{ATP}]_c\) in the absence of glucose, sucralose perhaps mobilizes substrate(s) from a source(s) outside the glycolytic pathway and generates ATP (Kojima et al. 2015). In addition, sucralose enhances the elevation of \([\text{ATP}]_c\) induced by methylsuccinate. This suggests that metabolism in mitochondria may also be activated by the glucose-sensing receptor T1R3 (Kojima et al. 2015).

The present results show that lactisole inhibited the action of glucose on the glucose-sensing receptor. Thus, lactisole attenuated glucose-induced increases in \([\text{NADH}]_c\) and \([\text{ATP}]_c\). These results are consistent with the notion that lactisole inhibits the glucose-sensing receptor and thereby attenuated the facilitation of the glucose metabolism (Medina et al. 2014, Nakagawa et al. 2014). More importantly, lactisole inhibited insulin secretion induced by glucose. As shown in Fig. 6C, lactisole attenuated the elevation of \([\text{ATP}]_c\) induced by glucose. In addition, lactisole also reduced elevation of \([\text{Ca}^{2+}]_c\) induced by glucose. Given that glucose increases \([\text{Ca}^{2+}]_c\) by inhibiting the \(\text{KATP}\) channel and causing \(\text{Ca}^{2+}\) entry via the voltage-dependent \(\text{Ca}^{2+}\) channel, the \(\text{KATP}\) channel dependent pathway is inhibited partially by lactisole. As shown in Fig. 7B, lactisole inhibited insulin secretion induced by glucose in the presence of diazoxide and high concentration of KCl. Hence, the \(\text{KATP}\) channel independent pathway or the amplifying pathway is also inhibited partially by lactisole. These results are consistent with the report by Aizawa et al. (1994). Collectively, the glucose-sensing receptor is involved in both \(\text{KATP}\) channel-dependent and independent pathways. This is not surprising because the glucose-sensing receptor modulates glucose metabolism and promotes ATP generation (Kojima et al. 2015).

Lactisole is commercially available and may be useful to assess the function and physiological role of the glucose-sensing receptor T1R3 expressed in β-cells. Again, we should keep in mind that lactisole inhibits the calcium signaling system activated by the glucose-sensing receptor, but activation of the cAMP pathway is not affected by the compound. We may underestimate the role of the glucose-sensing receptor. In any event, further studies are necessary to elucidate the role of the glucose-sensing receptor in the action of glucose in β-cells.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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