K_{ATP} channel as well as SGLT1 participates in GIP secretion in the diabetic state

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP), a gut hormone secreted from intestinal K-cells, potentiates insulin secretion. Both K-cells and pancreatic β-cells are glucose-responsive and equipped with a similar glucose-sensing apparatus that includes glucokinase and an ATP-sensitive K^+ (K_{ATP}) channel comprising KIR6.2 and sulfonylurea receptor 1. In absorptive epithelial cells and enteroendocrine cells, sodium glucose co-transporter 1 (SGLT1) is also known to play an important role in glucose absorption and glucose-induced incretin secretion. However, the glucose-sensing mechanism in K-cells is not fully understood. In this study, we examined the involvement of SGLT1 (SLC5A1) and the K_{ATP} channels in glucose sensing in GIP secretion in both normal and streptozotocin-induced diabetic mice. Glimepiride, a sulfonylurea, did not induce GIP secretion and pretreatment with diazoxide, a K_{ATP} channel activator, did not affect glucose-induced GIP secretion in the normal state. In mice lacking K_{ATP} channels (*Kir6.2*$^{-/-}$ mice), glucose-induced GIP secretion was enhanced compared with control (*Kir6.2*$^{+/+}$) mice, but was completely blocked by the SGLT1 inhibitor phlorizin. In *Kir6.2*$^{-/-}$ mice, intestinal glucose absorption through SGLT1 was enhanced compared with that in *Kir6.2*$^{+/+}$ mice. On the other hand, glucose-induced GIP secretion was enhanced in the diabetic state in *Kir6.2*$^{+/+}$ mice. This GIP secretion was partially blocked by phlorizin, but was completely blocked by pretreatment with diazoxide in addition to phlorizin administration. These results demonstrate that glucose-induced GIP secretion depends primarily on SGLT1 in the normal state, whereas the K_{ATP} channel as well as SGLT1 is involved in GIP secretion in the diabetic state *in vivo*.

Key Words
- K_{ATP} channel
- SGLT1
- GIP secretion

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Introduction

Incretins, the gut hormones such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP1), which are secreted from enteroendocrine K-cells and L-cells, respectively, following meal ingestion stimulate insulin secretion in a glucose-concentration-dependent manner to prevent postprandial hyperglycemia (Baggio & Drucker 2007, Seino *et al*. 2010). A recent study has shown that an alternative form of GIP lacking the C-terminus is also secreted and expressed in pancreatic α-cells (Fujita *et al*. 2010). The beneficial effect of incretins on insulin secretion has provided the basis for incretin-based diabetes therapies, including dipeptidyl peptidase 4 (DPP4) inhibitors and GLP1 receptor agonists, which are currently in use worldwide (Drucker & Nauck 2006). Incretins are known to be secreted through stimulation of nutrients including carbohydrates, fats, and amino acids (Diakogiannaki *et al*. 2012). Whereas GLP1 secretion is regulated by hormonal and neural inputs as well as by nutrients, GIP secretion is mainly due to direct stimulation of these nutrients (Brubaker 1991, Rocca & Brubaker 1999, Jang *et al*. 2007).

In absorptive epithelial cells in the intestine, there are two glucose transport systems: sodium-dependent glucose transport mediated by a sodium-glucose co-transporter 1 (SGLT1) present in the brush-border (Takata *et al*. 1992, Martín *et al*. 1996, Wright 1998) and facilitated glucose transport mediated by facilitative glucose transporter 2 (GLUT2) in the basolateral membrane (Kellett 2001). Glucose enters the absorptive cells by SGLT1 and exits into the blood by GLUT2. It has been reported that GLUT2 knockout mice exhibited reduced GLP1 secretion but did not show a change in GIP secretion in response to oral glucose loading (Cani *et al*. 2007). In contrast, studies of SGLT1-deficient mice (Gorboulev *et al*. 2012) and SGLT1 inhibitors (Sykes *et al*. 1980, Powell *et al*. 2013) revealed that only the early-phase GLP1 secretion at 5 min after glucose loading is significantly reduced, whereas GIP secretion is almost completely abolished. The results from these studies indicate that SGLT1 is critical in glucose-induced incretin secretion, especially GIP secretion. It has been shown that K-cells express GLUT2, glucokinase, and an ATP-sensitive K (K_{ATP}) channel consisting of KIR6.2 and sulfonylurea receptor 1 (SUR1) subunits, identical to those expressed in pancreatic β-cells (Parker *et al*. 2009). These findings indicate that the glucose-sensing apparatus in K-cells may be similar to that expressed in β-cells.

We previously reported that *Kir6.2*-deficient (*Kir6.2*$^{-/-}$) mice entirely lack glucose-induced insulin secretion (GIIS) as assessed by perifusion of pancreatic islets (Miki *et al*. 1998). In contrast to GIIS, GIP secretion in response to oral glucose loading in *Kir6.2*$^{-/-}$ mice is somewhat enhanced, compared with that in *Kir6.2*$^{+/+}$ mice (Miki *et al*. 2005). This indicates that the K_{ATP} channels in K-cells are activated and involved in the suppression of glucose-induced GIP secretion in the physiological state. However, the link between SGLT1 and K_{ATP} channels in GIP secretion in the normal state and diabetic state is not understood.

In this study, we demonstrate that in mice in the normal state, SGLT1 is principally responsible for glucose-induced GIP secretion; however, in the diabetic state, the K_{ATP} channel is also involved in its secretion.

Materials and methods

Materials

Standard rodent normal chow, dextrin, and high-sucrose diet were used as previously reported (Sakamoto *et al*. 2012, Seino *et al*. 2013). Dextrin hydrate, glimepiride, diazoxide, α-methyl-D(+)-glucoside (αMG) were purchased from Wako, Osaka, Japan. Streptozotocin (STZ), phlorizin, and D-xylose were purchased from Sigma–Aldrich.

Mice

Kir6.2$^{-/-}$ mice were generated as described previously (Miki *et al*. 1998). As the *Kir6.2*$^{-/-}$ mice had been backcrossed with the C57BL/6 mouse strain for more than five generations, WT (*Kir6.2*$^{+/+}$) C57BL/6J mice were used as control mice. All animal experiments were carried out according to the protocol approved by the Nagoya University Institutional Animal Care and Use Committee.

Induction of diabetes

Two-month-old male *Kir6.2*$^{+/+}$ mice were rendered diabetic by i.p. injection of STZ (150 mg/kg body weigh) after a 16 h fast. Control mice received injections of an equal volume of saline. Two months after STZ injection, the mice with plasma glucose concentrations higher than 11 mmol/l under the fasted state were selected and used for the experiments together with the saline-injected controls.

Plasma biochemical analyses

Blood glucose levels were measured with ANTSENSE II (Bayer Medical). Plasma insulin levels were determined using an ELISA Kit (Morinaga, Tokyo, Japan). Plasma total

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GIP and GLP1 levels were determined using a GIP (total) ELISA Kit (Merck Millipore, Billerica, MA, USA) and an electrochemiluminescent sandwich immunoassay (Meso Scale Discovery, Gaithersburg, MD, USA) respectively.

Oral glucose, αMG, and saccharin administration

Procedure-naïve mice were deprived of food for 16 h and oral administration of 6 g/kg body weight D-glucose or 6.5 g/kg αMG (an equimolar amount to 6 g/kg glucose) or 0.05 g/kg sodium saccharin dehydrate by gavage was used in these experiments. After the oral administration, blood was collected at 0 and 15 min to measure blood glucose, plasma insulin, and plasma GIP levels.

Oral phlorizin administration

Mice were deprived of food for 16 h and then administrated 6 g/kg body weight D-glucose with or without 500 mg/kg body weight phlorizin administered orally.

Diazoxide and glucose administration

After food deprivation for 16 h, 40 or 240 mg/kg body weight diazoxide was administered orally. Ninety minutes after diazoxide administration, 6 g/kg body weight D-glucose was administered orally.

Voluntary feeding

As described previously (Seino *et al.* 2013), both *Kir6.2*$^{+/+}$ and *Kir6.2*$^{-/-}$ mice were trained for the experiments of voluntary feeding with standard chow or dextrin. They were deprived of food for 16 h and then given free access to standard chow or dextrin for the voluntary feeding experiments.

Oral D-xylose administration

Blood samples were collected 20, 60, 90, and 120 min after xylose (1 g/kg) was administered orally to determine xylose concentration. Xylose assay was carried out as previously described by Conarello *et al.* (2007).

Immunohistochemistry

The animals were killed and the duodenum from each animal was removed, fixed in 4% paraformaldehyde (PFA), and embedded in Tissue-Tek O.C.T. compound (Sakura Finetechnical, Tokyo, Japan). The tissue was frozen and stored at −80 °C. The sections were cut at 10 μm thickness and incubated overnight at 4 °C with primary antibodies against SGLT1 (1:200, sc-20582, Santa Cruz Biotechnology) and GIP (1:500, Y101, Yanaihara, Shizuoka, Japan), followed by 90 min incubation in Alexa Fluor-conjugated secondary antibody (1:1000, Alexa Fluor 546 or Alexa Fluor 647, Invitrogen) at room temperature. Images were taken using an HS BZ-9000 fluorescent microscope system (Keyence Corporation, Osaka, Japan).

Isolation of tissue RNA and quantitative real-time RT-PCR

Total RNA was extracted from the duodenum or the jejunum using RNAiso Plus (Takara Bio, Inc., Otsu, Shiga, Japan) as previously reported (Seino *et al.* 2008). One microgram of total RNA was reverse transcribed using the ReverTra Ace qPCR Kit (Toyobo, Osaka, Japan). After cDNA synthesis, quantitative real-time PCR was carried out in 25 μl reactions containing THUNDERBIRD qPCR Mix (Toyobo) using Mx3000 (Stratagene, La Jolla, CA, USA). The following oligonucleotide primers were used: mouse SGLT1, 5′-CATTCCAGACGTGCACCTGTAC-3′ (sense) and 5′-TCCAGGTCGATTCGCTCTTC-3′ (antisense). The mRNA levels were normalized with respect to those of β-actin mRNA.

Statistical analysis

Data are presented as mean±S.E.M. Statistical analysis was performed by unpaired, two-tailed Student's *t*-test or one-way or two-way ANOVA with Holm-Sidak multiple

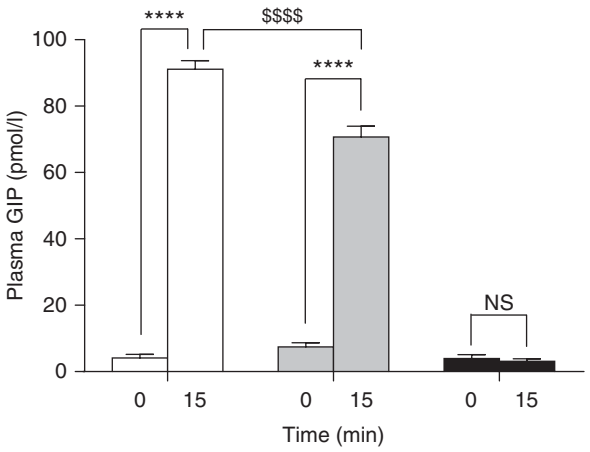

Figure 1
GIP secretion by metabolizable and nonmetabolizable sugar and artificial sweetener. Plasma GIP levels upon the oral administration of 6 g/kg glucose (white bars; $n=5$), 6.5 g/kg α-methyl-D(+)-glucoside (gray bars; $n=6$) or 0.05 g/kg sodium saccharin dihydrate (black bars; $n=5$) in *Kir6.2*$^{+/+}$ mice ($^{\$\$\$\$}P<0.0001$ comparing α-methyl-D(+)-glucoside administration with glucose administration at 15 min) (****$P<0.0001$ relative to 0 min). Data are expressed as means±S.E.M.

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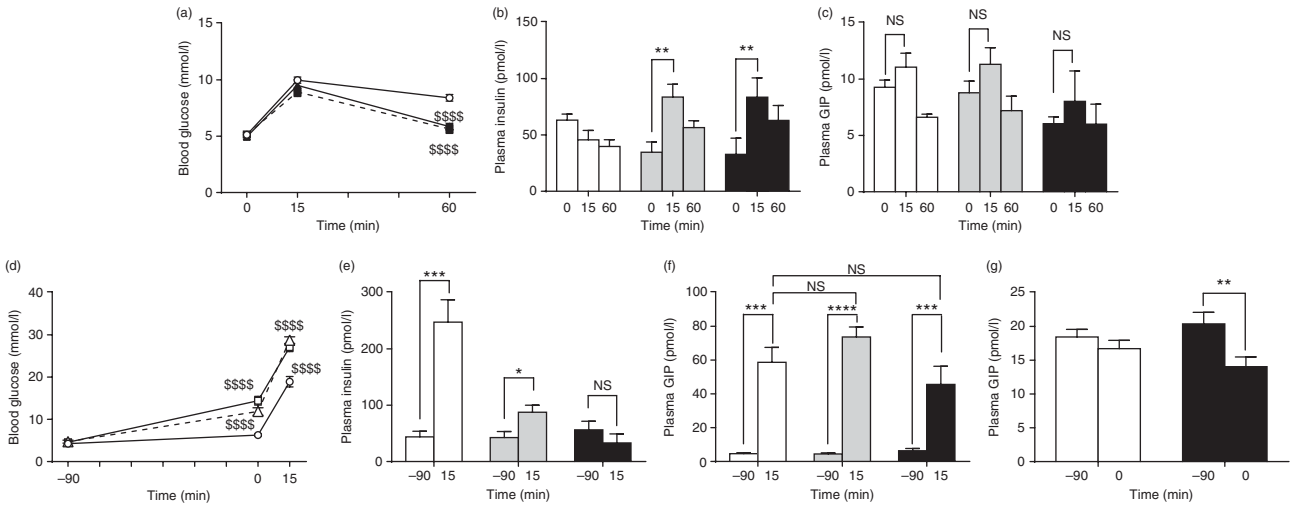

Figure 2
Effects of K_{ATP} channel on GIP secretion. (a) Blood glucose, (b) plasma insulin, and (c) plasma GIP levels upon the oral administration of vehicle (open circles and white bars; $n=6$), glimepiride 1 mg/kg (filled squares and grey bars; $n=7$) or glimepiride 10 mg/kg (filled triangles and black bars; $n=5$) to *Kir6.2*$^{+/+}$ mice. ($^{\$\$\$\$}P<0.0001$ comparing glimepiride administration with vehicle administration at 60 min) (**$P<0.01$ relative to 0 min). (d) Blood glucose, (e) plasma insulin, and (f) plasma GIP levels during OGTT, pretreated with vehicle (open circles and white bars; $n=6$), diazoxide 40 mg/kg (open squares and grey bars; $n=7$) or 240 mg/kg (open triangles and black bars; $n=10$) in *Kir6.2*$^{+/+}$ mice. (g) Plasma GIP levels in *Kir6.2*$^{+/+}$ mice pretreated with vehicle (white bars; $n=6$) or diazoxide 240 mg/kg (black bars; $n=6$). ($^{\$\$\$\$}P<0.0001$ comparing diazoxide pretreatment with vehicle pretreatment) (*$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ relative to −90 min). Data are expressed as means±S.E.M.

comparison. Differences between groups were considered statistically significant when P values were <0.05. GraphPad Prism for Windows version 6.03 (GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analysis.

Results

Metabolism of glucose in K-cells is not essential for GIP secretion

We first examined GIP secretion by oral administration of metabolizable (glucose), nonmetabolizable sugar (αMG), or artificial sweetener (sodium saccharin dehydrate) (Fig. 1). Both glucose and αMG stimulated GIP secretion significantly, whereas saccharin did not induce GIP secretion. Plasma GIP levels induced by glucose administration were higher than those induced by αMG administration at 15 min. These results indicate that metabolism of glucose in K-cells is not essential for sugar-induced GIP secretion and that stimulation of the sweet taste receptor is not involved in GIP secretion.

K_{ATP} channels are not involved in glucose-induced GIP secretion in the normal state

To investigate whether K_{ATP} channels are involved in GIP secretion *in vivo*, we examined the effects of sulfonylurea and the K_{ATP} channel activator diazoxide on GIP secretion. Oral administration of glimepiride (1 or 10 mg/kg), a sulfonylurea, did not change plasma GIP levels at 15 and 60 min, although plasma insulin levels were significantly elevated and blood glucose concentrations were decreased (Fig. 2a, b and c). Blood glucose levels during oral glucose tolerance test (OGTT) were significantly elevated by pretreatment with diazoxide (Fig. 2d). GIIS was partially or completely suppressed by pretreatment with diazoxide (40 or 240 mg/kg) respectively (Fig. 2e). Glucose-induced GIP secretion was not blocked at all by pretreatment with diazoxide (Fig. 2f). However, the basal level of GIP was not decreased by pretreatment with vehicle, but was decreased significantly by pretreatment with diazoxide (Fig. 2g).

GIP secretion by carbohydrates is enhanced in *Kir6.2*$^{-/-}$ mice

We then estimated the role of K_{ATP} channels in carbohydrate-induced GIP secretion. We examined GIP secretion in *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice deficient in the Kir6.2 component of K_{ATP} channels. Oral administration of glucose elicited significant GIP secretion in both *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice at 15 min. Plasma GIP levels in *Kir6.2*$^{-/-}$ mice at 15 min after glucose loading were significantly higher than those in *Kir6.2*$^{+/+}$ mice ($P=0.021$) (Fig. 3a), and are consistent with results described in a previous report (Miki *et al.* 2005). Plasma GIP levels at 15 min with voluntary

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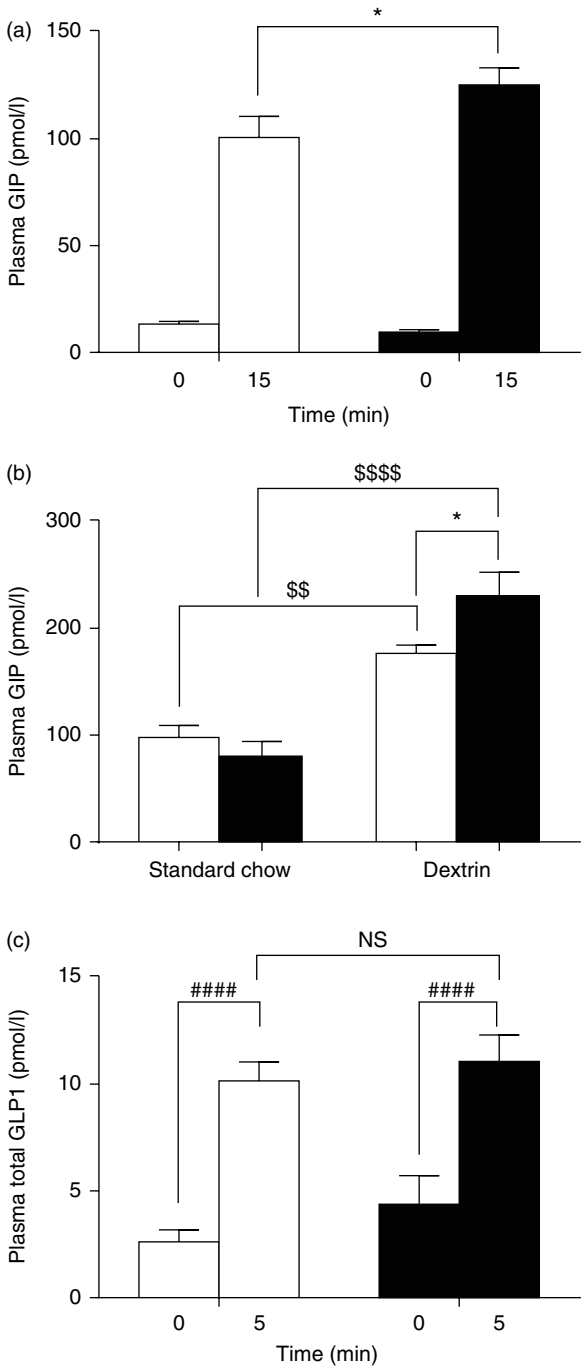

Figure 3
GIP secretion with oral administration of carbohydrate in *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice. (a) Plasma GIP levels during OGTT in *Kir6.2*$^{+/+}$ mice (white bars; $n=7$) and *Kir6.2*$^{-/-}$ mice (black bars; $n=9$). (*$P<0.05$ compared *Kir6.2*$^{+/+}$ mice with *Kir6.2*$^{-/-}$ mice at 15 min). (b) Plasma GIP levels with voluntary feeding of standard chow or dextrin at 15 min in *Kir6.2*$^{+/+}$ mice (white bars; $n=9$) and *Kir6.2*$^{-/-}$ mice (black bars; $n=7$–10) ($^{\$\$}P<0.01$, $^{\$\$\$\$}P<0.0001$ comparing standard chow with dextrin) (*$P<0.05$ comparing *Kir6.2*$^{+/+}$ mice with *Kir6.2*$^{-/-}$ mice). (c) Plasma GLP1 levels during OGTT in *Kir6.2*$^{+/+}$ mice (white bars; $n=12$) and *Kir6.2*$^{-/-}$ mice (black bars; $n=10$) ($^{\#\#\#\#}P<0.0001$ relative to 0 min). Data are expressed as means±S.E.M.

feeding on dextrin were higher than those with voluntary feeding on standard chow in both *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice, indicating that carbohydrate is a powerful GIP secretagogue. Interestingly, plasma GIP levels at 15 min in *Kir6.2*$^{-/-}$ mice with voluntary feeding on dextrin were significantly higher than those in *Kir6.2*$^{+/+}$ mice (Fig. 3b). These results indicate that glucose-induced GIP secretion is enhanced in *Kir6.2*$^{-/-}$ mice compared with that in *Kir6.2*$^{+/+}$ mice. Plasma GLP1 levels in *Kir6.2*$^{-/-}$ mice at 5 min after oral glucose loading did not differ from those in *Kir6.2*$^{+/+}$ mice (Fig. 3c).

Phlorizin completely blocks glucose-induced GIP secretion in both *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice in the normal state

We investigated the involvement of SGLT1 in glucose-induced GIP secretion by using phlorizin, a well-known inhibitor of SGLT1 (Alvarado & Crane 1962). Phlorizin inhibited the elevation of blood glucose levels in both *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice (Fig. 4a). Phlorizin completely blocked glucose-induced GIP secretion at 15 min not only in *Kir6.2*$^{+/+}$ mice but also in *Kir6.2*$^{-/-}$ mice (Fig. 4b).

SGLT1 expression and function are enhanced in *Kir6.2*$^{-/-}$ mice

Immunohistochemistry reveled that SGLT1 was distributed in the apical side of the duodenal absorptive epithelial cells and K-cells in both in *Kir6.2*$^{+/+}$ mice and in *Kir6.2*$^{-/-}$ mice (Fig. 5a and b), as previously reported (Gorboulev *et al.* 2012). Interestingly, the expression levels of *Sglt1* mRNA in the duodena of *Kir6.2*$^{-/-}$ mice were significantly higher than those in the duodena of *Kir6.2*$^{+/+}$ mice under fasted conditions (Fig. 5c). On the other hand, the expression levels of *Sglt1* mRNA in the jejuna were not different between *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice (Fig. 5c). Since xylose is absorbed through SGLT1 in the intestine and is not metabolized, the xylose absorption test reflects the activity of SGLT1 (Fujita *et al.* 1998). We then measured plasma xylose concentrations. Plasma xylose concentrations after oral administration of xylose in *Kir6.2*$^{-/-}$ mice were higher than those in *Kir6.2*$^{+/+}$ mice (Fig. 5d), indicating that xylose is absorbed through SGLT1 in *Kir6.2*$^{-/-}$ mice at a higher rate than that in *Kir6.2*$^{+/+}$ mice.

Long-term exposure to a high-sugar diet is known to increase the expression levels of *Sglt1* mRNA (Miyamoto *et al.* 1993). To clarify whether the K_{ATP} channel is required for the adaptive increase in *Sglt1* mRNA, mice were fed with high-sucrose diet for 5 weeks. The high-sucrose diet

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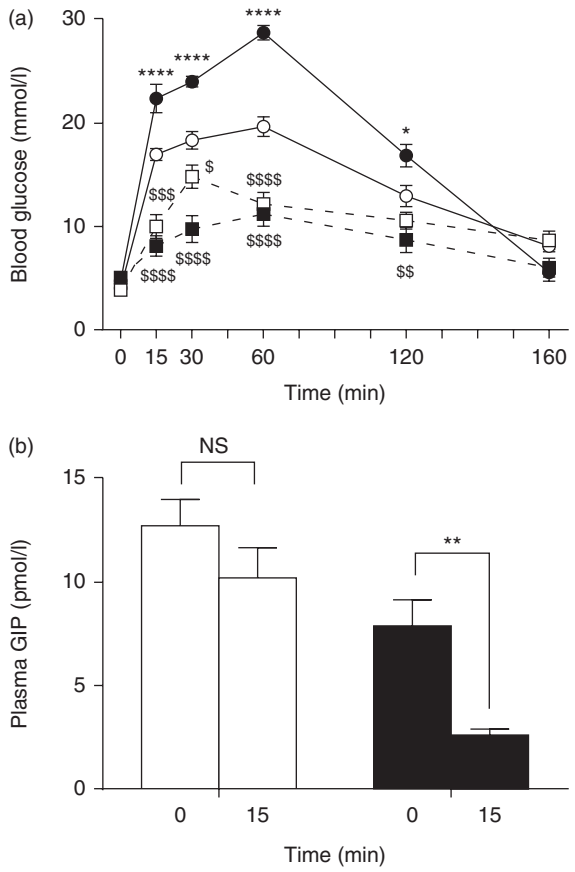

Figure 4
Effects of phlorizin on glucose-induced GIP secretion. (a) Blood glucose levels during OGTT without phlorizin administration in *Kir6.2*$^{+/+}$ mice (open circles; $n=5$) or with phlorizin administration in *Kir6.2*$^{+/+}$ mice (open squares; $n=6$) and without phlorizin administration in *Kir6.2*$^{-/-}$ mice (filled circles; $n=6$) or with phlorizin administration in *Kir6.2*$^{-/-}$ mice (filled squares; $n=6$). (*$P<0.05$, ****$P<0.0001$ comparing *Kir6.2*$^{+/+}$ mice without phlorizin administration, with *Kir6.2*$^{-/-}$ mice without phlorizin administration) ($^{\$}P<0.05$, $^{\$\$}P<0.01$, $^{\$\$\$}P<0.001$, $^{\$\$\$\$}P<0.0001$ comparing *Kir6.2*$^{+/+}$ mice or *Kir6.2*$^{-/-}$ mice with phlorizin administration, with *Kir6.2*$^{+/+}$ mice or *Kir6.2*$^{-/-}$ mice without phlorizin administration). (b) Plasma GIP levels during OGTT with phlorizin administration in *Kir6.2*$^{+/+}$ mice (white bars; $n=7$) and *Kir6.2*$^{-/-}$ mice (black bars; $n=7$) (**$P<0.01$ relative to 0 min). Data are expressed as means ± S.E.M.

increased the expression levels of *Sglt1* mRNA in the duodenum of both *Kir6.2*$^{+/+}$ mice (Fig. 5e) and *Kir6.2*$^{-/-}$ mice (Fig. 5f). This result indicates that regulation of the expression of *Sglt1* by intraluminal carbohydrate concentrations is K_{ATP}-channel-independent.

Both SGLT1 and K_{ATP} channels are involved in GIP secretion in the diabetic state

In STZ diabetic *Kir6.2*$^{+/+}$ mice with severe hyperglycemia, plasma insulin levels were decreased markedly under fasted conditions (Fig. 6a and b). The expression levels of *Sglt1* mRNA in the duodenum were higher in the STZ diabetic mice than those in control mice (treated with saline) (Fig. 6c).

In accordance with the increased *Sglt1* mRNA expression, GIP secretion after glucose loading was significantly enhanced at 15 min in STZ-treated mice (Fig. 6d). In contrast to the normal state (Fig. 4b), plasma GIP levels were significantly elevated 15 min after concomitant administration of glucose and phlorizin in the diabetic mice (Fig. 6e). To determine whether the K_{ATP} channel is involved in glucose-induced GIP secretion in diabetic mice, they were pretreated with diazoxide (240 mg/kg) and then administered glucose and phlorizin concomitantly.

Interestingly, phlorizin completely blocked the glucose-induced GIP secretion in the diabetic mice after pretreatment with diazoxide (Fig. 6f).

Discussion

In the present study, we show that SGLT1 is critical for glucose-induced GIP secretion in the diabetic state, as well as in the normal state, while the K_{ATP} channels participate in GIP secretion only in the diabetic state.

The incretins GIP and GLP1 are essential for maintianing systemic glucose homeostasis by amplifying insulin secretion. Enhancement of incretin action in insulin secretion is a therapeutic strategy to improve glycemic control in type 2 diabetes. It has been shown that in type 2 diabetes, the insulin secretory response to GLP1 is somewhat retained whereas the response to GIP is severely impaired (Nauck *et al.* 1993, Seino *et al.* 2010). Thus, it has been thought that GLP1-induced insulin secretion is the main contributor to improvement of glycemic control by incretin therapy. However, a recent study has indicated that GIP-induced insulin secretion is also involved in the therapeutic effect in type 2 diabetes (Woerle *et al.* 2012). In addition, GIP-overexpressing mice (Kim *et al.* 2012) and mice with GIP hypersecretion (Fukami *et al.* 2012) both exhibit good glycemic control due to excessive early-phase insulin secretion. These findings indicate that GIP-induced insulin secretion also is a contributor to improvement of glycemic control by incretin therapy.

GIP secretion is regulated by various nutrients, among which glucose is particularly important (Baggio & Drucker 2007). Glucose is transported across the apical membrane by SGLT1 (Wright 1998). SGLT1 is expressed at high levels in the upper intestine, especially the duodenum, and at moderate to low levels in the lower intestine, with an expression pattern similar to that of GIP-secreting K-cells

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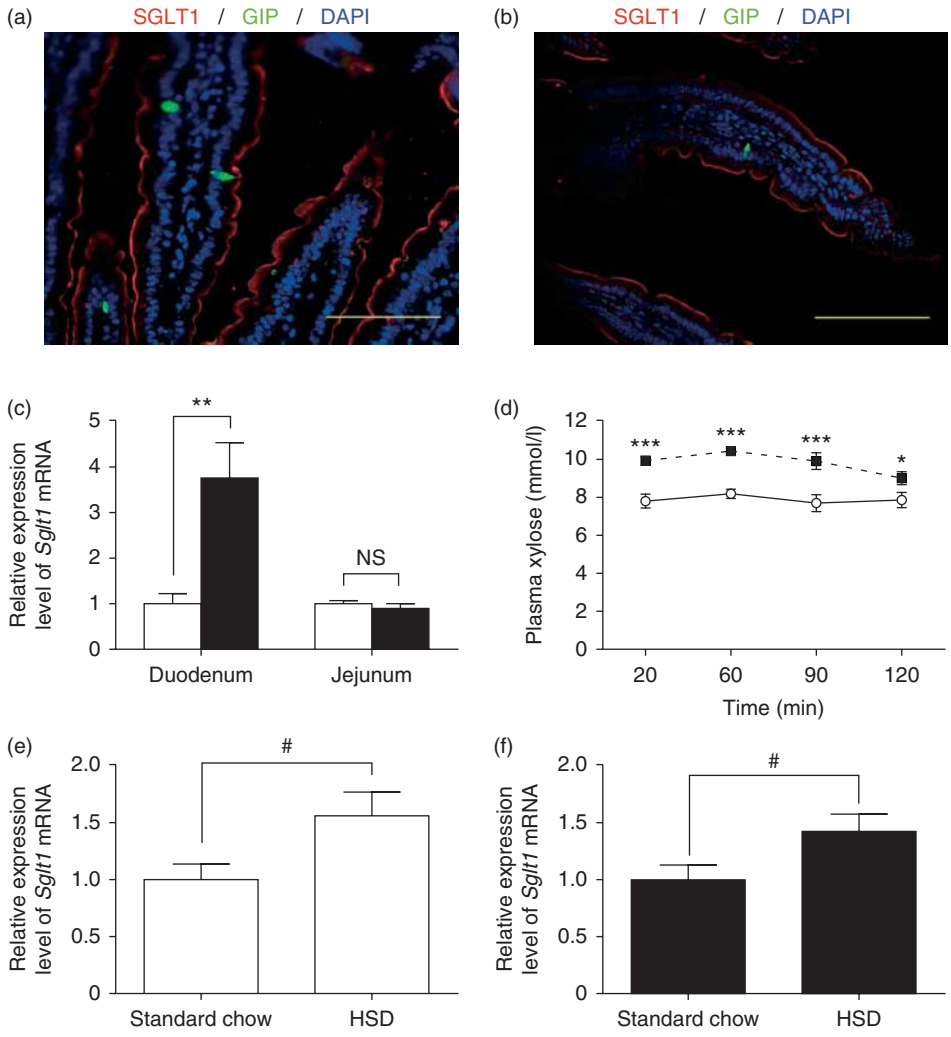

Figure 5

SGLT1 expression and activity in *Kir6.2*$^{+/+}$ mice and *Kir6.2*$^{-/-}$ mice. Representative immunohistochemistry for SGLT1 (red), GIP (green) in the duodenum in (a) *Kir6.2*$^{+/+}$ mice and (b) *Kir6.2*$^{-/-}$ mice (scale bars; 100 μm). (c) mRNA expression of *Sglt1* in the duodenum and jejunum in *Kir6.2*$^{+/+}$ mice (white bars; duodenum $n=9$, jejunum $n=5$) and *Kir6.2*$^{-/-}$ mice (black bars; duodenum $n=11$, jejunum $n=4$) under fasted conditions. (d) Plasma xylose concentrations during the oral D-xylose administration test in *Kir6.2*$^{+/+}$ mice (open circles; $n=5$) and *Kir6.2*$^{-/-}$ mice (filled squares; $n=12$). (e) mRNA expression of *Sglt1* in the duodenum in the fed state after 5 weeks loading with standard chow ($n=6$) or a high-sucrose diet (HSD) ($n=6$) in *Kir6.2*$^{+/+}$ mice. (f) mRNA expression of *Sglt1* in the duodenum in the fed state after 5 weeks loading with standard chow ($n=12$) or HSD ($n=12$) in *Kir6.2*$^{-/-}$ mice. (*$P<0.05$, **$P<0.01$, ***$P<0.001$ comparing *Kir6.2*$^{+/+}$ mice with *Kir6.2*$^{-/-}$ mice) ($^{\#}P<0.05$ relative to standard chow). Data are expressed as means ± S.E.M.

(Damholt *et al.* 1999, Yoshikawa *et al.* 2011). It has been shown recently that entry of glucose into intestinal epithelia is required for glucose-induced GIP secretion (Moriya *et al.* 2009, Gorboulev *et al.* 2012). The results of these studies indicate that activation of SGLT1 induces GIP enhancement and/or increases glucose uptake into epithelia.

GIP-secreting K-cells and pancreatic β-cells are both glucose-responsive. In fact, both K-cells and β-cells are equipped with a similar glucose-sensing apparatus including GLUT2, glucokinase, and K_{ATP} channels comprising KIR6.2 and SUR1 (Parker *et al.* 2009). In pancreatic β-cells, glucose is transported through GLUT2 and metabolized to generate ATP. The increase in ATP concentration closes the K_{ATP} channels, depolarizing the β-cell membrane, inducing Ca^{2+} influx through voltage-dependent calcium channels thereby triggering insulin secretion. The closure of the K_{ATP} channels is essential for GIIS (Seino *et al.* 2011). It has been shown that the K_{ATP} channel inhibitor sulfonylurea directly stimulates GIP secretion in isolated K-cells (Parker *et al.* 2009) and that SGLT1 is required for glucose-induced GIP secretion in a study of SGLT1 knockout mice *in vivo* (Gorboulev *et al.* 2012). However, the link between SGLT1

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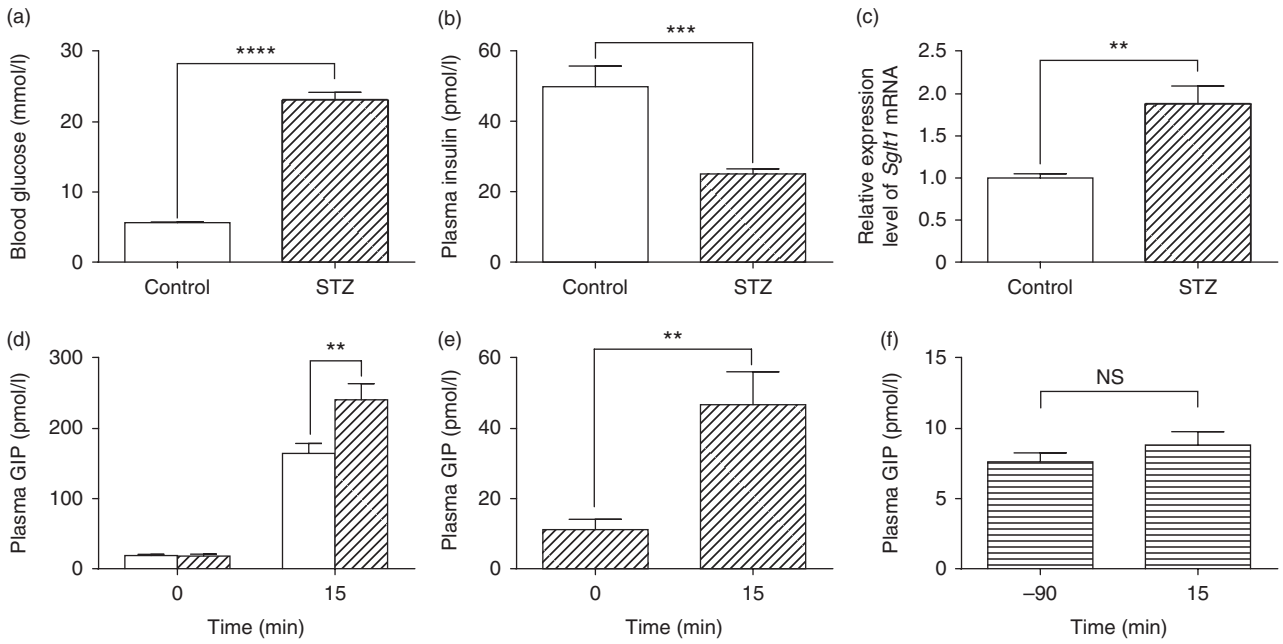

Figure 6

Effects of diazoxide and phlorizin on glucose-induced GIP secretion under the diabetic hyperglycemic condition. (a) Blood glucose, (b) plasma insulin, and (c) mRNA expression of *Sglt1* in the duodenum under fasted conditions in control mice (white bars; $n=8$) or the STZ diabetic mice (diagonally striped bars; $n=10$). (d) Plasma GIP levels during OGTT in control mice (white bars; $n=8$) or the diabetic mice (diagonally striped bars; $n=10$). (**$P<0.01$, ***$P<0.001$, ****$P<0.0001$ comparing control mice with the diabetic mice). (e) Plasma GIP levels during OGTT with phlorizin administration in the diabetic mice ($n=11$) (**$P<0.01$ relative to 0 min). (f) Plasma GIP levels during OGTT with phlorizin administration, in the diabetic mice pretreated with diazoxide ($n=8$). Data are expressed as means±S.E.M.

and K_{ATP} channels in glucose-induced GIP secretion is not known. It has also been reported that the expression levels of SGLT1 in small intestine were increased in STZ-induced diabetic rats (Burant *et al.* 1994), but the relationship between SGLT1 expression and glucose-induced GIP secretion was not investigated. In this study, we clarify for the first time, to our knowledge, *in vivo* that glucose-induced GIP secretion in the normal state is dependent primarily on an SGLT1-dependent mechanism, while glucose-induced GIP secretion in the diabetic state involves both SGLT1- and K_{ATP} channel-dependent mechanisms.

There have been a few reports showing that sulfonylureas have no effects on GIP secretion in patients with type 2 diabetes. However, the patients examined in these studies exhibited mild-to-moderate hyperglycemia (El-Ouaghlidi *et al.* 2007, Yabe *et al.* 2010, Stephens *et al.* 2011). Considering these together with the present findings, the stimulatory effect of K_{ATP} channels on GIP secretion may well require a threshold level of glycemia. The mechanism of involvement of the K_{ATP} channels in GIP secretion in K-cells is unknown, but deserves consideration. Given the rapid turnover of intestinal epithelia (Ferraris *et al.* 1992), it is possible that ATP consumption is greatly increased and causes the K_{ATP} channels to remain in the closed state under normal conditions. On the other hand, in the hyperglycemic state, glucose metabolism in K-cells is impaired, reducing ATP production to levels similar to those in diabetic β-cells. Under such conditions, the K_{ATP} channels are in the open state, in which inhibition of the channels is effective.

We have shown that in the normal state, the K_{ATP} channels are not involved in glucose-induced GIP secretion. *Kir6.2*$^{-/-}$ mice are the animal model of loss-of-function mutation of the K_{ATP} channel. However, we found that in *Kir6.2*$^{-/-}$ mice, glucose-induced GIP secretion was enhanced compared with that in *Kir6.2*$^{+/+}$ mice in the normal state. The K_{ATP} channel participates in the basal level of GIP secretion in *Kir6.2*$^{+/+}$ mice (Fig. 2g). On the other hand, in *Kir6.2*$^{-/-}$ mice, the basal level of GIP secretion is considered to be maintained independently of the K_{ATP} channel. In fact, as shown in Fig. 4b, the basal level of GIP is significantly reduced by phlorizin treatment, indicating that SGLT1 is involved in basal GIP secretion in *Kir6.2*$^{-/-}$ mice.

K-cells and L-cells are present predominantly in the upper and lower intestine respectively (Polak *et al.* 1973, Thomas *et al.* 1977, Damholt *et al.* 1999, Baggio & Drucker 2007, Egerod *et al.* 2012). Increased expression levels and

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activity in the duodenum in *Kir6.2*$^{-/-}$ mice could thus account for the enhanced glucose-induced GIP secretion. On the other hand, glucose-induced GLP1 secretion was not altered in *Kir6.2*$^{-/-}$ mice, most probably due to the unchanged expression levels of SGLT1 in the jejunum. Results from recent studies have indicated that the nutrient-sensing mechanism differs in the duodenum and in the jejunum (Seino *et al*. 2008, Breen *et al*. 2013), and that the nutrient-sensing system in K-cells and L-cells differs in the upper and lower intestine.

Gain-of-function mutations of the K_{ATP} channel cause neonatal diabetes and loss-of-function mutations of the K_{ATP} channel cause persistent hyperinsulinemic hypoglycemia of infancy (McTaggart *et al*. 2010). In patients carrying gain-of-function mutations of the K_{ATP} channel, sulfonylurea stimulates insulin secretion by closing the K_{ATP} channels of the pancreatic β-cells, but neither glucose-induced GIP nor GLP1 secretion is changed after sulfonylurea treatment (Pearson *et al*. 2006). It is of great interest to examine whether hypersecretion of GIP through SGLT1 in patients with loss-of function mutations contributes to hyperinsulinism in the disease.

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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Author contribution statement
H O, Y S, N H, T M, N I, S T, Y H, S S, and Y O contributed to the study design and data analyses. H O, Y S, N H, A I, and K S performed the research. H O, Y S, N H, A I, T I, K I, E U, Y H, T M, N I, S T, Y H, S S, and Y O contributed to the discussion. H O, Y S, and S S wrote the manuscript. S T, Y H, S S, and Y O reviewed the manuscript.

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