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The effects of different concentrations of glucose on glucose sensors and GLP-1 secretion in the enteroendocrine cell line STC-1

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Abstract. Glucose triggers glucagon-like peptide (GLP)-1 secretion from L cells involving several glucose sensors including sodium-glucose transporter (SGLT)1, glucose transporter (GLUT)2, and sweet taste receptors (STRs). This study investigated the effects of different glucose concentrations on GLP-1 secretion, intracellular concentrations of Ca^{2+} and cAMP, glucose uptake, and protein levels of SGLT1, GLUT2, and STRs in STC-1 cells. Low glucose (5.6 mM) increased GLP-1 secretion, intracellular Ca^{2+} concentration, and SGLT1 protein level compared with glucose-free group. GLP-1 secretion and intracellular Ca^{2+} concentration triggered by low glucose were inhibited by the SGLT1 inhibitor. GLP-1 secretion or intracellular Ca^{2+} concentration in high-glucose (25, 100, 200 mM) groups was significantly higher than that of low-glucose group. Elevation of cAMP level was observed in concentration-dependent manner, and decreased glucose uptake was observed in 100 or 200 mM glucose group. High glucose increased protein levels of STRs and GLUT2 in comparison to low-glucose group. GLP-1 secretion and intracellular levels of Ca^{2+} and cAMP triggered by high glucose were inhibited in the presence of the GLUT2 or STR inhibitor. These results suggest that SGLT1 is dominantly responsible for GLP-1 secretion triggered by low glucose, and that STRs and GLUT2 are involved in GLP-1 secretion induced by high glucose.

Key words: Glucose-induced GLP-1 secretion — Sodium-glucose transporter 1 — Glucose transporter 2 — Sweet taste receptor

Introduction

Oral intake of nutrients results in the secretion of gut incretin hormones including glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 and GIP together account for 50–70% of prandial insulin secretion (Shuster et al. 2017). In terms of the treatment of type 2 diabetes mellitus (T2DM), GLP-1 is being a special interest for its broad role in glucose homeostasis. GLP-1 postprandially secreted by intestinal enteroendocrine L-cells potentiates glucose-induced insulin release from pancreatic beta-cells (Doyle and Egan 2007). Glucose-lowering effect of GLP-1 also exerts *via* increasing insulin-independent glucose disposal, for example, reducing glucagon secretion, inhibiting the appetite, and delaying gastric emptying (Holst 2007). GLP-1-based agents, namely stable GLP-1 analogs and dipeptidyl peptidase 4 inhibitors, are used for T2DM therapy (Drucker and Nauck 2006; Kim and Egan 2008). Additionally, there is now generally accepted that the metabolic benefits of the Roux-en-Y gastric bypass and vertical sleeve gastrectomy are partly attributable to the postprandially increased GLP-1 level (Benaiges et al. 2011). These new therapeutic strategies highlight the importance of endogenous GLP-1 in maintaining glucose homeostasis.

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As glucose is the most important energy source and its ambient level needs to be maintained, many cell types, including pancreatic beta-cells and enteroendocrine L-cells, are equipped with a glucose-sensing apparatus. Accumulating evidence indicated that luminal glucose exposure potently stimulates GLP-1 secretion from isolated perfused rat or human small intestine (Kuhre et al. 2015; Xu et al. 2016; Sun et al. 2017). From studies of the GLP-1-expressing cell lines (Gribble et al. 2003; Parker et al. 2012) including STC-1, GLUTag and NCI-H716, primary murine intestinal mixed cell cultures (Frank et al. 2008), *in vivo* transgenic mouse models (Cani et al. 2007; Gorboulev et al. 2012), an *ex vivo* rat model (Kuhre et al. 2015), and an *ex vivo* human model (Sun et al. 2017), several glucose sensors have suggested to link glucose exposure to GLP-1 secretion. They include electrogenic glucose transport sodium-glucose transporter (SGLT)1 and facilitative glucose transporter (GLUT)2. It has been proposed that glucose induces GLP-1 release through SGLT1, and to a lesser extent, GLUT2. Studies also indicated that sweet taste receptors (STRs, TAS1R2-TAS1R3) signaling is also involved in glucose-induced GLP-1 release (Jang et al. 2007; Kokrashvili et al. 2009; Xu et al. 2016).

After a meal, although the exact concentration of luminal glucose is the big unknown, it estimates range from 50 to 300 mM (Kellett and Brot-laroche 2006). In this study, using enteroendocrine STC-1 cells, we investigated the effect of luminal glucose concentration (5.6, 25, 100, and 200 mM) on GLP-1 secretion, intracellular concentrations of Ca^{2+} and cAMP, glucose uptake, and the protein expression levels of SGLT1, GLUT2, and STRs. The SGLT1, GLUT2 and STRs inhibitor were used as an intervention reagent to evaluate the role of SGLT1, GLUT2, and STRs in GLP-1 secretion triggered by different concentrations of glucose, respectively.

Materials and Methods

Reagents

Phloretin, lactisole, and phloridzin dihydrate purchased from Sigma-Aldrich (St. Louis, MO, USA) were prepared as a stock solution in DMSO. For all conditions tested, the final concentration of DMSO, phloretin, lactisole, and phloridzin dihydrate was adjusted to 0.05%, 0.1 mM, 5 mM, and 0.5 mM, respectively.

Cell culture

STC-1 cells, a murine enteroendocrine intestinal cell line, were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 25 mM glucose supplemented with 10% fetal bovine serum (FBS; Zhejiang Tianhang Biological Technology Co., Ltd., Huzhou, China) and antibiotics (100 units/ml penicillin and 0.1 ng/ml streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2. The cells were grown to 85% confluence for the experiments.

Measurement of GLP-1 secretion

STC-1 cells were seeded into 24-well plates at a density of 1.5×10^5 cells/well for 48 h. The cells were washed with phosphate buffer saline (PBS) for three times, then incubated with glucose-free DMEM for 3 h. The medium was subsequently removed, and the cells were treated with the indicated reagents for variable times (see figure legends). After the incubation, the medium was collected and centrifuged at 12 000 × *g* for 5 min at 4°C. The GLP-1 concentration (total) in the supernatant was measured using a GLP-1 Total ELISA kit (Millipore, MA, USA).

Determination of intracellular Ca^{2+} levels

STC-1 cells were plated at a density of 1.5×10^5 cells/well in 6-well plates for 48 h. The cells were washed with PBS for three times, then incubated with glucose-free DMEM for 3 h. The cells were incubated with different concentrations of glucose in the presence and absence of various agents for variable times (see figure legends). 1 μl Fluo-3/AM (Beyotime, Jiangsu, China) was added in each well at room temperature and then incubated for 30 min. Then the cells were washed three times with PBS. Mean fluorescence ratios of the cells were determined by flow cytometry (BD Accuri C6, Ann Arbor, USA) with excitation/emission, 488/525-530, and analyzed by BD software 1.0.

Determination of intracellular cAMP levels

STC-1 cells were seeded into 6-well plates at a density of 1.5×10^5 cells/well for 48 h. The cells were washed three times with PBS. Then the cells were incubated with glucose-free DMEM for 3 h. The cells were incubated with different concentrations of glucose in the presence and absence of various agents for variable times (see figure legends). After the incubation, cells were collected and cell lysates were prepared for the measurement of cAMP levels using an ELISA kit (Shanghai BangYi Bio-Technology Co., Ltd., Shanghai, China).

Measurement of glucose uptake

The glucose uptake was measured by using 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxyglucose (2-NBDG) (Cayman Chemical Co., Ann Arbor, MI, USA),

as previously described (Wang et al. 2017). The STC-1 cells were plated in 96-well plate for 48 h and then washed with Krebs-Ringer bicarbonate (KRb) buffer (4A Biotech Co. Ltd., Beijing, China). Different concentration of glucose (at 0, 5.6, 25, 100, and 200 mM) was added into the medium. After 1-h incubation, the cells were washed twice with KRb buffer. Then 100 μl serum-free KRb buffer containing 160 μM 2-NBDG was added and continued to incubate for 30 min at 37°C. The medium was subsequently removed, and the fluorescence intensity in the cells was measured using a fluorescence microplate reader (excitation/emission, 488/520; Model 680, Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Western blot analysis

After treatment with glucose-free DMEM for 3 h, STC-1 cells were incubated with different concentrations of glucose (0, 5.6, 25, 100, and 200 mM) for 1 h. The cells were harvested, and cell lysates were prepared. BCA Protein Assay Kit (Beyotime, Jiangsu, China) was used to determine the protein content in the lysates. For Western blot analysis, 50–100 μg of protein from each sample was subjected to separate on a SDS-PAGE gel. The blots of proteins in polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany) were incubated with the appropriate primary antibody and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody. The blots were detected with chemiluminescence (ECL-kit, Beyotime, Jiangsu, China) followed by autoradiography. The antibodies used for Western blot were as follows: anti-SGLT1 (Abcam, Cambridge, UK), anti-GLUT2 (Abcam, Cambridge, UK), anti-STR subunit TAS1R2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-STR subunit TAS1R3 (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Quantification of protein bands was performed using ImageJ software version 1.42 (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All of the data shown are representative of at least three independent experiments performed in duplicates and are expressed as mean ± SD. Statistical significance of differences was analyzed by the Student's *t*-test or analysis of variance followed by the Bonferroni or Dunnett's post hoc tests (Graph Pad Prism Software, San Diego, CA, USA). All *p*-values ≤ 0.05 were considered statistically significant.

Results

Effects of different concentrations of glucose on GLP-1 secretion and intracellular Ca^{2+} level

As shown in Fig. 1A, exposure to glucose (at 5.6, 25, 100, and 200 mM) significantly stimulated GLP-1 secretion from STC-1 cells compared with the glucose-free control group, with a maximum stimulation of ~1.36-fold. Compared with the group of 5.6 mM glucose stimulation, GLP-1 secretion from STC-1 cells was significantly increased by approximately 1.15-fold in the groups of high-glucose (25, 100, and 200 mM) stimulation (Fig. 1A). There was not a significant difference in GLP-1 secretion among the groups of 25, 100, and 200 mM glucose (Fig. 1A). We then examined the effect

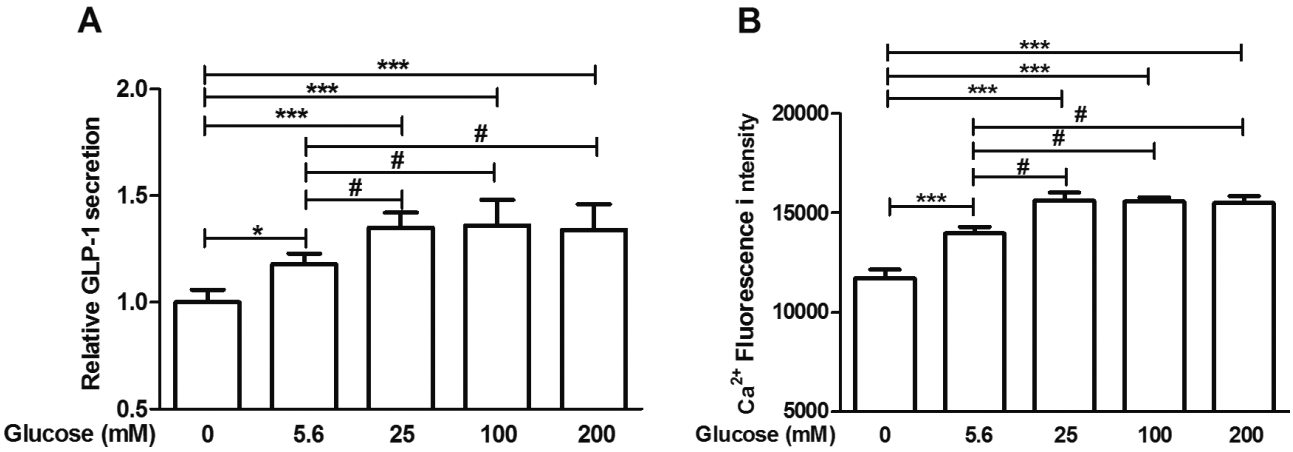

Figure 1. Effects of different concentrations of glucose on GLP-1 secretion and intracellular Ca^{2+} level in STC-1 cells. STC-1 cells were incubated with different concentrations of glucose (0, 5.6, 25, 100, and 200 mM) for 1 h. **A.** The GLP-1 concentration in the supernatant was measured by ELISA. All data were expressed as the relative dimensionless number of the glucose-free control group. * $p < 0.05$, *** $p < 0.001$, # $p < 0.05$ ($n = 6$). **B.** The Ca^{2+} concentrations in STC-1 cells were measured at the 488/525-530 nm fluorescence ratio in the cells loaded with fluo-3/AM. *** $p < 0.001$, # $p < 0.05$ ($n = 6$).

of different concentrations of glucose on intracellular Ca^{2+} concentration in STC-1 cells. As shown in Fig. 1B, the groups of 5.6, 25, 100, and 200 mM glucose stimulation exhibited a significant increase in intracellular Ca^{2+} concentration compared with the glucose-free control group, with a maximum stimulation of approximately 1.34-fold. Intracellular Ca^{2+} concentration in the group of 25 mM glucose stimulation was dramatically higher than that of the 5.6 glucose group, but no significant difference in intracellular Ca^{2+} concentration was observed among the groups of 25, 100, and 200 mM groups (Fig. 1B). In terms of GLP-1 secretion and intracellular Ca^{2+} concentration, glucose ranged from 5.6 to 200 mM appeared to have a similar effect.

Effects of different concentrations of glucose on intracellular cAMP level and glucose uptake

The elevation of intracellular cAMP level was reported to increase GLP-1 secretion in several models of intestinal enteroendocrine cells, associated with the release of Ca^{2+} from intracellular stores (Simpson et al. 2007). Thus we further examined the effect of different concentrations of glucose on intracellular cAMP levels. As shown in Fig. 2A, compared with the glucose-free control group, glucose at 5.6 mM concentration did not alter intracellular cAMP levels. Following treatment with 25, 100 and 200 mM glucose, intracellular cAMP levels were markedly elevated to approximately 1.5-fold, 3.5-fold, and 4.6-fold, respectively. Furthermore, glucose ranged from 5.6 to 200 mM increased intracellular cAMP levels in a concentration-dependent manner (Fig. 2A). L-cell function depends on glucose uptake, and the subsequent signaling pathways influence the concentration of intracellular cAMP. Thus, we examined the effect of different concentrations of glucose on glucose uptake. 2-NBDG was used for the determination of glucose uptake. Compared with the glucose-free control group, no significant difference in 2-NBDG uptake was observed between 5.6 mM glucose group and 25 mM glucose group. 2-NBDG uptake was significantly attenuated by either 100 or 200 mM glucose treatment (Fig. 2B), which reflects that the ability of cells to uptake glucose decreases after exposure to either 100 or 200 mM glucose stimulation.

Effects of different concentrations of glucose on the protein levels of SGLT1, GLUT2 and STR subunits

To determine the involvement of SGLT1, GLUT2 or STRs in GLP-1 secretion induced by different concentrations of glucose, we treated STC-1 cells with different concentrations of glucose (at 0, 5.6, 25, 100, and 200 mM) for 1 h and compared the protein levels of SGLT1, GLUT2, and STRs. As shown in Fig. 3, SGLT1 expression gradually increased when glucose concentration in the medium was reduced from 100 to 5.6 mM. No significant difference in SGLT1 expression was observed between the group of 100 mM glucose and the group of 200 mM glucose. Importantly, 5.6 mM glucose treatment induced a significant increase in SGLT1 protein level in comparison to the glucose-free control group (Fig. 3). By contrast, no significant difference in GLUT2 protein expression was observed between the 5.6 mM glucose group and glucose-free control group (Fig. 3). While glucose ranged from 5.6 to 200 mM increased GLUT2 expression in a concentration-dependent manner (Fig. 3). The expression of STR subunits (TAS1R2, TAS1R3) was significantly increased in the groups of 25, 100, and 200 mM glucose in comparison to the group of 5.6 mM glucose (Fig. 3).

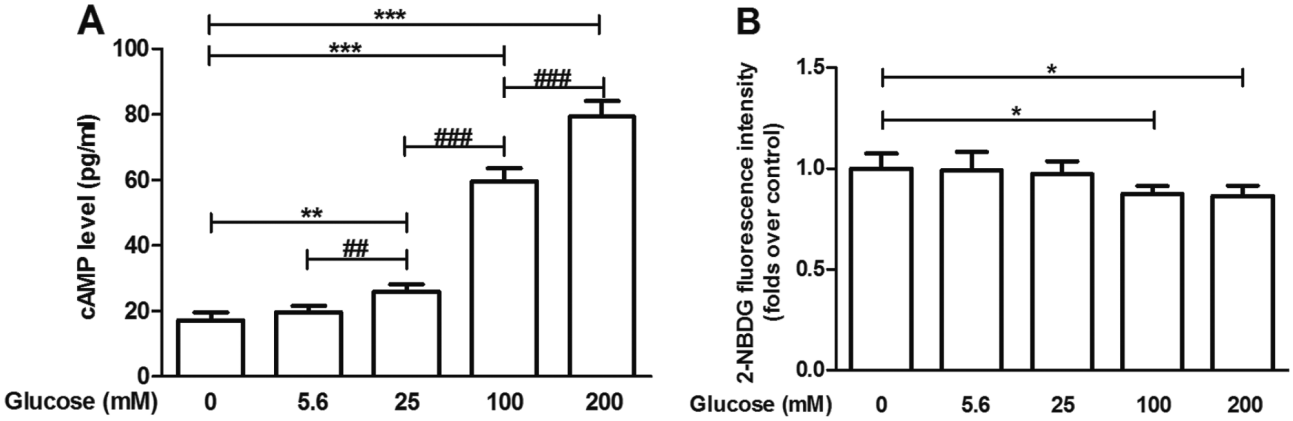

Figure 2. Effects of different concentrations of glucose on intracellular cAMP level and glucose uptake in STC-1 cells. STC-1 cells were incubated with different concentrations of glucose (0, 5.6, 25, 100, and 200 mM) for 1 h. **A.** Intracellular cAMP levels were measured by ELISA. ** $p < 0.01$, *** $p < 0.001$, ## $p < 0.01$, ### $p < 0.001$ ($n = 6$). **B.** 2-NBDG fluorescence intensity incorporated into the cells was measured on a fluorescence microplate reader. All data were expressed as relative dimensionless number of glucose-free control group. * $p < 0.05$ ($n = 6$).

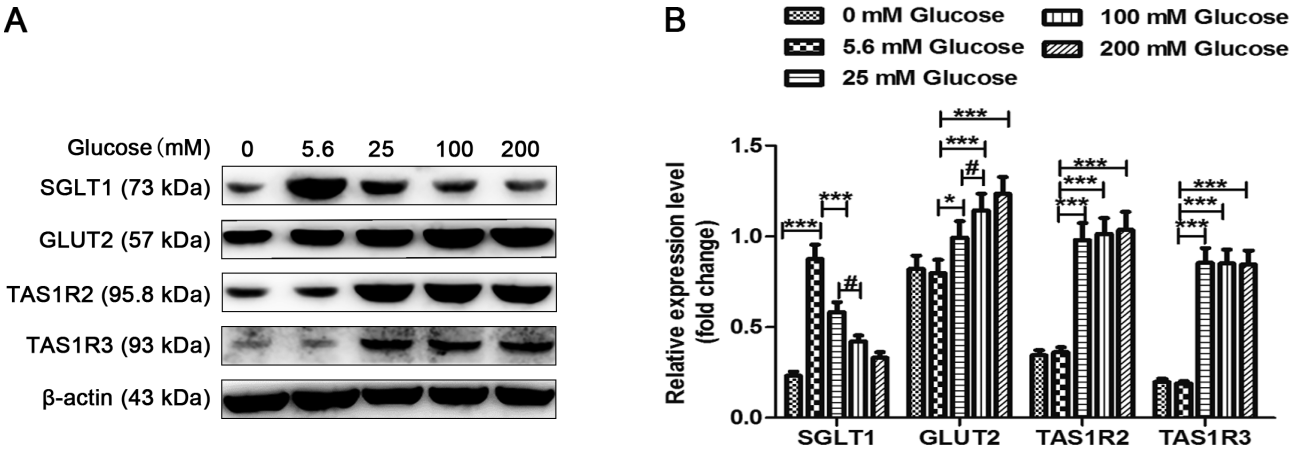

Figure 3. Effects of different concentrations of glucose on the protein level of SGLT1, GLUT2 and STR subunits in STC-1 cells. **A.** The protein expression of SGLT1, GLUT2 and STR subunits (TAS1R2 and TAS1R3) in STC-1 cells cultured in different concentrations of glucose (0, 5.6, 25, 100, and 200 mM) for 1 h were measured by Western blot. ($n = 3$). **B.** The protein levels of SGLT1, GLUT2, TAS1R2, and TAS1R3 were analyzed by Image-J software. * $p < 0.05$, *** $p < 0.001$, # $p < 0.01$ ($n = 3$).

Effects of pharmacological interference with SGLT1, GLUT2 or STRs on GLP-1 secretion and intracellular levels of cAMP and Ca^{2+}

To further investigate the role of SGLT1, GLUT2 and STRs in GLP-1 secretion from STC-1 cells induced by different concentrations of glucose, we exposed STC-1 cells to different concentrations of glucose (5. 6, 25, 100, and 200 mM) in the presence or absence of the SGLT1 inhibitor phloridzin, the GLUT2 inhibitor phloretin or the STR inhibitor lactisole for 1 h and evaluated GLP-1 secretion. GLP-1 secretion triggered by 5.6 mM glucose was significantly decreased to approximately 85% in the presence of phloridzin (Fig. 4A). In the presence of phloridzin, GLP-1 secretion stimulated by high concentrations of glucose (25, 100, and 200 mM) appeared to also have a tendency to decrease (Fig. 4A). In the presence of phloretin, GLP-1 secretion induced by glucose at 25, 100 and 200 mM was significantly reduced to 88%, 90%, and 90%, respectively (Fig. 4B). In the presence of lactisole phloretin, GLP-1 secretion induced by glucose at 25, 100 and 200 mM was significantly reduced to 83%, 82%, and 83%, respectively (Fig. 4B). GLP-1 secretion induced by 5.6 mM glucose in lactisole-treated STC-1 cells exhibited a tendency to decrease (Fig. 4C). We further examined the effects of phloridzin on intracellular levels of cAMP and Ca^{2+} under 5.6 mM glucose condition. As shown in Fig. 4D, 5.6 mM glucose-induced elevation of intracellular Ca^{2+} level was significantly decreased approximately 26% by phloridzin. No significant decrease in intracellular cAMP level was observed in the presence of phloridzin (Fig. 4E). The effects of phloretin or lactisole on intracellular levels of cAMP and Ca^{2+} under 25 mM glucose condition were also examined. Following treatment with phloretin and lactisole, increases in intracellular Ca^{2+} induced by 25 mM glucose was significantly decreased approximately 16% and 17%, respectively (Fig. 4D). Under 25 mM glucose condition, intracellular cAMP levels were significantly reduced approximately 27% by phloretin and approximately 42% by lactisole (Fig. 4E).

Possible involvements of SGLT1, GLUT2 or STRs in GLP-1 secretion in response to 3-h low-glucose exposure

We incubated STC-1 cells with 5.6 mM glucose for 3 h and then assessed GLP-1 secretion and intracellular levels of cAMP and Ca^{2+}. Both GLP-1 secretion and intracellular Ca^{2+} level were significantly increased after 3 h compared to 1 h (Fig. 5A and C). 3-h exposure to 5.6 mM glucose unaffected intracellular cAMP levels (Fig. 5B). Then, we exposed STC-1 cells to 5.6 mM glucose in the presence or absence of the SGLT1 inhibitor phloridzin, the GLUT2 inhibitor phloretin or the STR inhibitor lactisole and then assessed GLP-1 secretion after 1-h and 3-h treatment. As shown in Fig. 5D, GLP-1 secretion induced 5.6 mM glucose for 3 h was impaired by inhibition of SGLT1 with phloridzin, which is consistent with the observations in 1-h treatment with phloridzin. Interestingly, inhibition of STRs with lactisole for 3 h significantly decreased GLP-1 secretion triggered by 5.6 mM glucose (Fig. 5D). As expected, exposure to phloretin for 1 h or 3 h unaffected GLP-1 secretion in response to 5.6 mM glucose stimulation (Fig. 5D).

Discussion

The development of new therapeutic strategies that aim to increase endogenous GLP-1 secretion makes the study of

the GLP-1 stimulus-secretion pathways of growing interest. Using the STC-1 enteroendocrine cell model, the current study confirmed that GLP-1 secretion triggered by 5.6 mM glucose was impaired by the SGLT1 inhibitor phloridzin. The observations were compatible with the results that this relatively low concentration of glucose upregulated SGLT1 expression. These results supported the idea that the involvement of SGLT1 in GLP-1 secretion induced by low concentrations of glucose. We also further established that glucose concentration (25, 100, and 200 mM), similar to the postprandially luminal glucose concentration, stimulates GLP-1 secretion from STC-1 cells and this effect was attenuated by an addition of GLUT2 inhibitor phloretin or STR inhibitor lactisole. Furthermore, high glucose increased the protein levels of GLUT2 and STR subunits in comparison to low-glucose group. These results also indicated the im-

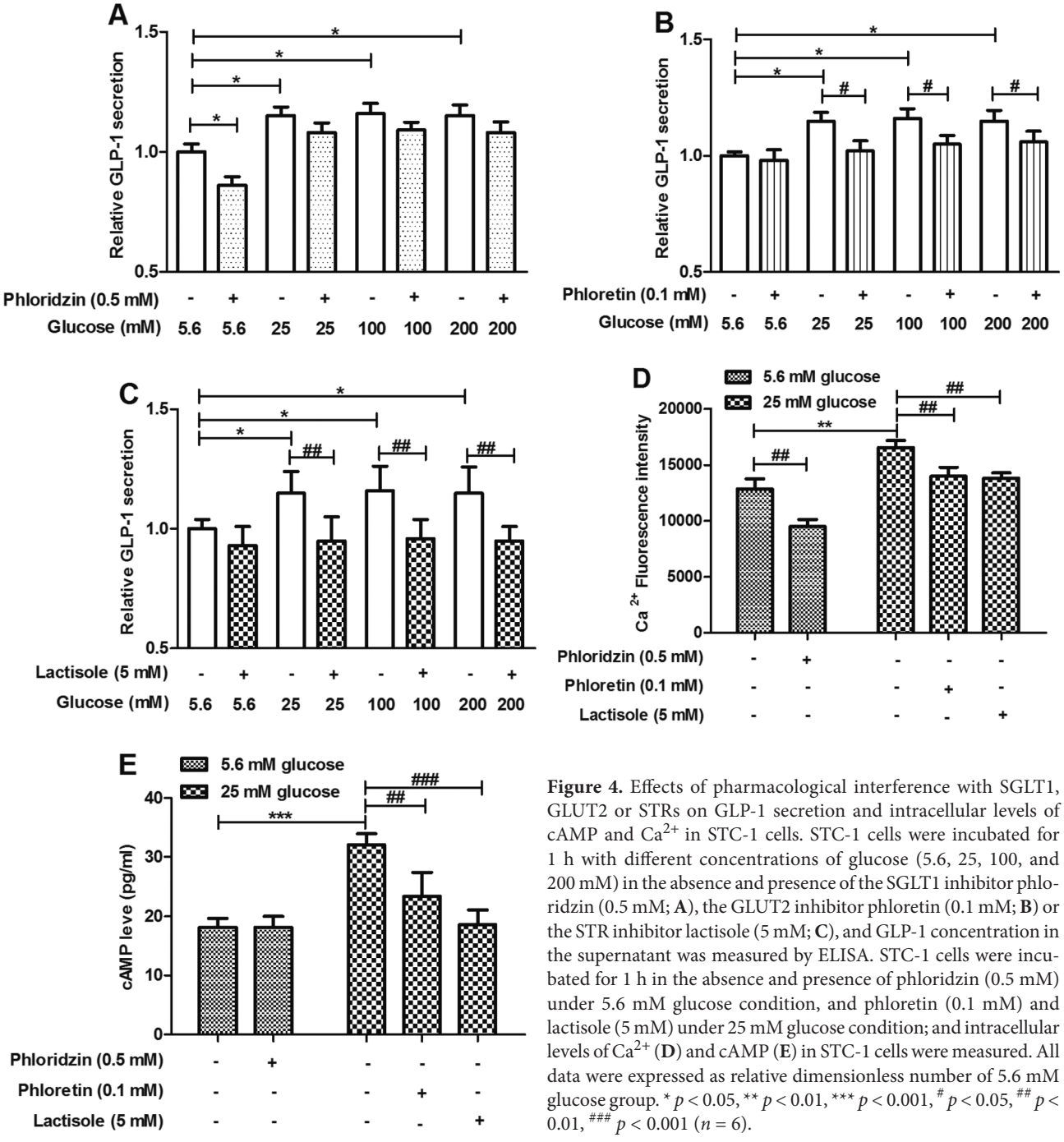

Figure 4. Effects of pharmacological interference with SGLT1, GLUT2 or STRs on GLP-1 secretion and intracellular levels of cAMP and Ca^{2+} in STC-1 cells. STC-1 cells were incubated for 1 h with different concentrations of glucose (5.6, 25, 100, and 200 mM) in the absence and presence of the SGLT1 inhibitor phloridzin (0.5 mM; **A**), the GLUT2 inhibitor phloretin (0.1 mM; **B**) or the STR inhibitor lactisole (5 mM; **C**), and GLP-1 concentration in the supernatant was measured by ELISA. STC-1 cells were incubated for 1 h in the absence and presence of phloridzin (0.5 mM) under 5.6 mM glucose condition, and phloretin (0.1 mM) and lactisole (5 mM) under 25 mM glucose condition; and intracellular levels of Ca^{2+} (**D**) and cAMP (**E**) in STC-1 cells were measured. All data were expressed as relative dimensionless number of 5.6 mM glucose group. $^{*}\ p < 0.05$, $^{**}\ p < 0.01$, $^{***}\ p < 0.001$, $^{\#}\ p < 0.05$, $^{\#\#}\ p < 0.01$, $^{\#\#\#}\ p < 0.001$ ($n = 6$).

portance of STRs and GLUT2 in GLP-1 secretion triggered by high glucose.

It has reported that GLUTag and primary murine L-cells express both SGLT1 and GLUT2 transporters (Frank et al. 2008). Our results also presented evidence for the protein expression of SGLT1 and GLUT2 on mouse enteroendocrine cell line STC-1. It is well known that SGLT1 and GLUT2 are the two types of glucose transporters, active and facilitative, respectively. Recently, using the pharmacological interference with GLUT2, studies have demonstrated that GLUT2 largely dominate intracellular glucose concentrations in GLUTag cells and primary intestinal cultures (Parker et al. 2012). Our results showed that GLUT2, but not SGLT1, was gradually increased when glucose concentration was raised from 5.6 to 200 mM, which is in agreement with the previous report (Kellett and Helliwell 2000). These results observed in STC-1 cells may suggest that increased GLUT2 expression led to elevation of intracellular glucose concentrations. Thus, the decreased 2-NBDG uptake ability could be observed in STC-1 cells after exposure to a high concentration of glucose. Indeed, Teusink et al. (1998) observed that when the intracellular glucose concentration is high, the glucose influx would be reduced. In addition, since Sun et al. (2017) reported that 300 mM mannitol failed to induce GLP-1 secretion, we believe that the decreased glucose uptake is not secondary to osmotic stress.

The relative roles of SGLT1 and GLUT2 in glucose-induced GLP-1 secretion were investigated in seminal studies using pharmacological and genetic interference with SGLT1 and GLUT2 (Cani et al. 2007; Gorboulev et al. 2012; Parker et al. 2012), suggesting some SGLT1 are required for glucose-induced GLP-1 secretion. Electrogenic SGLT1

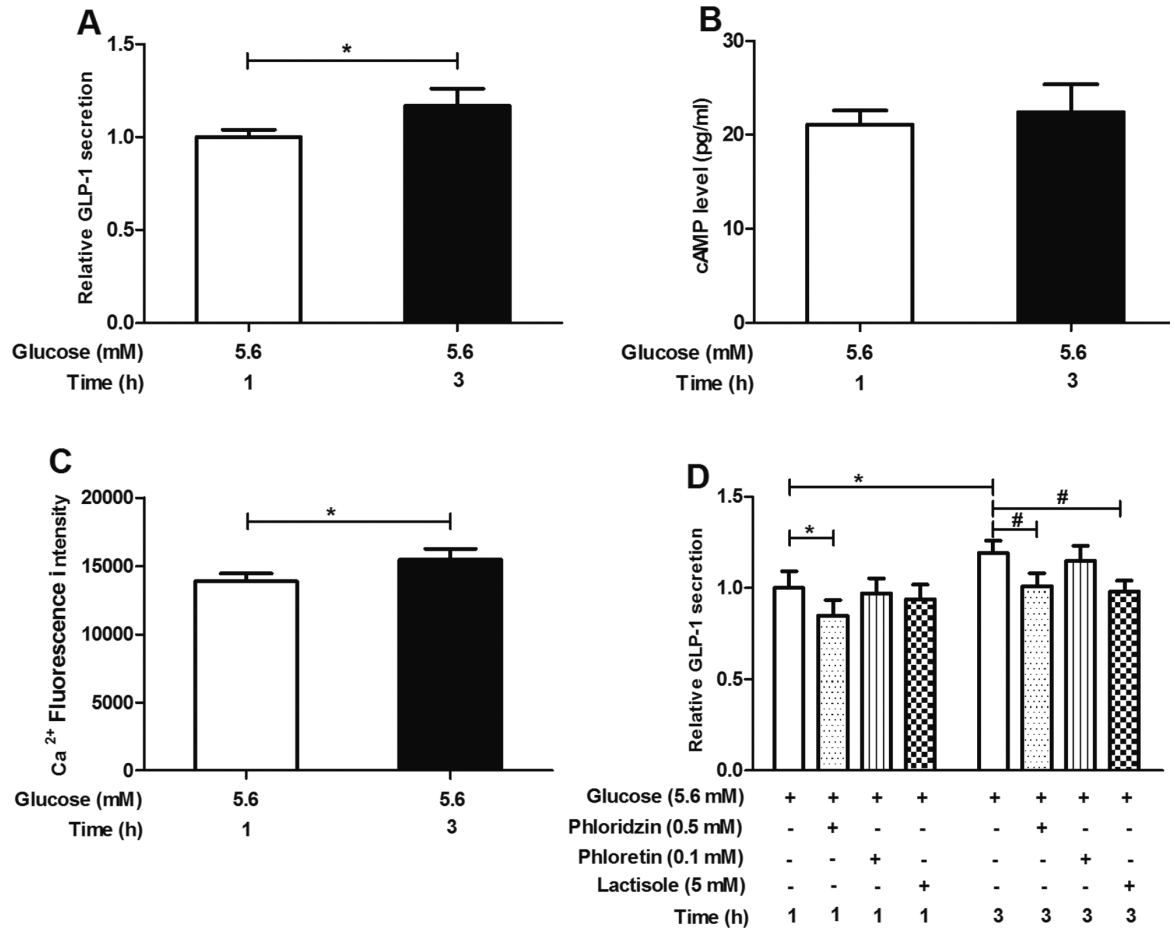

Figure 5. Possible involvements of SGLT1, GLUT2 or STRs in GLP-1 secretion in response to 3-h low-glucose exposure. STC-1 cells were incubated at 5.6 mM glucose in the presence or absence of the SGLT1 inhibitor phloridzin, the GLUT2 inhibitor phloretin or the STR inhibitor lactisole for 1 h or 3 h. (**A, D**) The GLP-1 concentration in the supernatant was measured by ELISA. The data were expressed as relative dimensionless number of 1-h group. **B.** Intracellular cAMP levels were measured by ELISA. **C.** The Ca^{2+} concentrations in STC-1 cells were measured at the 488/525-530 nm fluorescence ratio in the cells loaded with fluo-3/AM. * $p < 0.05$, # $p < 0.05$ ($n = 6$).

triggers electrical activity and the subsequent elevation of intracellular Ca^{2+}, which in turn evokes GLP-1 secretion especially in the presence of low concentrations of glucose (Gribble et al. 2003). Our results also identified the pivotal roles of electrogenic SGLT1 in GLP-1 secretion from STC-1 cells induced by low concentrations of glucose: 1) 5.6 mM glucose increased GLP-1 secretion and intracellular Ca^{2+} level as well as SGLT1 protein expression; 2) GLP-1 secretion and intracellular Ca^{2+} concentration under 5.6 mM glucose condition was decreased by the SGLT1 inhibitor phloridzin. The role of GLUT2 for glucose-induced GLP-1 secretion is mediated by an amplifying pathway at a given Ca^{2+} level and this amplifying pathway is also dependent on the SGLT1-mediated triggering pathway (Tagliavini and Pedersen 2017). In the current study, as a result of upregulation of GLUT2 expression, cAMP level is increased with glucose-dependent manner. Importantly, inhibition of GLUT2 decreased GLP-1 secretion and intracellular levels of Ca^{2+} and cAMP under high-glucose conditions. These results suggested the involvement of GLUT2 in GLP-1 secretion triggered by high concentrations of glucose, associated with cAMP and Ca^{2+} signaling system. While elevation of intracellular levels of Ca^{2+} and cAMP secondary to GLUT2-mediated glucose transport under high glucose conditions can increase GLP-1 secretion, but the increment is limited, which could explain the results that glucose at 100 and 200 mM did not further augment intracellular Ca^{2+} and GLP-1 secretion.

STR activation mobilizes Ca^{2+} from intracellular stores by the activation of phospholipase C and inositol triphosphate (Roper 2007). The increase in intracellular Ca^{2+} opens the Transient Receptor Potential Cation Channel Subfamily M Member 5, leading to Na^{+} influx, depolarization of the cell, and eventually GLP-1 secretion (Liu and Liman 2003). STR activation also activates adenylate cyclase and thereby leads to the formation of cAMP (Roper 2007). In MIN-6 beta cells, activation of STRs also facilitates the metabolic pathway in mitochondria and augments ATP production (Nakagawa et al. 2014). Glucose is a bona fide ligand for STRs and glucose-induced GLP-1 secretion is inhibited using pharmacological and genetic interference with STRs (Jang et al. 2007). Lactisole is usually used to assess the function and physiological role of the STRs in human. Some recent reports suggest that lactisole may be useful in assessing the role of STRs in rodents (Hamano et al. 2015). In the present study, co-administration of the STR inhibitor lactisole reduced GLP-1 secretion triggered by high glucose, which provides a useful pharmacological tool to assess the function of STRs in STC-1 cells. Moreover, high glucose increased STR subunits expression. These results also support the high-glucose-induced elevation of the intracellular cAMP and Ca^{2+} levels observed in our study. Taken together, STR signaling is also involved in GLP-1 secretion stimulated by high glucose. Interestingly, while STC-1 cells upon 1-h lactisole exposure exhibited a tendency to decrease in GLP-1 secretion triggered by 5.6 mM glucose, 3-h lactisole treatment obviously decreased GLP-1 secretion induced by 5.6 mM glucose. It may account for the result that 3-h exposure to 5.6 mM glucose-induced the sustained increase in GLP-1 secretion and intracellular Ca^{2+} levels. These results indicate the possible involvement of STRs in GLP-1 secretion induced by low glucose under certain conditions, but it requires being further investigated.

The existence of different physiological states should be considered when we study molecular function, in addition to the structure. In the current study, we pay attention to the role of glucose sensors, including SGLT1, GLUT2, and STR, in GLP-1 secretion under low-glucose and high-glucose conditions. Our data from STC-1 cells show that GLP-1 secretion is predominantly controlled by SLGT1 whereas GLUT2 and STR are involved when glucose concentrations in the medium are high. On the basis of the study and the above-mentioned reports, it was concluded that SGLT1 signals for glucose-stimulated GLP-1 secretion act in synergy with STR signaling and GLUT2 signaling, but it requires being further investigated.

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