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

Hidetada Ogata¹, Yusuke Seino^{1,2}, Norio Harada³, Atsushi Iida¹, Kazuyo Suzuki³, Takako Izumoto^{1,4}, Kota Ishikawa¹, Eita Uenishi¹, Nobuaki Ozaki^{1,5}, Yoshitaka Hayashi⁶, Takashi Miki⁷, Nobuya Inagaki³, Shin Tsunekawa¹, Yoji Hamada², Susumu Seino⁸ and Yutaka Oiso¹

Departments of ¹Endocrinology and Diabetes and ²Metabolic Medicine, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

³Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan

⁴Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

⁵Research Center of Health, Physical Fitness, and Sports and ⁶Division of Stress Adaptation and Recognition, Department of Genetics, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

⁷Department of Medical Physiology, Graduate School of Medicine, Chiba University, Chiba, Japan

⁸Division of Molecular and Metabolic Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Correspondence should be addressed to Y Seino
Email
yusuke@med.nagoya-u.ac.jp

Abstract

Glucose-dependent insulintropic 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

Journal of Endocrinology
(2014) 222, 191–200

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 perfusion 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 (α MG) 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 weight) 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

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, α MG, 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 α MG (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 administered 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 RNeasy 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'-CATTC-CAGACGTGCACCTGTAC-3' (sense) and 5'-TCCAGGTC-GATTCGCTCTTC-3' (antisense). The mRNA levels were normalized with respect to those of β -actin mRNA.

Statistical analysis

Data are presented as mean $\pm$ 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 $\pm$ S.E.M.

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 $\pm$ 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 (α MG), or artificial sweetener (sodium saccharin dehydrate) (Fig. 1). Both glucose and α MG stimulated GIP secretion significantly, whereas saccharin did not induce GIP secretion. Plasma GIP levels induced by glucose administration were higher than those induced by α MG 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). GIS 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

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 $\pm$ 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 revealed 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 jejunum 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

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 maintaining 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

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 GIIIS (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

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; $n=10$).

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-Ouaghli *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

(** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ 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 $\pm$ S.E.M.

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

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.

Funding

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sport, Science and Technology, Japan.

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.

Acknowledgements

The authors thank Michiko Yamada and Mayumi Katagiri (Nagoya University Graduate School of Medicine, Nagoya, Japan) for their technical assistance.

References

- Alvarado F & Crane RK 1962 Phlorizin as a competitive inhibitor of the active transport of sugars by hamster small intestine, *in vitro*. *Biochimica et Biophysica Acta* **56** 170–172. (doi:10.1016/0006-3002(62)90543-7)
- Baggio LL & Drucker DJ 2007 Biology of incretins: GLP-1 and GIP. *Gastroenterology* **132** 2131–2157. (doi:10.1053/j.gastro.2007.03.054)

- Breen DM, Rasmussen BA, Côté CD, Jackson VM & Lam TKT 2013 Nutrient-sensing mechanisms in the gut as therapeutic targets for diabetes. *Diabetes* **62** 3005–3013. (doi:10.2337/db13-0523)
- Brubaker PL 1991 Regulation of intestinal proglucagon-derived peptide secretion by intestinal regulatory peptides. *Endocrinology* **128** 3175–3182. (doi:10.1210/endo-128-6-3175)
- Burant CF, Flink S, DePaoli AM, Chen J, Lee WS, Hediger MA, Buse JB & Chang EB 1994 Small intestine hexose transport in experimental diabetes. Increased transporter mRNA and protein expression in enterocytes. *Journal of Clinical Investigation* **93** 578–585. (doi:10.1172/JCI117010)
- Cani PD, Holst JJ, Drucker DJ, Delzenne NM, Thorens B, Burcelin R & Knauf C 2007 GLUT2 and the incretin receptors are involved in glucose-induced incretin secretion. *Molecular and Cellular Endocrinology* **276** 18–23. (doi:10.1016/j.mce.2007.06.003)
- Conarello SL, Jiang G, Mu J, Li Z, Woods J, Zychband E, Ronan J, Liu F, Roy RS, Zhu L *et al.* 2007 Glucagon receptor knockout mice are resistant to diet-induced obesity and streptozotocin-mediated β cell loss and hyperglycaemia. *Diabetologia* **50** 142–150. (doi:10.1007/s00125-006-0481-3)
- Damholt AB, Kofod H & Buchan AM 1999 Immunocytochemical evidence for a paracrine interaction between GIP and GLP-1-producing cells in canine small intestine. *Cell and Tissue Research* **298** 287–293. (doi:10.1007/s004419900093)
- Diakogiannaki E, Gribble FM & Reimann F 2012 Nutrient detection by incretin hormone secreting cells. *Physiology & Behavior* **106** 387–393. (doi:10.1016/j.physbeh.2011.12.001)
- Drucker DJ & Nauck MA 2006 The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* **368** 1696–1705. (doi:10.1016/S0140-6736(06)69705-5)
- Egerod KL, Engelstoft MS, Grunddal KV, Nøhr MK, Secher A, Sakata I, Pedersen J, Windeløv JA, Füchtbauer E-M, Olsen J *et al.* 2012 A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY, and neurotensin but not somatostatin. *Endocrinology* **153** 5782–5795. (doi:10.1210/en.2012-1595)
- El-Ouaghilidi A, Rehling E, Holst JJ, Schweizer A, Foley J, Holmes D & Nauck MA 2007 The dipeptidyl peptidase 4 inhibitor vildagliptin does not accentuate glibenclamide-induced hypoglycemia but reduces glucose-induced glucagon-like peptide 1 and gastric inhibitory polypeptide secretion. *Journal of Clinical Endocrinology and Metabolism* **92** 4165–4171. (doi:10.1210/jc.2006-1932)
- Ferraris RP, Villenas SA & Diamond J 1992 Regulation of brush-border enzyme activities and enterocyte migration rates in mouse small intestine. *American Journal of Physiology* **262** G1047–G1059.
- Fujita Y, Kojima H, Hidaka H, Fujimiya M, Kashiwagi A & Kikkawa R 1998 Increased intestinal glucose absorption and postprandial hyperglycaemia at the early step of glucose intolerance in Otsuka Long-Evans Tokushima Fatty rats. *Diabetologia* **41** 1459–1466. (doi:10.1007/s001250051092)
- Fujita Y, Wideman RD, Asadi A, Yang GK, Baker R, Webber T, Zhang T, Wang R, Ao Z, Warnock GL *et al.* 2010 Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet α -cells and promotes insulin secretion. *Gastroenterology* **138** 1966–1975. (doi:10.1053/j.gastro.2010.01.049)
- Fukami A, Seino Y, Ozaki N, Yamamoto M, Sugiyama C, Sakamoto-Miura E, Himeno T, Takagishi Y, Tsunekawa S, Ali S *et al.* 2012 Ectopic expression of GIP in pancreatic β -cells maintains enhanced insulin secretion in mice with complete absence of proglucagon-derived peptides. *Diabetes* **62** 510–518. (doi:10.2337/db12-0294)
- Gorboulev V, Schürmann A, Vallon V, Kipp H, Jäschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R *et al.* 2012 Na^{+} -D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes* **61** 187–196. (doi:10.2337/db11-1029)
- Jang H, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim B-J, Zhou J, Kim HH, Xu X, Chan SL, Juhaszova M *et al.* 2007 Gut-expressed

- gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *PNAS* **104** 15069–15074. (doi:10.1073/pnas.0706890104)
- Kellett GL 2001 The facilitated component of intestinal glucose absorption. *Journal of Physiology* **531** 585–595. (doi:10.1111/j.1469-7793.2001.0585h.x)
- Kim S-J, Nian C, Karunakaran S, Clee SM, Isaacs CM & McIntosh CHS 2012 GIP-overexpressing mice demonstrate reduced diet-induced obesity and steatosis, and improved glucose homeostasis. *PLoS ONE* **7** e40156. (doi:10.1371/journal.pone.0040156)
- Martín MG, Turk E, Lostao MP, Kerner C & Wright EM 1996 Defects in Na^+ /glucose cotransporter (SGLT1) trafficking and function cause glucose-galactose malabsorption. *Nature Genetics* **12** 216–220. (doi:10.1038/ng0296-216)
- McTaggart JS, Clark RH & Ashcroft FM 2010 The role of the K_{ATP} channel in glucose homeostasis in health and disease: more than meets the islet. *Journal of Physiology* **588** 3201–3209. (doi:10.1113/jphysiol.2010.191767)
- Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gonoi T, Iwanaga T, Miyazaki J & Seino S 1998 Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. *PNAS* **95** 10402–10406. (doi:10.1073/pnas.95.18.10402)
- Miki T, Minami K, Shinozaki H, Matsumura K, Saraya A, Ikeda H, Yamada Y, Holst JJ & Seino S 2005 Distinct effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 on insulin secretion and gut motility. *Diabetes* **54** 1056–1063. (doi:10.2337/diabetes.54.4.1056)
- Miyamoto K, Hase K, Takagi T, Fujii T, Taketani Y, Minami H, Oka T & Nakabou Y 1993 Differential responses of intestinal glucose transporter mRNA transcripts to levels of dietary sugars. *Biochemical Journal* **295** 211–215.
- Moriya R, Shirakura T, Ito J, Mashiko S & Seo T 2009 Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. *American Journal of Physiology. Endocrinology and Metabolism* **297** E1358–E1365. (doi:10.1152/ajpendo.00412.2009)
- Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R & Creutzfeldt W 1993 Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *Journal of Clinical Investigation* **91** 301–307. (doi:10.1172/JCI116186)
- Parker HE, Habib AM, Rogers GJ, Gribble FM & Reimann F 2009 Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* **52** 289–298. (doi:10.1007/s00125-008-1202-x)
- Pearson ER, Flechtner I, Njølstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V *et al.* 2006 Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *New England Journal of Medicine* **355** 467–477. (doi:10.1056/NEJMoa061759)
- Polak JM, Bloom SR, Kuzio M, Brown JC & Pearse AG 1973 Cellular localization of gastric inhibitory polypeptide in the duodenum and jejunum. *Gut* **14** 284–288. (doi:10.1136/gut.14.4.284)
- Powell DR, Smith M, Greer J, Harris A, Zhao S, DaCosta C, Mseeh F, Shadoan MK, Sands A, Zambrowicz B *et al.* 2013 LX4211 increases serum glucagon-like peptide 1 and peptide YY levels by reducing sodium/glucose cotransporter 1 (SGLT1)-mediated absorption of intestinal glucose. *Journal of Pharmacology and Experimental Therapeutics* **345** 250–259. (doi:10.1124/jpet.113.203364)
- Rocca AS & Brubaker PL 1999 Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. *Endocrinology* **140** 1687–1694. (doi:10.1210/endo.140.4.6643)
- Sakamoto E, Seino Y, Fukami A, Mizutani N, Tsunekawa S, Ishikawa K, Ogata H, Uenishi E, Kamiya H, Hamada Y *et al.* 2012 Ingestion of a moderate high-sucrose diet results in glucose intolerance with reduced liver glucokinase activity and impaired glucagon-like peptide-1 secretion. *Journal of Diabetes Investigation* **3** 432–440. (doi:10.1111/j.2040-1124.2012.00208.x)
- Seino Y, Miki T, Kiyonari H, Abe T, Fujimoto W, Kimura K, Takeuchi A, Takahashi Y, Oiso Y, Iwanaga T *et al.* 2008 Isx participates in the maintenance of vitamin A metabolism by regulation of β -carotene 15,15'-monooxygenase (Bcmo1) expression. *Journal of Biological Chemistry* **283** 4905–4911. (doi:10.1074/jbc.M707928200)
- Seino Y, Fukushima M & Yabe D 2010 GIP and GLP-1, the two incretin hormones: similarities and differences. *Journal of Diabetes Investigation* **1** 8–23. (doi:10.1111/j.2040-1124.2010.00022.x)
- Seino S, Shibasaki T & Minami K 2011 Dynamics of insulin secretion and the clinical implications for obesity and diabetes. *Journal of Clinical Investigation* **121** 2118–2125. (doi:10.1172/JCI45680)
- Seino Y, Miki T, Fujimoto W, Young-Lee E, Takahashi Y, Minami K, Oiso Y & Seino S 2013 Cephalic phase insulin secretion is K_{ATP} channel independent. *Journal of Endocrinology* **218** 25–33. (doi:10.1530/JOE-12-0579)
- Stephens JW, Bodvarsdottir TB, Wareham K, Prior SL, Bracken RM, Lowe GD, Rumley A, Dunseath G, Luzio S, Deacon CF *et al.* 2011 Effects of short-term therapy with glibenclamide and repaglinide on incretin hormones and oxidative damage associated with postprandial hyperglycaemia in people with type 2 diabetes mellitus. *Diabetes Research and Clinical Practice* **94** 199–206. (doi:10.1016/j.diabres.2011.07.014)
- Sykes S, Morgan LM, English J & Marks V 1980 Evidence for preferential stimulation of gastric inhibitory polypeptide secretion in the rat by actively transported carbohydrates and their analogues. *Journal of Endocrinology* **85** 201–207. (doi:10.1677/joe.0.0850201)
- Takata K, Kasahara T, Kasahara M, Ezaki O & Hirano H 1992 Immuno-histochemical localization of Na^+ -dependent glucose transporter in rat jejunum. *Cell and Tissue Research* **267** 3–9. (doi:10.1007/BF00318685)
- Thomas FB, Shook DF, O'Dorisio TM, Cataland S, Mekhjian HS, Caldwell JH & Mazzaferri EL 1977 Localization of gastric inhibitory polypeptide release by intestinal glucose perfusion in man. *Gastroenterology* **72** 49–54.
- Woerle HJ, Carneiro L, Derani A, Göke B & Schirra J 2012 The role of endogenous incretin secretion as amplifier of glucose-stimulated insulin secretion in healthy subjects and patients with type 2 diabetes. *Diabetes* **61** 2349–2358. (doi:10.2337/db11-1701)
- Wright EM 1998 I. Glucose galactose malabsorption. *American Journal of Physiology* **275** G879–G882.
- Yabe D, Kuroe A, Lee S, Watanabe K, Hyo T, Hishizawa M, Kuroe T, Deacon CF, Holst JJ, Hirano T *et al.* 2010 Little enhancement of meal-induced glucagon-like peptide 1 secretion in Japanese: comparison of type 2 diabetes patients and healthy controls. *Journal of Diabetes Investigation* **1** 56–59. (doi:10.1111/j.2040-1124.2010.00010.x)
- Yoshikawa T, Inoue R, Matsumoto M, Yajima T, Ushida K & Iwanaga T 2011 Comparative expression of hexose transporters (SGLT1, GLUT1, GLUT2 and GLUT5) throughout the mouse gastrointestinal tract. *Histochemistry and Cell Biology* **135** 183–194. (doi:10.1007/s00418-011-0779-1)

Received in final form 10 May 2014

Accepted 29 May 2014

Accepted Preprint published online 2 June 2014