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Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice

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Moriya R, Shirakura T, Ito J, Mashiko S, Seo T. Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. *Am J Physiol Endocrinol Metab* 297: E1358–E1365, 2009. First published October 6, 2009; doi:10.1152/ajpendo.00412.2009.—Glucose ingestion stimulates the secretion of the incretin hormones, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). Despite the critical role of incretins in glucose homeostasis, the mechanism of glucose-induced incretin secretion has not been established. We investigated the underlying mechanism of glucose-induced incretin secretion in vivo in mice. Injection of glucose at 1 g/kg in the upper intestine significantly increased plasma GIP and GLP-1 levels, whereas injection of glucose in the colon did not increase GIP or GLP-1 levels. This finding indicates that the glucose sensor for glucose-induced incretin secretion is in the upper intestine. Coadministration of a sodium-glucose cotransporter-1 (SGLT1) inhibitor, phloridzin, with glucose in the upper intestine blocked glucose absorption and glucose-induced incretin secretion. α-methyl-D-glucopyranoside (MDG), an SGLT1 substrate that is a nonmetabolizable sugar, significantly increased plasma GIP and GLP-1 levels, whereas phloridzin blocked these increases, indicating that concomitant transport of sodium ions and glucose (substrate) via SGLT1 itself triggers incretin secretion without the need for subsequent glucose metabolism. Interestingly, oral administration of MDG significantly increased plasma GIP, GLP-1, and insulin levels and reduced blood glucose levels during an intraperitoneal glucose tolerance test. Furthermore, chronic MDG treatment in drinking water (3%) for 13 days reduced blood glucose levels after a 2-h fast and in an oral glucose tolerance test in diabetic *db/db* mice. Our findings indicate that SGLT1 serves as the intestinal glucose sensor for glucose-induced incretin secretion and that a noncalorigenic SGLT1 substrate ameliorates hyperglycemia by stimulating incretin secretion.

glucagon-like peptide-1; glucose-dependent insulinotropic peptide; α-methyl-D-glucopyranoside; insulin resistance

GLUCOSE-DEPENDENT INSULINOTROPIC peptide (GIP) and glucagon-like peptide-1 (GLP-1) are insulinotropic gut hormones secreted from K cells, which are abundantly detected in the upper intestine, and L cells, which are abundantly detected in the lower intestine. GIP and GLP-1 play an important role in the regulation of blood glucose levels in humans and rodents via several mechanisms, including amplification of glucose-induced insulin secretion. Research has shown that incretin-based therapies, such as GLP-1 analogs and agents that inhibit GIP and GLP-1 degradation, have a therapeutic advantage over current therapies in the treatment of type 2 diabetes (1, 5). Because patients with type 2 diabetes have lower postprandial incretin levels (10, 18, 21), a therapeutic strategy that has been receiving increasing interest involves the use of oral drugs that increase circulating bioactive GIP and GLP-1 levels to stimulate secretion of endogenous incretin from intestinal K and L cells.

Nutrient intake and delivery to the gut is the primary stimulator of incretin secretion. Several nutrients, including triglycerides, fatty acids, protein (peptone), and carbohydrate (glucose) have been shown to stimulate incretin secretion (8, 15, 19). Among these nutrients, glucose is one of the most potent stimulators of incretin secretion in rodents and humans (6, 15, 16, 19). Interestingly, plasma GIP and GLP-1 levels increase after oral ingestion of glucose, whereas intravenous injection of glucose has no effect on GIP or GLP-1 levels (20), suggesting that there is a luminal glucose sensor that triggers glucose-induced incretin secretion.

From studies of gut hormone-secreting cell lines, including GLUTag, STC-1, and NCI-H716 cells, various candidate intestinal glucose sensors for glucose-induced incretin secretion have been proposed, including ATP-sensitive potassium (K_{ATP}) channel closure, sodium glucose cotransporter-1 (SGLT1) activity, and activation of sweet taste receptors (14, 16, 19, 20). Recently, Reimann et al. (13) and Parker et al. (11) identified and purified primary mouse K and L cells and showed that these cells express SGLT1 and that glucose and α-methyl-D-glucopyranoside (MDG), an SGLT1 substrate that is a nonmetabolizable sugar, but not artificial sweeteners, stimulated GIP and GLP-1 secretion (11, 13). SGLT1 transports sodium ions and glucose concomitantly, and therefore produces electrogenic signals (23). These in vitro studies using primary mouse K and L cells indicate that SGLT1 is an intestinal glucose sensor, and electrogenic coupling of sodium ions and glucose uptake via SGLT1, at least in part, triggers glucose-induced incretin secretion. However, whether SGLT1 signaling is important for stimulation of incretin secretion by glucose in vivo is unknown.

The aim of this study was *1*) to determine whether SGLT1 mediates glucose-induced incretin secretion in vivo and *2*) to clarify the therapeutic utility of stimulating SGLT1-dependent incretin secretion by a noncalorigenic SGLT1 substrate for treatment of type 2 diabetes. To this aim, we examined the effects of the SGLT1 substrates glucose MDG, and 3-*O*-methyl-D-glucose (3-OMG) on plasma GIP, GLP-1, and glucose levels in mice in vivo.

MATERIALS AND METHODS

Materials

Phloridzin dihydrate, MDG, and glucose were purchased from Sigma (St. Louis, MO). 3-OMG, sucralose, and saccharin sodium dihydrate were purchased from Wako (Osaka, Japan). Gastrin-releasing peptide (GRP) was purchased from the Peptide Institute (Osaka, Japan).

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Animals

Male C57B6 mice (9–13 wk old; CLEA Japan, Tokyo, Japan) and *db/db* mice (6–9 wk old; Charles River Japan, Tokyo, Japan) were used. Animals were housed individually in plastic cages kept at 23 ± 2°C, 55 ± 15% relative humidity, and maintained on a light-dark cycle with the lights on from 0700–1900. Water and regular chow (CE-2; CLEA Japan) were available ad libitum unless otherwise stated. All experimental procedures were approved by our Institutional Animal Care Committee and followed the Japanese Pharmacological Society Guidelines for Animal Use.

Study 1: Intraluminal Administration

Before the study, mice were fasted for 18 h and then anesthetized with intraperitoneal pentobarbital (80 mg/kg). Through a midline incision, a polyethylene catheter (2 mm in diameter) was placed in the upper small intestine (1 cm below the pylorus), middle small intestine (15 cm above the ileocecal junction), or colon (0.5 cm below the cecocolic junction). The abdominal wound was covered with a saline gauze, and the animal body temperature was maintained at 37°C with a heating pad. After at least 30 min of stabilization, mice were used for the study.

Study a. Mice were randomly divided into the following six groups (n = 6–8): vehicle in the upper small intestine, glucose at 1.0 g/kg (5.5 mmol/kg) in the upper small intestine, vehicle in the middle small intestine, glucose at 1.0 g/kg in the middle small intestine, vehicle in the colon, and glucose at 1.0 g/kg in the colon. Mice received intraluminal administration of vehicle [distilled water (DW)] or glucose in the upper small intestine, middle small intestine, or colon for 10 $ml \cdot kg^{-1} \cdot animal^{-1} \cdot min^{-1}$. Blood samples were collected from the portal vein at 5 and 15 min after the administration, and plasma glucose GIP and GLP-1 were measured.

Study b. Mice were randomly divided into the following four groups (n = 6–10): vehicle (30% polyethylene glycol 400), glucose at 1.0 g/kg (5.5 mmol/kg), glucose plus 0.5 g/kg phloridzin (1 mmol/kg), and 0.5 g/kg phloridzin (1 mmol/kg) alone. Mice received intraluminal administration of vehicle, glucose alone, glucose plus phloridzin, or phloridzin alone in the upper small intestine for 10 $ml \cdot kg^{-1} \cdot animal^{-1} \cdot min^{-1}$ (n = 6–10). Blood samples were collected from the portal vein at 5 min after the administration, and plasma glucose GIP and GLP-1 were measured.

Study c. Mice were randomly divided into the following five groups (n = 6–10): vehicle (30% polyethylene glycol 400), 3-OMG at 1.1 g/kg (5.5 mmol/kg), 3-OMG plus phloridzin at 0.5 g/kg (1 mmol/kg), MDG at 1.1 g/kg (5.5 mmol/kg), and MDG plus phloridzin at 0.5 g/kg (1 mmol/kg). Mice received intraluminal administration of vehicle, 3-OMG alone, 3-OMG plus phloridzin, MDG, or MDG plus phloridzin for 10 $ml \cdot kg^{-1} \cdot animal^{-1} \cdot min^{-1}$ (n = 6–10). Blood samples were collected from the portal vein at 5 min after the administration, and plasma GIP and GLP-1 were measured.

Study d. Mice were randomly divided into the following three groups (n = 6–10): vehicle (DW), saccharin at 1.0 g/kg (5.5 mmol/kg), and sucralose at 2.2 g/kg (5.5 mmol/kg). Mice received intraluminal administration of vehicle, saccharin, or sucralose for 10 $ml \cdot kg^{-1} \cdot animal^{-1} \cdot min^{-1}$ (n = 6–10). Blood samples were collected from the portal vein at 5 min after the administration, and plasma GIP and GLP-1 were measured.

Study 2: Intravenous Administration

Mice were fasted for 18 h and then anesthetized with intraperitoneal pentobarbital (80 mg/kg). Saline, glucose (1 g/kg), MDG (1.1 g/kg), 3-OMG (1.1 g/kg), or GRP (30 μg/kg) were administered intravenously (10 ml/kg, n = 6–8). Because GRP in the circulation is well known to stimulate GLP-1 secretion (9), we used GRP as a positive control in this assay. After intravenous administration (2 min), blood was collected from portal vein, and plasma insulin, GIP, and GLP-1 levels were measured.

Study 3: Oral Administration

Study a. In an intraperitoneal glucose tolerance test (IPGTT), mice were fasted for 4 h and then received either oral DW or MDG (1.1 g/kg) or intraperitoneal saline or MDG (1.1 g/kg) (n = 7–8). Just after administration, mice received glucose (2 g/kg ip). Blood samples were collected from the tail at 0, 15, 30, 60, and 90 min after administration, and blood glucose levels were measured by a glucometer (Lifescan, Milpitas, CA). In another set of mice (n = 6–8), blood was collected from the portal vein under isoflurane anesthesia for 2 min at 5 and 15 min after administration of agents. For all blood samples, plasma insulin, GIP, and GLP-1 levels were measured.

Study b. Mice were fasted for 18 h and orally administered DW, glucose (1 g/kg), or MDG (1.1 g/kg). Blood samples were collected from the tail at 0, 15, 30, 60, and 90 min after administration, and blood glucose levels were measured by a glucometer (Lifescan). In another set of mice, blood was collected from the portal vein under isoflurane anesthesia for 2 min at 5 and 15 min after administration of agents. With the use of this blood sample, plasma insulin, GIP, and GLP-1 levels were measured.

Study 4: Chronic Administration

Male *db/db* mice were treated with DW or 3% MDG in drinking water for 13 days (n = 5 each). Water intake, food intake, and body weight were measured daily. Blood was collected from the tail after a 2-h fast (0800–1000) at 3-day intervals, and blood glucose levels were measured by a glucometer (Lifescan). At the end of the study (*day 13*), mice were fasted for 18 h and orally administered glucose at 2 g/kg. Blood was collected from the tail at 0, 15, 30, 60, and 90 min after glucose administration, and plasma glucose and insulin levels were measured. In another set of mice, blood was collected from the portal vein under isoflurane anesthesia at 5 min after administration of MDG (1.1 g/kg po). Plasma GIP and GLP-1 levels were measured by enzyme-linked immunosorbent assay (ELISA).

Measurement of Plasma Parameters

Plasma glucose was measured using commercial kits (Determiner GL-E kit; Kyowa Medex, Tokyo, Japan). Plasma insulin, GIP (total form), and GLP-1 (active form) levels were measured by ELISA (Morinaga, Yokohama, Japan or, Millipore, Billerica, MA). Active forms of GIP and GLP-1 secreted from K and L cells are rapidly degraded by dipeptidyl-peptidase 4 (DPP4) and became inactive (degraded) forms in plasma. We used total GIP ELISA, which detects both active and inactive form of GIP, and active GLP-1 ELISA, which detects only the active form of GLP-1. Thus, when measuring portal active GLP-1 levels, collected blood was rapidly mixed with a DPP4 inhibitor (Millipore).

Measurement of mRNA Levels in the Intestine

Male C57B6 mice (10–11 wk) were used (n = 2). After decapitation, gastrointestinal tracts were excised and divided into the upper small intestine (1–2 cm below the pylorus), middle small intestine (14–15 cm above the ileocecal junction), lower small intestine (4–5 cm above the ileocecal junction), or large intestine (1–2 cm below the cecocolic junction). Each region was isolated, and the muscle layer was carefully peeled off. Next, the mucosa of each region was isolated. Total RNA was isolated form each region using the RNeasy 96 Universal Tissue Kit (QIAGEN, Venlo, Netherlands). cDNA was synthesized from 5 μg of total RNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). Gene expression levels were determined by real-time quantitative polymerase chain reaction with an ABI7900HT and QTaq TM DNA Polymerase Mix (Clontech, Palo Alto, CA). Primers and probe sets to

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detect the expression of SGLT1 (Mm00451203_m1), GIP (Mm00433601_m1), and preproglucagon (Mm00801712_m1) were purchased from Applied Biosystems. The expression data were normalized to β-actin expression levels using the following primers: forward: AGGTCATCACTATTGGCAACGA; reverse: CACAGGATTCCATACCCAAGAAG; and probe: ATGCCCTGAGGCTCTTTTCCAGCCTT.

Statistical Analysis

Data are expressed as means ± SE. Data were analyzed by Student's *t*-test or one-way ANOVA followed by Dunnett's test. *P* values <0.05 were considered significant.

RESULTS

Administration of glucose at 1 g/kg (5.5 mmol/kg) in the upper small intestine significantly increased levels of portal blood glucose (vehicle, 89.0 ± 6.2 mg/dl; glucose, 218.7 ± 11.8 mg/dl), GIP (vehicle, 52.8 ± 6.7 pg/ml; glucose, 450 ± 48.3 pg/ml), and GLP-1 (vehicle, 1.4 ± 0.3 pM; glucose, 10.3 ± 1.8 pM) compared with vehicle controls 5 min after administration (Fig. 1, *A–C*). Glucose-induced incretin elevations were also observed at 15 min, but these increases were less than those seen at 5 min after administration of glucose in the upper small intestine (GIP: vehicle, 54.1 ± 6.8 pg/ml; glucose, 419.2 ± 43.0 pg/ml; GLP-1: vehicle, 3.1 ± 1.6 pM; glucose, 4.9 ± 0.9 pM, $n = 4–6$). Glucose administered in the middle small intestine also increased levels of portal blood glucose (vehicle, 95.6 ± 7.7 mg/dl; glucose, 141.3 ± 10.7 mg/dl), GIP (vehicle, 66.8 ± 4.9 pg/ml; glucose, 156.6 ± 31.8 pg/ml), and GLP-1 (vehicle, 2.0 ± 0.6 pM; glucose, 7.4 ± 1.4 pM) (Fig. 1, *A–C*). On the other hand, glucose administered in the colon did not increase portal glucose, GIP, or GLP-1 levels at 5 (Fig. 1, *A–C*) or 15(data not shown) min after administration.

SGLT1 mRNA was highly detected in the upper and middle small intestine, moderately detected in the lower small intestine, and barely detected in the colon (Fig. 1*D*). GIP mRNA was highly detected in the upper and middle small intestine but barely detected in the lower small intestine and colon (Fig. 1*E*). Conversely, preproglucagon mRNA, a precursor form of GLP-1 mRNA, was highly detected in the colon and barely detected in the small intestine (Fig. 1*F*).

An SGLT inhibitor, phloridzin, at 0.5 g/kg (1 mmol/kg), completely blocked the increase in portal blood glucose, GIP, and GLP-1 levels after intraluminal administration of glucose at 1 g/kg in the upper small intestine (Fig. 2, *A–C*). Intraluminal administration of the SGLT substrates MDG and 3-OMG at 1.1 g/kg (5.5 mmol/kg) in the upper small intestine also increased portal levels of GIP (vehicle, 58.1 ± 5.4 pg/ml; MDG, 367.4 ± 34.5 pg/ml; 3-OMG, 313.5 ± 61.8 pg/ml) and GLP-1 (vehicle, 3.0 ± 0.8 pM; MDG, 12.8 ± 1.8 pM; 3-OMG, 12.8 ± 3.8 pM); these effects were completely blocked by coadministration with phloridzin (Fig. 2, *D* and *E*). Because a report suggested that sweet taste receptor activation stimulates GIP and GLP-1 secretion, we examined the effect of intraluminal administration of artificial sweeteners in the upper intestine on portal plasma GIP and GLP-1 levels. Administration of saccharin at 1 g/kg (5.5 mmol/kg) or sucralose at 2.2 g/kg

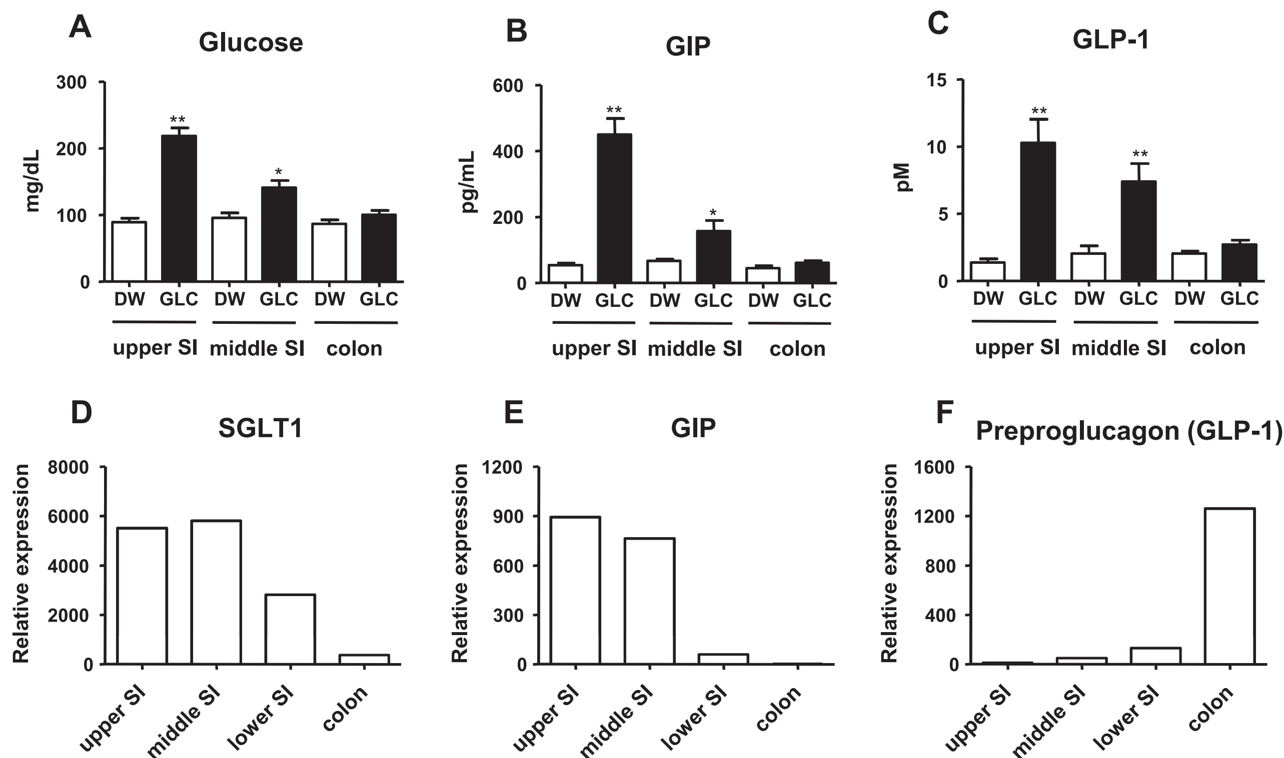

Fig. 1. Portal plasma glucose (*A*), glucose-dependent insulinotropic peptide (GIP; *B*), and glucagon-like peptide-1 (GLP-1; *C*) after single intraluminal administration of distilled water (DW) or glucose (1 g/kg) in the upper small intestine (upper SI), middle small intestine (middle SI), and colon in C57B6 mice ($n = 6–8$). Data are expressed as means ± SE. *$P < 0.05$ and **$P < 0.01$ compared with each DW-treated group and mRNA expression levels of mouse SGLT1 (*D*), GIP (*E*), and preproglucagon (*F*) in the upper small intestine, middle small intestine, lower small intestine (lower SI), and colon. Each column represents the mean value in duplicate.

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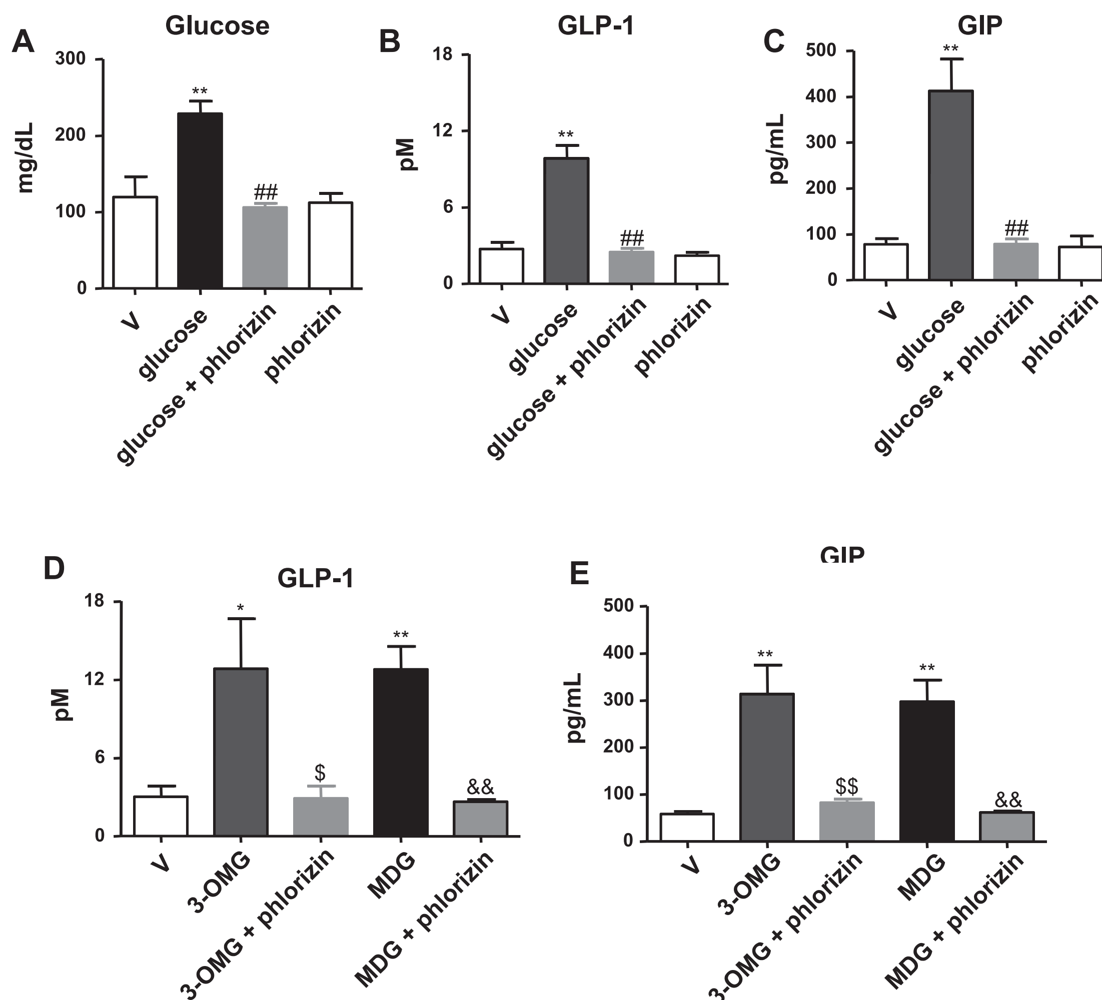

Fig. 2. Portal plasma glucose (*A*), GLP-1 (*B* and *D*), and GIP (*C* and *E*) after single intraluminal administration of vehicle [30% polyethylene glycol 400 (PEG400), V], glucose (1 g/kg), α-methyl-D-glucopyranoside (MDG, 1.1 g/kg), 3-*O*-methyl-D-glucose (3-OMG, 1.1 g/kg), or sodium-glucose cotransporter-1 (SGLT1) inhibitor phloridzin (0.5 g/kg) alone or in combination in the upper small intestine in C57B6 mice ($n = 6–10$). Data are expressed as means ± SE. *$P < 0.05$ and **$P < 0.01$ compared with vehicle-treated group. ##$P < 0.01$ compared with glucose-treated group. $\$P < 0.05$ and $\$\$P < 0.01$ compared with 3-OMG-treated group. &&$P < 0.01$ compared with MDG-treated group.

(5.5 mmol/kg) in the upper small intestine did not alter levels of plasma GIP (vehicle, 112.7 ± 27.9 pg/ml; saccharin, 103.0 ± 5.8 pg/ml; sucralose, 103.6 ± 12.3 pg/ml, $n = 6$) or GLP-1 (vehicle, 2.5 ± 0.7 pM; saccharin, 2.8 ± 0.7 pM; sucralose, 2.5 ± 0.2 pM, $n = 6$) at 5 min after administration.

To show that MDG and 3-OMG are nonmetabolizable sugars in vivo, as reported in vitro, and to confirm that MDG and 3-OMG in the circulation do not stimulate incretin secretion or glucose, we investigated the effect of intravenous administration of glucose, MDG, and 3-OMG on portal plasma insulin, GIP, and GLP-1 levels. Intravenous administration of glucose at 1 g/kg significantly increased plasma insulin levels, whereas MDG and 3-OMG at 1.1 g/kg showed no effects on plasma insulin levels (vehicle, 0.6 ± 0.1 ng/ml; glucose, 1.5 ± 0.3 ng/ml; MDG, 0.7 ± 0.1 ng/ml; 3-OMG, 0.4 ± 0.1 ng/ml; Fig. 3*A*). Intravenous administration of GRP at 30 μg/kg significantly increased portal plasma GLP-1 levels 2 min after administration (vehicle, 1.3 ± 0.4 pM; GRP at 30 μg/kg, 30.5 ± 1.1 pM, $n = 6$). On the other hand, intravenous administration of MDG and 3-OMG at 1.1 g/kg did not increase plasma GIP or GLP-1 levels. Glucose under these condition did not cause GIP and GLP-1 release as expected (Fig. 3, *B* and *C*).

The present findings led us to speculate whether oral ingestion of noncalorigenic SGLT1 substrate would reduce blood glucose levels by stimulating incretin release, which in turn stimulates glucose-induced insulin secretion. To explore this possibility, we used IPGTTs to evaluate the acute antihyperglycemic efficacy of the SGLT1 substrate MDG administered orally to 4-h-fasted mice. Oral administration of MDG at 1.1 g/kg significantly reduced blood glucose levels, whereas intraperitoneal administration of MDG did not (Fig. 4, *A* and *B*). Oral administration of MDG at 1.1 g/kg significantly increased levels of portal plasma GIP, GLP-1, and insulin compared with vehicle control (Fig. 4, *C–E*), whereas intraperitoneal administration of MDG did not alter levels of GIP (vehicle, 99.8 ± 12.2 pg/ml; MDG, 73.4 ± 7.4 pg/ml, $n = 4$) or GLP-1 (vehicle, 3.5 ± 0.7 pM; MDG, 4.6 ± 0.5 pM, $n = 4$) 5 min after administration.

In overnight-fasted mice, oral administration of glucose at 1 g/kg increased blood glucose during an OGTT (0–90 min),

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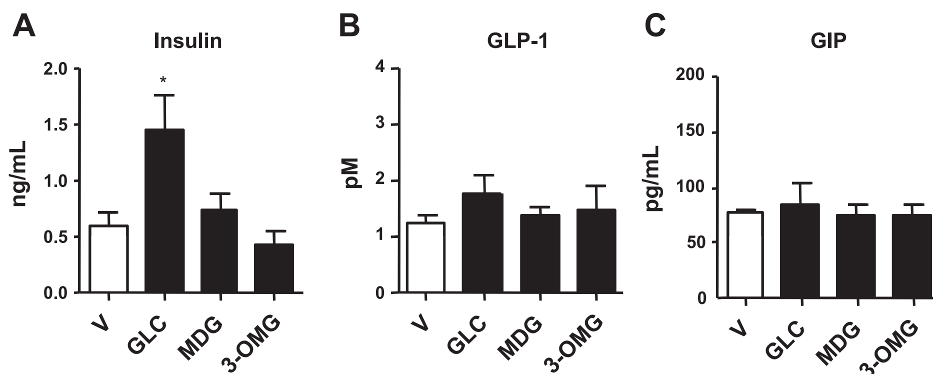

Fig. 3. Portal plasma insulin (*A*), GLP-1 (*B*), and GIP (*C*) after single iv administration of vehicle (saline), glucose (1 g/kg), MDG (1.1 g/kg), or 3-OMG (1.1 g/kg) in C57B6 mice ($n = 6-8$). Data are expressed as means ± SE. $^*P < 0.05$ compared with vehicle-treated group.

whereas a similar administration of MDG at 1.1 g/kg did not alter blood glucose levels (Fig. 5*A*). Glucose and MDG significantly increased portal GLP-1 levels by 2.9-fold and 2.3-fold 5 min after administration. The elevation of plasma active GLP-1 levels was transient and not observed 15 min after administration (Fig. 5*B*). Glucose and MDG also increased portal GIP levels by 5.6-fold and 4.1-fold, respectively, at 5 min after administration and by 5.8-fold and 5.4-fold, respectively, at 15 min after administration (Fig. 5*C*). Oral glucose increased plasma insulin levels 15 min after administration (vehicle, 0.6 ± 0.1 ng/ml; glucose, 1.5 ± 0.3 ng/ml; Fig. 5*D*), whereas MDG did not alter plasma insulin levels at the tested time points.

Oral administration of MDG at 1.1 g/kg significantly increased plasma GIP (vehicle, 123.3 ± 7.6 pg/ml; MDG, 431.3 ± 46.9 pg/ml, $n = 6$) and GLP-1 (vehicle, 36.3 ± 5.9 pM; MDG, 72.6 ± 12.3 pM, $n = 6$) levels in the portal vein 5 min after administration in diabetic *db/db* mice. Chronic treatment with MDG at 3% in drinking water significantly reduced blood glucose levels after a 2-h fast at 4 (vehicle, 446 ± 16 mg/dl; MDG, 401 ± 18 mg/dl), 8 (vehicle, 471 ± 17 mg/dl; MDG, 405 ± 16 mg/dl), and 12 (vehicle, 443 ± 18 mg/dl; MDG, 350 ± 14 mg/dL) days after initiation of MDG treatment in *db/db* mice (Fig. 6*D*). Chronic treatment with MDG did not produce significant changes in body weight or food intake (Fig. 6, *A* and *B*), although it tended to increase daily water intake (vehicle, 21.6 ± 3.5 mg/dl; MDG, 29.1 ± 1.0 mg/dl; $P = 0.07$; Fig. 6*C*). After 13 days, MDG-treated mice showed lower glucose excursion than DW-treated mice in an oral glucose tolerance test

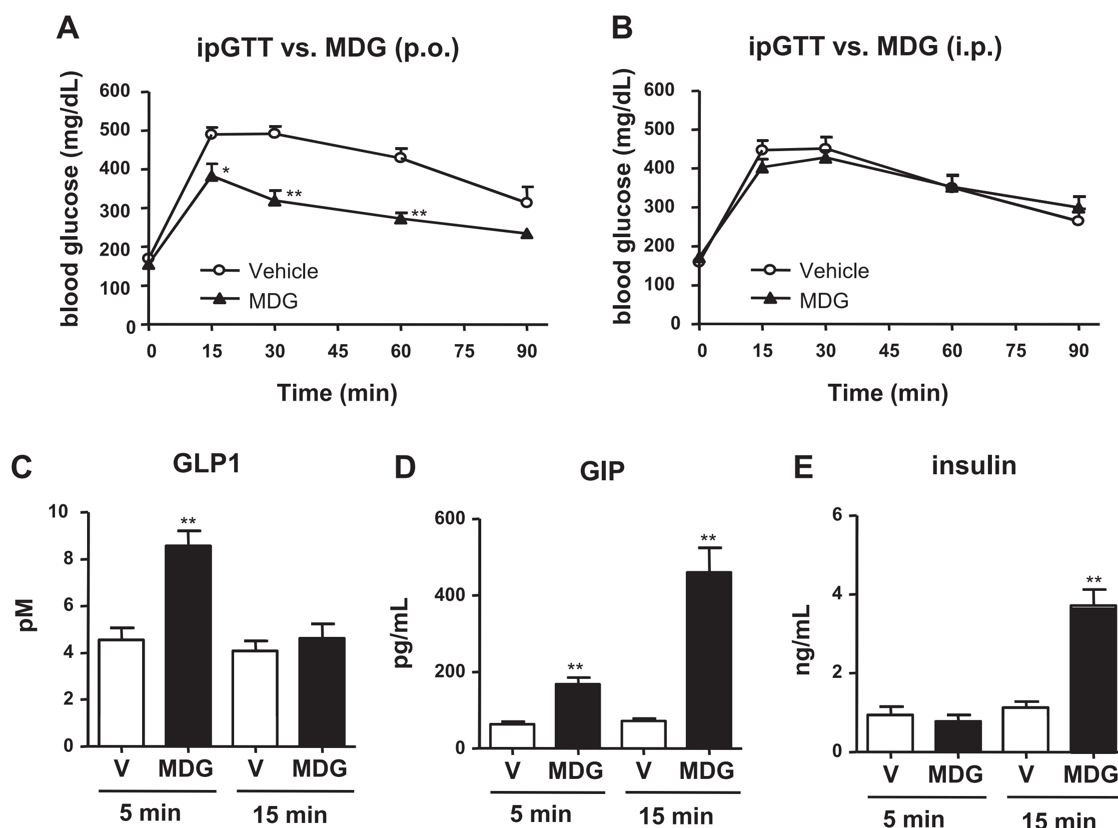

Fig. 4. Blood glucose (*A* and *B*), portal plasma GLP-1 (*C*), GIP (*D*), and insulin (*E*) levels after single oral (*A*, *C*, *D*, and *E*) or ip (*B*) administration of saline (V) or MDG (1.1 g/kg) during an ip glucose tolerance test in C57B6 mice ($n = 6-8$). Data are expressed as means ± SE. $^*P < 0.05$ and $^{**}P < 0.01$ compared with vehicle-treated group.

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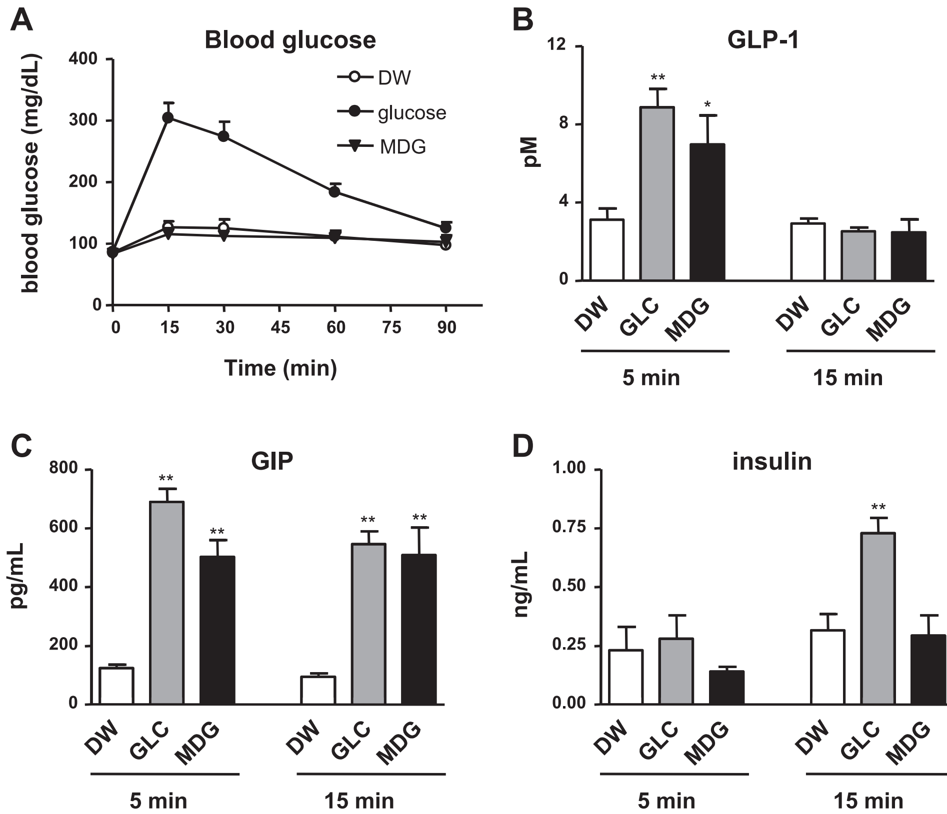

Fig. 5. Blood glucose (*A*), portal plasma GLP-1 (*B*), GIP (*C*), and insulin (*D*) levels after single oral administration of DW, glucose (1 g/kg), and MDG (1.1 g/kg) in overnight-fasted C57B6 mice (n = 8). Data are expressed as means ± SE. *P < 0.05 and **P < 0.01 compared with DW-treated group.

(OGTT) (Fig. 6*E*). The area under the curve of blood glucose during the OGTT significantly decreased in MDG-treated animals (vehicle, 64,257 ± 5,623 $mg\cdot dl^{-1}\cdot min^{-1}$; MDG, 47,282 ± 1,874 $mg\cdot dl^{-1}\cdot min^{-1}$; $P < 0.05$). In MDG-treated mice, oral administration of glucose caused significant elevation of plasma insulin levels from *time 0* to *15 min* after glucose treatment (start point, 4.1 ± 0.6 ng/ml; 15 min, 9.0 ± 1.4 ng/ml; $P < 0.05$), whereas, in DW-treated mice, no

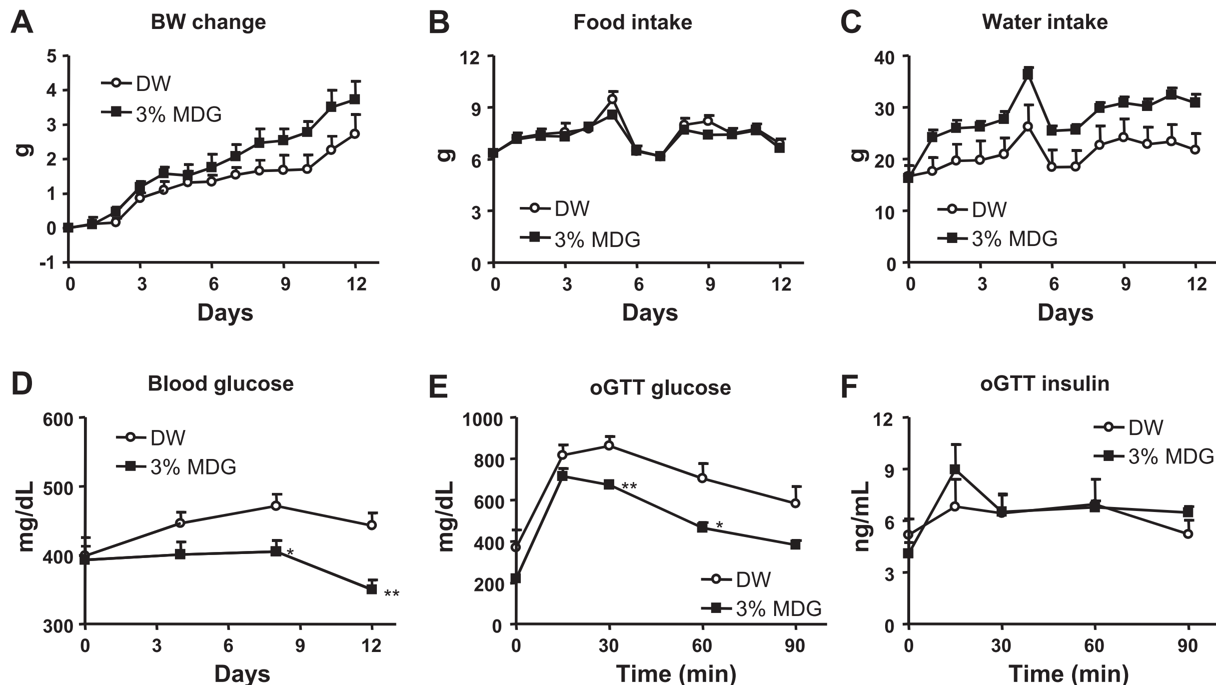

Fig. 6. Chronic effects of MDG at 3% in drinking water on body weight change (*A*), food intake (*B*), water intake (*C*), blood glucose (*D*), plasma glucose during an oral glucose tolerance test (*E*), and insulin levels during an oral glucose tolerance test (*F*) in *db/db* mice (n = 5). Data are expressed as means ± SE. *P < 0.05 and **P < 0.01 compared with DW-treated group.

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significant elevation of plasma insulin levels in response to oral glucose administration were observed (Fig. 6*F*).

DISCUSSION

We showed that SGLT1-mediated glucose transport triggers incretin secretion in mice in vivo. Intestinal distribution of SGLT1 mRNA is consistent with its proposed role as the glucose sensor for glucose-induced incretin secretion. Coadministration of the SGLT1 inhibitor phloridzin with glucose in the upper intestine completely inhibited glucose absorption and glucose-induced incretin secretion, demonstrating that glucose absorption via SGLT1 was essential for glucose-induced incretin secretion and that glucose contact with the intestinal mucosa is not sufficient to stimulate incretin secretion. These findings support the previous reports by Holst (8), whose studies showed a correlation between the ability of luminal sugars to stimulate GIP release and the sugar specificity of the intestinal glucose uptake pathway in mice. Similar correlation was also documented for GLP-1 in rats (18). Our data are also consistent with previous findings that coadministration of phloridzin with glucose prevented intestinal glucose absorption and glucose-induced GIP secretion in rats (17). Administration of the SGLT1 substrates MDG and 3-OMG in the upper intestine also increased plasma incretin levels comparable with the effects of glucose, whereas phloridzin completely inhibited these stimulatory effects of MDG and 3-OMG on incretin secretion. Unlike circulating glucose, intravenous administration of MDG and 3-OMG did not increase plasma insulin levels. These results confirm that MDG and 3-OMG are nonmetabolizable sugars in vivo, since glucose-induced insulin secretion depends on glucose metabolism. These data also indicate that glucose (substrate) transport through SGLT1 triggers incretin secretion without the need for subsequent glucose metabolism. SGLT1 transports sodium ions and glucose (substrate) concomitantly, and therefore produces electrogenic signals related to sodium ion flux (23).

SGLT3, like SGLT1, is also found in the intestinal mucosa (6, 9) and is suggested to act as an intestinal glucose sensor that regulates postprandial gastrointestinal secretion and motility (9). However, 3-OMG, which is not transported by SGLT3 (27), stimulated incretin secretion in a phrolidzin-sensitive manner, and its effect on incretin secretion was comparable to that of glucose. These results argue against the involvement of SGLT3 as an intestinal glucose sensor for glucose-induced incretin secretion in mice.

Although recent reports suggest that sweet taste receptors are an intestinal glucose sensor and that their activation stimulates GLP-1 secretion (9), this is unlikely to explain our finding that glucose contact with the intestinal mucosa was not sufficient to stimulate incretin secretion. In addition, we were unable to demonstrate effects of saccharin and sucralose on either GIP or GLP-1 secretion, consistent with a recent report that sweeteners do not stimulate GLP-1 release in vivo in rats (7). Taken together, these data lead us to conclude that sweet taste receptors might not play a role in glucose-induced incretin secretion.

We show here that increases in plasma GIP levels were observed when glucose was injected in the upper intestine, but not in the lower intestine, which is consistent with the distribution of the proposed glucose sensor, SGLT1, and GIP mRNA in the intestine. A recent study demonstrated that isolated mouse K cells express high levels of mRNA for SGLT1 and release GIP in response to the SGLT1 substrates glucose and MDG (11). The sodium ion flux through SGLT1 generates a small inward current that is sufficient to depolarize the cells, triggering calcium ion entry through L-type voltage-gated calcium ion channels (12, 13). Elevation of intracellular calcium levels is known to trigger several hormone secretions from endocrine cells, including GIP (2, 4, 14, 22). The present in vivo results support the idea that K cells directly sense luminal glucose and release GIP in response to intestinal glucose.

The underlying mechanism of glucose-induced GLP-1 secretion is still debated, in terms of the relative contributions of direct vs. indirect glucose-sensing SGLT1 pathways. We report here that an increase in plasma GLP-1 was observed only when glucose was injected in the upper intestine, whereas similar glucose injection to the lower intestine had no effect. These results demonstrate that the glucose sensor for glucose-induced GLP-1 secretion is mainly located in the upper intestine, in keeping with SGLT1 mRNA distribution. However, the distribution pattern of the glucose sensor is not correlated with preproglucagon (the precursor of GLP-1 and other glucagon family hormones) mRNA distribution and previously reported GLP-1 peptide distribution (18). The present findings suggest that glucose-induced GLP-1 secretion may be largely mediated via an indirect glucose-sensing pathway in mouse in a physiological condition. On the other hand, it has been suggested that multiple types of L cells exist (8) so that glucose-sensitive L cells could be located in the upper intestine, with glucose-insensitive L cells located in the lower intestine. Notwithstanding, it has been reported that L cells isolated from both the upper half of the small intestine and from the colon express SGLT1 and secrete GLP-1 in response to glucose and MDG (13). Hence, the possibility of distinct populations of L cells in the intestine with different glucose sensitivities seems to be low. Our studies do not completely address the possibility that direct sensing of glucose via SGLT1 in L cells leads to GLP-1 secretion under certain conditions as shown in in vitro studies (13). Nevertheless, our in vivo data using mice clearly demonstrate that glucose is sensed by an upper intestinal glucose sensor (SGLT1) and indirectly stimulates GLP-1 secretion probably from lower intestinal L cells. At this point, we can only speculate how indirect stimulation of GLP-1 release can be achieved, but several neuronal and hormonal stimuli have been indicated to play a role (8, 16).

In this study, we showed that the SGLT1 substrate, MDG, has glucose-lowering effects in mice. A single oral, but not intraperitoneal dose of MDG reduced blood glucose levels during an IPGTT and increased plasma levels of GIP, GLP-1, and insulin in normal mice. Moreover, chronic treatment of MDG for 13 days ameliorated basal hyperglycemia and reduced blood glucose excursions during an OGTT in *db/db* mice without concomitant reductions in body weight. Our data indicate that noncalorigenic SGLT1 substrates can be used to stimulate incretin secretion and lower blood glucose levels in the diabetic state. Interestingly, in fasted (i.e., normoglycemic) animals, MDG did not alter plasma glucose and insulin levels, although it increased GIP and GLP-1 levels, demonstrating that MDG stimulates insulin secretion in a glucose-dependent manner, in keeping with the characteristics of insulin secretory

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effects of incretins (3). These results support our proposal that the SGLT1 substrate, MDG, has glucose- lowering effects, at least in part, via the stimulation of GIP and GLP-1 secretion, and that an SGLT1-dependent mechanism stimulates meaningful quantities of incretin release for the regulation of blood glucose.

In humans, plasma levels of incretin increase rapidly within just few minutes after oral glucose, suggesting that a glucose sensor for glucose-induced incretin secretion is located in the upper intestine where SGLT1 highly exists (24, 30). Moreover, glucose and galactose, which are substrates for SGLT1, increased plasma GIP levels, whereas fructose and mannose, which do not bind to SGLT1, did not change plasma GIP levels in nonobese humans (11, 24). Although species difference has been suggested in the area of incretin secretion (14), these studies indicate that SGLT1-mediated glucose (substrate) transport may trigger incretin secretion in humans, as shown in the present mouse study. In addition, because type 2 diabetic patients have lower postprandial GLP-1 levels, noncalorigenic SGLT1 substrate may represent a novel therapeutic approach to stimulating incretin secretion in type 2 diabetes.

In conclusion, the present results indicate that SGLT1 is the intestinal glucose sensor for glucose-induced incretin secretion in vivo. Furthermore, we showed that the SGLT1 substrate, MDG, reduced blood glucose levels only in the hyperglycemic state, accompanied by an increase in plasma GIP and GLP-1 levels in mice. These data suggest that SGLT1 plays a role in the regulation of postprandial hyperglycemia by regulating not only glucose absorption but also incretin secretion. Further investigation of the mechanism of SGLT1-dependent incretin secretion would provide a novel therapeutic approach based on incretin for the treatment of type 2 diabetes.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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