A Novel Glucose-Sensing Mechanism Contributing to Glucagon-Like Peptide-1 Secretion From the GLUTag Cell Line

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Glucagon-like peptide 1 (GLP-1) secretion from intestinal L-cells is triggered by luminal nutrients. We reported previously that glucose-triggered GLP-1 release from the L-cell model GLUTag involves closure of ATP-sensitive K^+ (K_{ATP}) channels. We show here that GLP-1 secretion and electrical activity of GLUTag cells is triggered not only by metabolizable sugars (glucose or fructose) but also by the nonmetabolizable monosaccharide methyl-α-glucopyranoside. Responses to glucose and methyl-α-glucopyranoside were impaired by the sodium-glucose cotransporter (SGLT) inhibitor phloridzin. SLGT1 and 3 were detected in GLUTag cells by RT-PCR. Whereas fructose closed K_{ATP} channels, methyl-α-glucopyranoside increased the membrane conductance and generated an inward current. Low concentrations of glucose and methyl-α-glucopyranoside also triggered small inward currents and enhanced the action potential frequency. We conclude that whereas low concentrations of metabolizable sugars trigger GLP-1 secretion via K_{ATP} channel closure, SGLT substrates generate small inward currents as a result of the electrogenic action of the transporter. This transporter-associated current can trigger electrical activity and secretion when the concentration of substrate is high or when outward currents are reduced by metabolic closure of the K_{ATP} channels. Electrogenic sugar entry via SGLTs provides a novel mechanism for glucose sensing by neuroendocrine cells. *Diabetes* 52:1147–1154, 2003

Glucagon-like peptide one (GLP-1) and peptide YY (PYY) are secreted from intestinal L-cells in response to dietary carbohydrate and fat (1–6). The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion, inhibition of glucagon release, and inhibition of gastric emptying and food intake (5,6). GLP-1 analogues are therefore currently under development as a new treatment for type 2 diabetes (7–9). Gut-derived PYY acts as a potent satiety signal and reduces food intake in mice and humans (10). Despite the physiological and pharmacological interest in L-cell–derived peptides, however, little is known about the molecular mechanisms underlying the stimulus-secretion coupling in these cells.

GLP-1 secretion after a meal can be divided into two phases: an early phase that begins within minutes and lasts for 30–60 min and an overlapping second phase that causes prolonged secretion for 1–3 h (1,2). L-cells are highly polarized with an apical surface facing into the gut lumen, suggesting that they sense nutrients directly. However, the majority of L-cells are found in the distal gut, starting in humans at the level of the jejunum and increasing in number throughout the ileum and colon (11–13). It has therefore been argued that the early phase of GLP-1 release does not reflect direct nutrient sensing by L-cells, because they are ill-placed to receive nutrients delivered from the esophagus. An alternative or additional mechanism for the early phase includes the involvement of humoral and neuronal factors (14). The magnitude of the late phase is enhanced by α-glucosidase inhibitors, suggesting that this reflects the increased delivery to and sensing of glucose by distal L-cells (15).

Using an L-cell model, GLUTag (16), we showed recently that low concentrations (0.5 mmol/l) of glucose stimulate GLP-1 secretion and the firing of action potentials, via a mechanism involving the closure of ATP-sensitive potassium (K_{ATP}) channels (17). Similar channels in pancreatic β-cells are believed to respond to changes in the metabolic rate, mediated through alterations in the concentrations of adenine nucleotides ATP and ADP. As K_{ATP} channels are inhibited by ATP and activated by MgADP, the increasing ATP/MgADP ratio in β-cells that accompanies a rise in the plasma glucose results in K_{ATP} channel closure, membrane depolarization, opening of voltage-gated calcium channels, calcium entry, and insulin release (18–20). For both the GLUTag cell line and the related STC-1 cell line, an involvement of voltage-gated L-type Ca^{2+} channels in the response to nutrients has been demonstrated (21).

The characteristics of sugar-dependent GLP-1 secretion have been investigated previously in human studies and perfused ileum preparations. Whereas metabolizable sugars, such as glucose, fructose, and galactose, are potent stimuli for GLP-1 release, the nonmetabolizable sugars methyl-α-glucopyranoside and 3-O-methylglucose also triggered secretion (2,22–25). These findings cannot all be explained by a mechanism involving K_{ATP} channel closure in response to increased metabolism in the L-cell. Additional studies on perfused ileum showed that the effects of

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GLP-1, glucagon-like peptide 1; SGLT, sodium-glucose cotransporter; PYY, peptide YY.

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glucose, galactose, methyl-α-glucopyranoside, and 3-O-methylglucose were Na^+-dependent, suggesting the involvement of sodium-glucose cotransporters (SGLTs) (23,24).

Here we provide evidence that GLP-1–releasing cells express SGLTs and that the electrogenic coupling of sodium and sugar uptake could account for the secretory responses seen with high concentrations of nonmetabolizable sugars. By contrast, lower concentrations of metabolizable sugars such as fructose, which is not a substrate for the sodium-dependent uptake mechanism, trigger GLP-1 release by closure of K_{ATP} channels in response to increased metabolism.

RESEARCH DESIGN AND METHODS

Cell culture. GLUTag cells (16) were cultured in DMEM (5.6 mmol/l glucose), supplemented with 10% (vol/vol) FCS, penicillin, and streptomycin. Medium was exchanged every 3 days, and cells were trypsinized and reseeded at a 1:5 dilution when ~70% confluence was reached.

GLP-1 secretion. For secretion experiments, cells were plated in 24-well cultures plates and allowed to reach 60–80% confluence. On the day of the experiment, cells were washed twice with 500 μl of glucose-free Krebs-Ringer medium containing (in mmol/l) 120 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 22 $NaHCO_3$, and 0.1 DiprotinA, bubbled with 95% O_2/5% CO_2 for 10 min and supplemented with 0.5% (wt/vol) BSA. Experiments were performed by incubating the cells with test reagents in 500 μl of Krebs-Ringer for 2 h at 37°C, 5% CO_2. Phloridzin (Sigma) was prepared as a stock solution in DMSO, and the final DMSO concentration was adjusted to 0.25% for all conditions tested. At the end of this incubation period, medium was collected and centrifuged to remove any floating cells. GLP-1 was assayed using an ELISA specific for GLP-1 (7-36 amide) and GLP-1 (7-37) (GLP-1 active ELISA-kit, Linco). Secretion was normalized to the baseline release in 0 mmol/l glucose measured in parallel on the same day. This baseline secretory rate in 0 mmol/l glucose was 20.6 ± 1.1 fmol per dish in 2 h (n = 36).

Electrophysiology. Cells were plated into 35-mm dishes for 1–3 days before use. Experiments were performed on single cells and well-defined cells in small clusters. Microelectrodes were pulled from borosilicate glass (GC150T, Harvard Apparatus), and the tips were coated with refined yellow beeswax (Sigma-Aldrich, UK) maintained at ~90°C. Electrodes were fire-polished using a microforge (Narishige) and had resistances of 2.5–3 MΩ when filled with pipette solution. Membrane potential and currents were recorded using an Axopatch 200B (Axon Instruments) linked through a Digidata 1320A interface and analyzed using pCLAMP software (Axon Instruments). Experiments were performed at 22–24°C. Current clamp recordings were filtered at 5 kHz and digitized at 10 kHz. Membrane conductance was measured in perforated patch voltage clamp experiments by applying a series of 1.2-s voltage ramps from −90 to −50 mV (holding potential −70 mV). The current voltage relation was linear in this voltage range, and the slope conductance was calculated from the average of 10 voltage ramps.

Solutions. The bath solution contained (in mmol/l) 5.6 KCl, 138 NaCl, 4.2 $NaHCO_3$, 1.2 NaH_2PO_4, 2.6 $CaCl_2$, 1.2 $MgCl_2$, and 10 HEPES (pH 7.4). Sugars and drugs were added to the bath solution as indicated. Osmolarity was maintained in experiments involving high (100 mmol/l) methyl-α-glucopyranoside by reducing the NaCl concentration to 95 mmol/l. Control solutions for these experiments contained 100 mmol/l sucrose or mannitol, neither of which affected electrical activity. The Na-free bath solution contained (in mmol/l) 100 CholineCl, 4.4 $KHCO_3$, 1.2 KH_2PO_4, 2.6 $CaCl_2$, 1.2 $MgCl_2$, and 10 HEPES (pH 7.4 with CholineOH). The perforated patch pipette solution contained (in mmol/l) 76 K_2SO_4, 10 KCl, 10 NaCl, 55 sucrose, 10 HEPES, and 1 $MgCl_2$, (pH 7.2), to which amphotericin B was added to a final concentration of 200 μg/ml. Phloridzin (Sigma) was prepared as 400× stock in DMSO.

Statistical analysis. Results are presented as mean ± SE. Statistical significance was tested in the first case by ANOVA and subsequently by one-sample, paired or unpaired Student's t tests (as indicated), using a threshold for significance of $P < 0.05$.

RT-PCR. RT-PCR was performed as described previously (17) using the following specific primers, which were designed using sequence information from www.ensembl.org/mus_musculus/(from 5′ to 3′): SLC2A1/GLUT1 (ENSMUSG00000028645) sense CACCGCTACGGAGAGCCC, antisense CCCATGATGGAGTCTAAGCC; SLC2A2/GLUT2 (ENSMUSG00000027690) sense GTCAATGGCACAGACACCCC, antisense GGTGACATCCTCAGTTCCTC; SLC2A5/GLUT5 (ENSMUSG00000028976) sense GCTGCCGTCAACTCTCCC, antisense CTTCTGGATCAGCAGGTAGC; SLC5A1/SGLT1 (ENSMUSG00000011034) sense TGCTACACACCGAGGGCTG, antisense GGTGAAGAGAGTACTGGCGC; SLC5A4a/SGLT3a/SAAT-1 (ENSMUSG00000020229) sense GATGCTGTCAGTCATGCTGGC, antisense TACAGAAGACGGCGACCAGG. The predicted sizes (bp) of fragments were (GLUT-1) 395, (GLUT-2) 627, (GLUT-5) 564, (SGLT-1) 456, and (SGLT-3a) 290. All primer pairs span intron/exon borders to distinguish amplification from genomic DNA, and PCR products were confirmed by direct sequencing.

RESULTS

Nonmetabolizable glucose analogues trigger GLP-1 secretion in GLUTag cells. As reported previously for the perfused ileum (23,24), the nonmetabolizable glucose analogue methyl-α-glucopyranoside (100 mmol/l) stimulated GLP-1 secretion from GLUTag cells 2.2 ± 0.1-fold (n = 15; Fig. 1), suggesting that mechanisms in addition to K_{ATP} channel closure contribute to sugar sensing in these cells. Lower concentrations (0.5, 5 mmol/l) of methyl-α-glucopyranoside were not effective. As methyl-α-glucopyranoside is a specific substrate for the SGLTs but not facilitative GLUTs, the results indicate a role for SGLTs in sugar transport by GLUTag cells, consistent with the finding that GLP-1 release from the perfused ileum, triggered by glucose or methyl-α-glucopyranoside, is sodium-dependent (23,24). An osmotic action of the sugar at high concentration is unlikely, as the nontransported sugar mannitol (100 mmol/l) was without effect, and methyl-α-glucopyranoside-triggered secretion was inhibited by the SGLT inhibitor phloridzin (see below). Methyl-α-glucopyranoside did not trigger additional secretion when tested in the presence of the K_{ATP} channel inhibitor glibenclamide (10 nmol/l; Fig. 1).

Fructose also triggers GLP-1 secretion from GLUTag cells. The demonstration that GLP-1 release from GLUTag cells can be stimulated by nonmetabolizable glucose analogues seems at odds with our previous finding that glucose triggers GLP-1 release as a consequence of its metabolism and K_{ATP}-channel closure. To investigate whether metabolizable sugars that are not substrates for SGLTs are also able to trigger release, we tested the effect of fructose, which enters cells via the facilitative fructose transporter GLUT5 (26). Fructose stimulated GLP-1 release from GLUTag cells at all concentrations tested (Fig. 1). The results are consistent with the finding that fructose stimulated GLP-1 release from perfused ileum by a Na^+-independent mechanism (24).

Expression of different glucose transporters in GLUTag cells. Intestinal epithelial sugar transport is believed to involve SGLT1, SLGT3, and GLUT5 on the apical surface and GLUT2 on the basolateral membrane (27). To investigate which of these glucose transporters (plus GLUT1) are expressed by GLUTag cells, we performed RT-PCR and confirmed the identity of the products by direct sequencing. The intestinal SGLTs SGLT1 and SGLT3 and the facilitative GLUTs GLUT5 and GLUT1 were detected (Fig. 2). By contrast, although GLUT2 was detectable in RNA isolated from mouse small intestine or the murine β-cell line Min6 (data not shown), no signal of the correct size or sequence was seen with the GLUTag cell line.

Relatively low glucose concentrations trigger GLP-1 secretion in GLUTag cells. The finding that sodium-glucose co-transporters operate in GLUTag cells suggests the possibility that the high glucose sensitivity of GLP-1 release that we reported previously (17) might be attrib-

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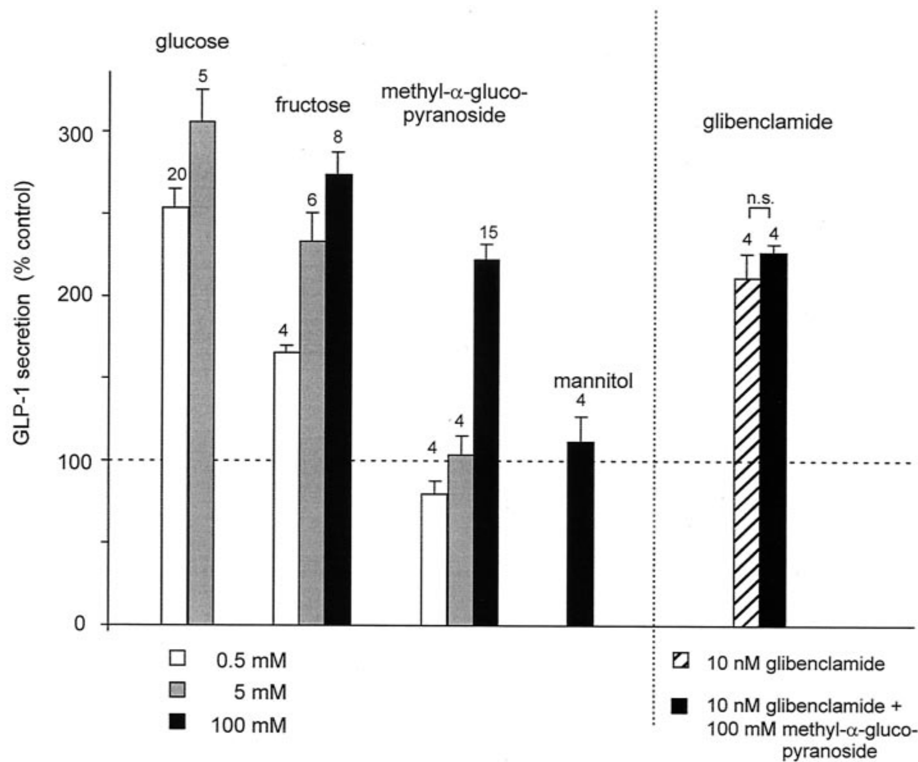

FIG. 1. Secretory responses of GLUTag cells to different carbohydrates. GLP-1 secretion from GLUTag cells cultured for 2 h in Krebs Ringer buffer under the conditions indicated. Error bars represent 1 SE, and the number of dishes is indicated above each bar. Secretion was normalized to the baseline secretion in 0 mmol/l glucose measured in parallel on the same day. ANOVA across all groups indicated significant differences at $P < 0.001$. Significant differences ($P < 0.001$) were also detected between GLP-1 secretion induced by maximum concentrations of glucose, methyl-α-glucopyranoside, fructose, and glibenclamide (by ANOVA).

utable to the low K_m for glucose of SGLT1 (0.3 mmol/l) (28). To investigate how closely the glucose sensitivity of GLUTag cells matches the affinity of SLGT1, we measured the dose-response curve for glucose-triggered GLP-1 release (Fig. 3). GLP-1 secretion was stimulated by glucose with an EC_{50} of 0.2 mmol/l, similar to the K_m of SGLT1 and consistent with our previous finding that the greatest fall in membrane conductance, reflecting the closure of K_{ATP} channels, occurred when the glucose concentration was raised from 0 to 1 mmol/l (17).

Glucose-triggered secretion and electrical activity are impaired by inhibition of SGLT. To investigate further the role of SGLTs in glucose sensing by GLUTag cells, we tested the effects of the SGLT inhibitor phloridzin on GLP-1 secretion and electrical activity. Glucose-triggered GLP-1 release was decreased ~40% by phloridzin (2, 20, or 50 μmol/l; Fig. 4), which competitively inhibits SGLT1 and SLGT3 with K_is of 0.22 and 9.0 μmol/l, respectively (28). Consistent with a role for SGLTs in the uptake of glucose and methyl-α-glucopyranoside but not fructose, 20 μmol/l phloridzin blocked GLP-1 release triggered by glucose or methyl-α-glucopyranoside but was without effect on secretion triggered by fructose or glibenclamide ($P < 0.05$ by ANOVA; Fig. 4). At a lower concentration (2 μmol/l), phloridzin inhibited GLP-1 secretion in the presence of 0.5 mmol/l glucose but did not significantly block the response to 100 mmol/l methyl-α-glucopyranoside, as predicted from the competitive nature of the drug. The highest concentration of phloridzin (500 μmol/l) completely abolished the response to methyl-α-glucopyranoside but also had nonspecific effects, as it significantly inhibited GLP-1 secretion triggered by fructose or glibenclamide (Fig. 4).

The effect of phloridzin on electrical activity was tested by monitoring the membrane potential of single GLUTag cells in perforated-patch recordings. As reported previously, GLUTag cells fired action potentials in response to raising the glucose concentration (17). In the presence of 5 mmol/l glucose, phloridzin (2 μmol/l) reversibly reduced the action potential frequency, from 1.4 ± 0.2 to 0.6 ± 0.1 Hz ($n = 6$; Fig. 4*B* and *C*).

Membrane events underlying sugar-triggered GLP-1 secretion. An electrophysiological approach was used to investigate further the mechanisms underlying sugar-triggered GLP-1 release. Perfusion with 20 mmol/l fructose, in the absence of glucose, triggered membrane depolarization and the generation of action potentials (Fig. 5). The effect of 100 mmol/l methyl-α-glucopyranoside was tested in both the absence and the presence of 1 mmol/l glucose. When glucose was removed from the bath solution, we observed a slow membrane hyperpolarization and continuous reduction in the action potential frequency, and against this changing background, 3/8 cells showed a clear (>40%) increase in action potential frequency in response to 100 mmol/l methyl-α-glucopyranoside. Action potentials in the presence of methyl-α-glucopyranoside were blocked by phloridzin (200 μmol/l; Fig. 4). The decline in action potential frequency after glucose removal is likely to reflect the opening of K_{ATP} channels as the ATP concentration falls, and we speculated that the increasing K_{ATP} channel conductance might be sufficient to counteract the effect of methyl-α-glucopyranoside in some cells. In the

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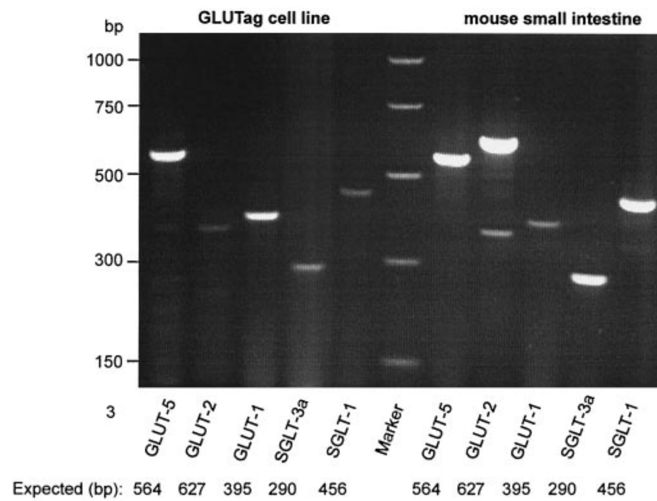

FIG. 2. Glucose transporter expression in the GLUTag cell line. RT-PCR detected SGLT1, SGLT3a, GLUT1, and GLUT5 mRNA in the GLUTag cell line but not GLUT2. Positive controls for all primer pairs tested, using RNA isolated from mouse small intestine, are shown on the right. No bands were amplified when H_2O was used instead of RNA. The predicted band sizes are indicated. Identity of amplified bands was confirmed by sequencing. The smaller band in the GLUT2 lane corresponds to an unrelated sequence and does not indicate GLUT2 expression.

presence of 1 mmol/l glucose, by contrast, addition of 100 mmol/l methyl-α-glucopyranoside dramatically increased the action potential frequency in all cells tested (Fig. 5). Fructose (20 mmol/l) triggered the generation of action potentials in the absence of glucose (Fig. 5).

We next measured changes in the membrane conductance by applying voltage ramps between −90 and −50 mV, from a holding potential of −70 mV. As described previously for 20 mmol/l glucose, 20 mmol/l fructose decreased the slope conductance (Fig. 6*A*) consistent with the closure of K_{ATP} channels (17). Methyl-α-glucopyranoside (100 mmol/l), applied in the presence of 1 mmol/l glucose, resulted in an increase in the membrane conductance (Fig. 6*A*) in the presence but not the absence of Na^+

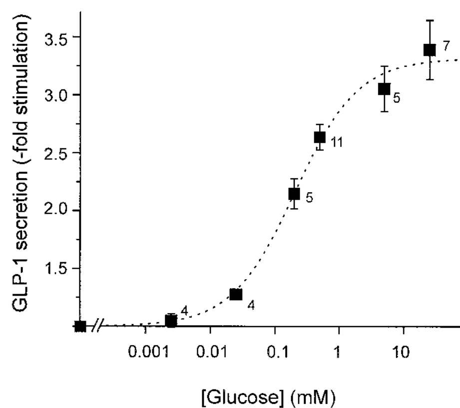

FIG. 3. Glucose dose-response of GLUTag cells. GLP-1 secretion from GLUTag cells cultured for 2 h in Krebs Ringer buffer containing different concentrations of glucose. Error bars represent 1 SE, and the number of dishes tested for each concentration is indicated next to the symbol. Secretion was normalized to the baseline secretion in 0 mmol/l glucose measured in parallel on the same day. The dotted line was fitted with the logistic equation $y = A2 + (A1 - A2)/(1 + (x/EC_{50})\wedge p)$ using Origin software (Microcal) and the resulting values were as follows: A1 = 1.0, A2 = 3.3, EC_{50} = 0.2 mmol/l, and *P* = 0.9.

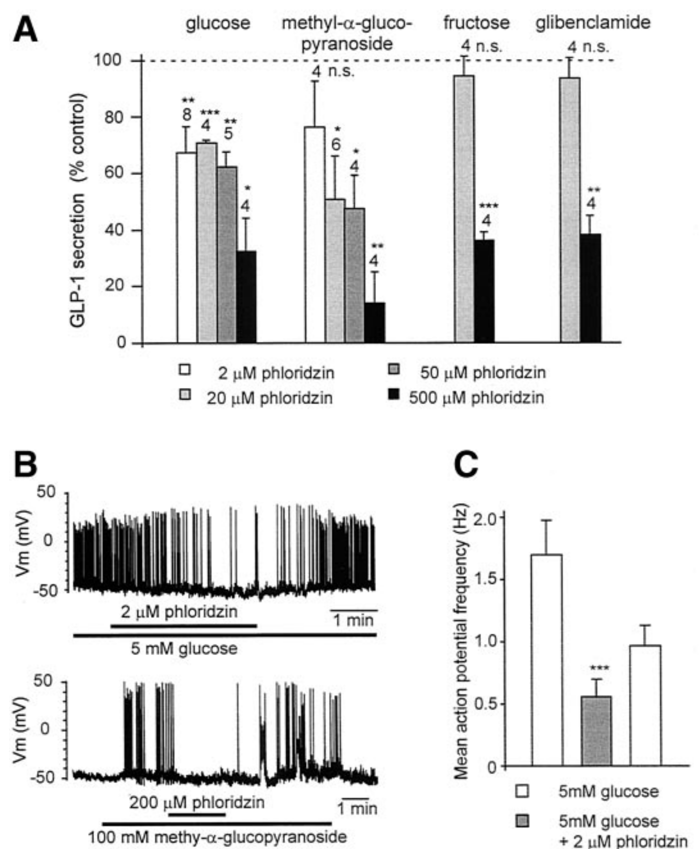

FIG. 4. Phloridzin suppresses glucose responses in GLUTag cells. *A*: GLP-1 secretion from GLUTag cells cultured for 2 h in Krebs Ringer buffer containing glucose (0.5 mmol/l), methyl-α-glucopyranoside (100 mmol/l), fructose (100 mmol/l), glibenclamide (100 nmol/l), and phloridzin, as indicated. Error bars represent 1 SE, and the numbers of dishes tested for each condition are indicated above the bars. Secretion was normalized to the control secretion seen with the same secretagogue in the absence of phloridzin measured in parallel on the same day; the control secretion is indicated by the dotted line. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s., non significant using a one-sample *t* test. Comparison (by ANOVA) of the inhibitory effect of phloridzin on different secretagogue stimuli indicated significant differences for 20 ($P < 0.05$) but not 500 μmol/l. *B*: Action potentials from individual GLUTag cells in current-clamp recording, using the perforated-patch whole-cell configuration of the patch clamp setup. Action potential frequency was reduced by perfusion with 2 μmol/l phloridzin in the presence of 5 mmol/l glucose, or 200 μmol/l phloridzin in the presence of 100 mmol/l methyl-α-glucopyranoside as indicated by the horizontal bars. *C*: Mean action potential frequency of five cells in 5 mmol/l glucose before, during, and after addition of 2 μmol/l phloridzin to the perfusate. The action potential frequency was determined by counting events crossing above a threshold of −10 mV. Statistical significance was tested by comparing the mean action potential frequency in the presence and absence of phloridzin, with a paired *t* test; ***$P < 0.001$.

ions, suggesting the involvement of an alternative mechanism.

This was further explored by measuring changes in the holding current at a membrane potential of −70 mV. At this voltage, which is close to the reversal potential for K^+ ions in GLUTag cells, changes in current resulting from altered activity of K_{ATP} channels are minimized. Consistent with a mechanism involving K_{ATP} channel closure, fructose did not alter the holding current at −70 mV (Fig. 6*B*). Methyl-α-glucopyranoside, by contrast, induced a dose-dependent inward current, with a magnitude of ~5 pA/cell at a concentration of 100 mmol/l. Smaller currents were induced by a lower concentration (20 mmol/l) of the sugar. The simplest explanation for the current triggered

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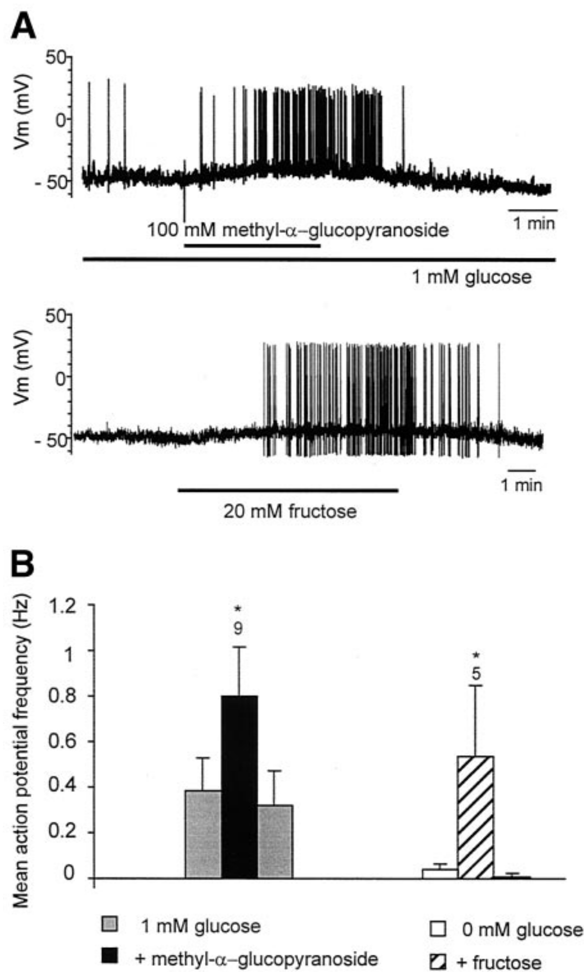

FIG. 5. Fructose and methyl-α-glucopyranoside trigger action potentials. ***A*****: Action potentials from an individual GLUTag cell in a current-clamp recording, using the perforated-patch whole-cell configuration of the patch clamp setup. Action potential frequency was increased by perfusion with either 100 mmol/l methyl-α-glucopyranoside or 20 mmol/l fructose as indicated by the horizontal bars.** ***B*****: Mean action potential frequency of GLUTag cells, as shown in** ***A*****, before, during, and after perfusion with 100 mmol/l methyl-α-glucopyranoside or 20 mmol/l fructose. Methyl-α-glucopyranoside was added in the continuous presence—and fructose in the absence—of 1 mmol/l glucose. The number of cells is given above each bar. Error bars represent 1 SE. Statistical significance was tested comparing the mean action potential frequency in the presence and absence of the added sugar with a paired** ***t*** **test; *$P < 0.05$.**

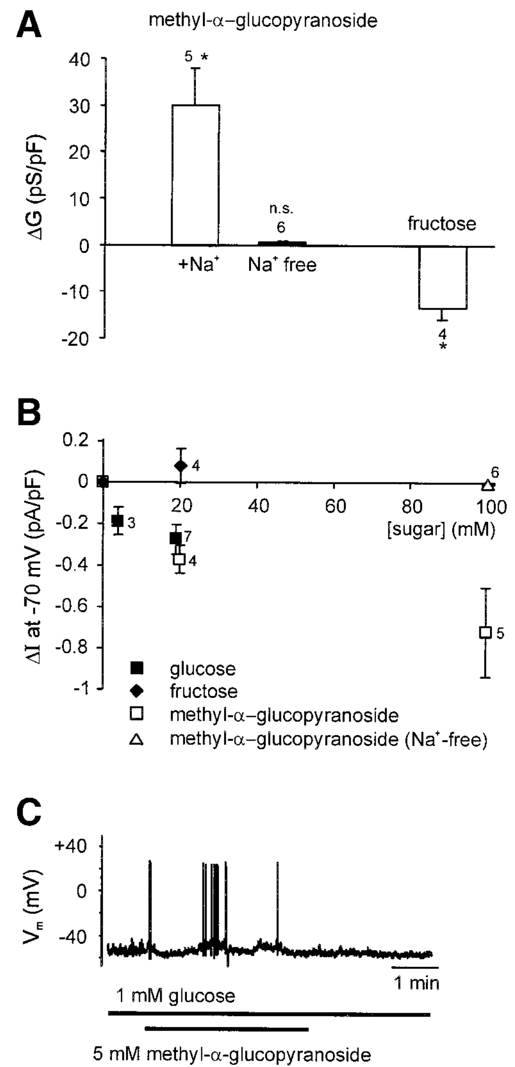

FIG. 6. Differences in the electrical responses to glucose, fructose, and methyl-α-glucopyranoside. ***A*****: Changes in slope conductance, ΔG, in response to 100 mmol/l methyl-α-glucopyranoside (applied in the presence of 1 mmol/l glucose; in the presence or absence of Na^+) or 20 mmol/l fructose (in the absence of glucose and presence of Na^+). ΔG was calculated by comparing the conductances measured in the presence of the test sugar with the mean conductance measured before addition and after removal of this sugar. For compensating for different cell sizes, the values were normalized to the cell capacitance. The numbers of cells are given next to the bars. Error bars represent 1 SE. Statistical significance was tested by comparing the conductance in the presence and absence of the test sugar with a paired** ***t*** **test; *$P < 0.05$.** ***B*****: Changes in the holding current at −70 mV (ΔI) were measured in perforated patch voltage clamp recordings, after addition of the test sugars at the concentrations indicated. The control solution for the fructose experiment contained 0 glucose, and that for glucose and methyl-α-glucopyranoside contained 1 mmol/l glucose. The currents have been normalized to the cell capacitance, and inward currents are denoted as negative. The bars indicate 1 SE, and the numbers of patches are shown next to each symbol.** ***C*****: Action potentials from an individual GLUTag cell in response to the addition of 5 mmol/l methyl-α-glucopyranoside, in the presence of 1 mmol/l glucose, as indicated by the horizontal bars.**

by methyl-α-glucopyranoside is the inward movement of Na^+ ions, coupled to the uptake of sugar. Consistent with this idea, no increase in the current was observed when methyl-α-glucopyranoside was applied in the absence of Na^+ ions. As the kinetics of glucose uptake by SGLTs are similar to those of methyl-α-glucopyranoside (28), we also investigated whether glucose induces in inward current, in addition to closing K_{ATP} channels. Small increases in the inward current were detected when the glucose concentration was increased from 1 mmol/l to 5 or 20 mmol/l (Fig. 6*B*). The importance of these small currents for the electrophysiological activity of GLUTag cells is exemplified by the finding that the action potential frequency was increased from 0.14 ± 0.07 to 0.22 ± 0.09 Hz ($n = 5$; $P < 0.05$) when 5 mmol/l methyl-α-glucopyranoside was applied in the presence of 1 mmol/l glucose (Fig. 6*C*).

DISCUSSION

GLP-1 release from GLUTag cells is triggered by low concentrations of glucose or fructose, consistent with a mechanism involving K_{ATP} channel closure, as suggested previously (17). The finding that secretion is also stimulated by high concentrations of the nonmetabolizable sugar methyl-α-glucopyranoside cannot, however, be explained by this pathway. We show here that methyl-α-

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glucopyranoside stimulates electrical activity and GLP-1 secretion by inducing an inward current that is most simply explained by the electrogenic action of SGLTs, as they couple the uptake of sugar to the influx of Na^+ ions. In addition to providing a depolarizing short-circuit current, the coupling of sodium and sugar transport enables cells to concentrate transported sugars within the cytoplasm.

Membrane potential is dependent on the balance between inward and outward currents. In the absence of glucose, GLUTag cells have a membrane potential of ~−50 mV, maintained by a slight dominance of the outward currents (carried largely by K^+) over the inward currents (carried, e.g., by Na^+, Ca^{2+}). We showed previously that low concentrations of glucose trigger GLP-1 secretion by closing K_{ATP} channels, thereby decreasing the magnitude of the outward K^+ current and allowing the membrane to depolarize. Our present results indicate that fructose also decreased the K^+ conductance, suggesting that a mechanism involving fructose metabolism and K_{ATP} channel closure also underlies the depolarizing effect of this sugar.

Methyl-α-glucopyranoside, by contrast, also triggered electrical activity and GLP-1 secretion but caused a net increase in the membrane conductance and induced a dose-dependent inward current at −70 mV, which was abolished by the removal of external Na^+ ions. This is not compatible with a model dominated by K_{ATP} channel closure and is explained instead by the increased inward current that causes depolarization when it is large enough to overcome the outward currents. The ability of SGLTs to carry large sugar-dependent inward currents has been demonstrated in heterologous expression studies using *Xenopus* oocytes (28). Consistent with the similar transport kinetics of glucose and methyl-α-glucopyranoside demonstrated in oocytes, glucose also induced small inward currents at −70 mV in GLUTag cells.

The effectiveness of SGLT-associated currents depends on the balance between the inward and outward currents. In the absence of glucose, when the K_{ATP} channel conductance is relatively high, a correspondingly large inward current is required to induce depolarization, explaining why GLP-1 release was triggered by 100 mmol/l but not 5 mmol/l methyl-α-glucopyranoside. When the majority of K_{ATP} channels were closed by 1 mmol/l glucose, however, the inward current produced by 5 mmol/l methyl-α-glucopyranoside was large enough to trigger a small increase in the action potential frequency. An increase in the inward current on raising the glucose concentration from 1 mmol/l to 5 or 20 mmol/l may similarly underlie the increasing action potential frequency observed at higher glucose concentrations (17).

Two types of SGLTs, SGLT1 and SGLT3a, were detected in GLUTag cells by RT-PCR. Expression studies in *Xenopus* oocytes have demonstrated that SGLT1 is a high-affinity glucose transporter (K_m ~0.2–0.3 mmol/l) and SGLT3 is a low-affinity glucose transporter (K_m ~2–6 mmol/l) (28). As SGLT1 should largely be saturated by 5 mmol/l glucose or methyl-α-glucopyranoside, the increments in electrical activity and secretion measured at higher substrate concentrations might reflect the activity of SGLT3. The importance of SGLTs in the GLUTag cells is indicated by the inhibitory effect of low concentrations of phloridzin on GLP-1 release triggered by glucose or methyl-α-glucopyranoside.

It is interesting that GLP-1 secretion triggered by 5 mmol/l glucose was significantly higher than that achieved with either 100 mmol/l methyl-α-glucopyranoside or 10 nmol/l glibenclamide. We initially speculated that the greater effectiveness of glucose might reflect the combination of the depolarizing Na^+-current and K_{ATP}-channel closure, providing a stronger stimulus than either of these components acting alone. This, however, was not borne out by subsequent experiments, which showed that secretion triggered by methyl-α-glucopyranoside plus glibenclamide was not greater than that induced by either agent alone. An alternative possibility is that glucose (and fructose) has additional effects on later stages of the secretory pathway, as described in pancreatic β-cells (29), as a result, for example, of the metabolic generation of ATP or other signals.

Characteristics of glucose uptake by GLUTag cells. In addition to the SGLTs, GLUTag cells express the facilitative GLUTs GLUT1 and GLUT5. The similarity between the glucose sensitivity of GLP-1 release from GLUTag cells (EC_{50} ~0.2 mmol/l) and the affinity of SGLT1 (K_m ~0.3 mmol/l) (28) suggested the possibility that glucose transport through SGLT1 might underlie the glucose dependencies of K_{ATP}-channel closure and hormone secretion. Indeed, the coupling of glucose and Na^+ uptake by SGLT1 could enable cells to use the electrochemical Na^+ gradient to concentrate glucose inside cells even at low extracellular levels, elevating the intracellular glucose concentration to a level appropriate for phosphorylation by glucokinase ($S_{0.5}$ ~8 mmol/l) (30). An alternative explanation for the high glucose sensitivity of GLUTag cells, however, is the expression in this cell line of high-affinity hexokinases I–III (K_m ~0.05–0.15 mmol/l), as has been reported in some insulinoma cell lines (31,32). Consistent with the latter hypothesis, we were unable to detect significant glucokinase activity in crude cytoplasmic extracts from GLUTag cells, using a glucose-6-phosphate dehydrogenase coupled assay over a glucose concentration range of 0.06–100 mmol/l (33), whereas >95% of the observed activity was due to hexokinase with a K_m for glucose of 0.17 ± 0.05 mmol/l ($n = 2$, data not shown). The activity of hexokinase in GLUTag cells, in combination with the expression of GLUT1 and GLUT5, might also explain why the SGLT inhibitor phloridzin only incompletely abolished the electrophysiological response to 5 mmol/l glucose and the secretory response to 0.5 mmol/l glucose. Although glucokinase activity was not detectable in the current study, we previously detected mRNA encoding glucokinase under the control of the upstream promoter in GLUTag cells by RT-PCR (17). The expression of glucokinase protein in native L-cells is suggested by the report that low-affinity hexokinase activity could be measured in jejunal extracts and that the upstream glucokinase promoter directed transgene expression in GLP-1–containing cells (34).

GLUT2 mRNA was not detectable by RT-PCR, suggesting the absence of this basolateral membrane transporter in the GLUTag cell line. This is an interesting finding because, if also true of native L-cells, it might account for their apparent blindness to changes in the plasma glucose

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concentration (7). Additional studies will be required to establish whether features observed in the GLUTag cells, such as the lack of GLUT2 and the predominance of high-affinity hexokinase I–III over glucokinase activity, are also features of native L-cells. Although GLUTag cells secrete GLP-1 in response to a range of physiological stimuli, immortalization has resulted in a loss of cell polarity and may have affected the expression of metabolic enzymes.

Conclusion.

Delivery of small quantities of glucose to the jejunum has been shown to occur rapidly after a meal, as a result of saturation of the duodenal absorptive capacity (35). At the level of the ileocecal valve, the glucose concentration in the human gut lumen was found to increase within ~30 min after meal ingestion (from 0 in the preprandial period) and remained elevated at 5–10 mmol/l for up to 6 h (36). The relative contributions of direct glucose detection by L-cells and indirect signals provided, for example, by the vagus are difficult to assess in vivo under physiological conditions. Direct glucose sensing by the L-cells, however, has been implicated under conditions that increase sugar delivery to the small intestine, such as partial gastrectomy and therapy with α-glucosidase inhibitors (15,37). The concentrations of luminal glucose necessary to trigger GLP-1 release in vivo are unclear, as concentrations ranging from 5 to 1,000 mmol/l have variously been reported to trigger or not to trigger GLP-1 release from the perfused ileum (23–25,38–40).

The finding that both electrogenic sugar uptake and sugar metabolism contribute to the glucose sensitivity of GLUTag cells resolves the paradox that although GLP-1 release stimulated by low glucose concentrations involves sugar metabolism and K_{ATP}-channel closure, secretion can also be triggered by higher concentrations of nonmetabolizable glucose analogues that act as substrates for SGLTs. Electrogenic substrate entry through SGLTs could also provide a K_{ATP} channel–independent glucose-sensing mechanism in other tissues. Expression of SGLT1 has been detected, for example, in populations of neurones (41). The functional importance of hypothalamic SGLTs is suggested by the finding that intracerebroventricular administration of phloridzin enhances food intake in rats and mice (42,43) and blocks the glucose responsiveness of neurons in the ventromedial hypothalamus (44). The idea that electrogenic substrate entry through SGLTs could provide an alternative glucose-sensing mechanism in some neurones is consistent with the recent report that cytosolic ATP concentrations are not altered by glucose in glucose-responsive neurones from the hypothalamus (45). The expression and function of SGLTs in different glucose-sensitive tissues will be an interesting area for future research.

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