

Glucose-Sensing in Glucagon-Like Peptide-1-Secreting Cells

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Glucagon-like peptide-1 (GLP-1) is released from intestinal L-cells in response to carbohydrate and fat in the diet. Despite the interest in GLP-1 as an antidiabetic agent, very little is known about the mechanism of stimulus-secretion coupling in L-cells. We investigated the electrophysiological events underlying glucose-induced GLP-1 release in the GLP-1-secreting cell line, GLUTag. Cells were studied using perforated-patch and standard whole-cell patch clamp recordings. GLUTag cells were largely quiescent and hyperpolarized in the absence of glucose. Increasing the glucose concentration between 0 and 20 mmol/l decreased the membrane conductance, caused membrane depolarization, and triggered the generation of action potentials. Action potentials were also triggered by tolbutamide (500 $\mu\text{mol/l}$) and were suppressed by diazoxide (340 $\mu\text{mol/l}$) or the metabolic inhibitor azide (3 mmol/l), suggesting an involvement of K_{ATP} channels. Large tolbutamide-sensitive washout currents developed in standard whole-cell recordings, confirming the presence of K_{ATP} channels. RT-PCR detected the K_{ATP} channel subunits Kir6.2 and SUR1 and glucokinase. GLP-1 secretion was also stimulated by glucose over the concentration range 0–25 mmol/l and by tolbutamide. Our results suggest that glucose triggers GLP-1 release through closure of K_{ATP} channels and action potential generation. *Diabetes* 51:2757–2763, 2002

Glucagon-like peptide-1 (GLP-1) (1,2) is an incretin hormone that enhances insulin release from pancreatic β -cells in both normal and type 2 diabetic subjects (3). Unlike the sulfonylureas, which stimulate insulin release at low as well as high glucose levels, the action of GLP-1 on β -cells is glucose dependent (4). As a potential treatment for type 2 diabetes, GLP-1 offers the advantages that it is associated with a low incidence of hypoglycemic side effects and may also not induce weight gain (5). GLP-1 receptor agonists and drugs that slow the degradation of active GLP-1 are therefore under development as novel treatments for type 2 diabetes

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ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GIP, glucose-dependent insulinotropic peptide; IBMX, isobutylmethylxanthine; GLP-1, glucagon-like peptide-1; GRP, gastrin-releasing peptide; K_{ATP} channel, ATP-sensitive K^+ channel; PKA, protein kinase A; PKC, protein kinase C.

(6,7). An alternative strategy for drug development would be to enhance endogenous GLP-1 secretion, but there are currently very few data on the pathways underlying GLP-1 release.

GLP-1 is secreted from specialized intestinal neuroendocrine cells (L-cells) in response to dietary nutrients, particularly carbohydrates and lipids (8,9). L-cells are distributed throughout the intestine but are found in greatest numbers in the jejunum, ileum, and colon (10–12). The cells face the gut lumen, suggesting that they sense the luminal concentration of lipids and carbohydrates directly. Oral administration of glucose and fat results in two overlapping phases of GLP-1 secretion. The early phase begins within minutes of a meal and continues for 30–60 min. The second phase causes prolonged secretion 1–3 h after a meal (8,9). This delayed phase of secretion has been suggested to involve the direct detection by L-cells of the luminal contents, as infusion of glucose into segments of rat, dog, and pig ileum stimulates GLP-1 release (13–15). The mechanism underlying the early phase of GLP-1 release is more controversial. As most of the L-cells lie distal to the duodenum, it has been argued that the onset of GLP-1 secretion is too rapid to be due to direct glucose sensing by the L-cells and may involve hormonal or neural signals, e.g., by glucose-dependent insulinotropic peptide (GIP) or gastrin-releasing peptide (GRP) (16–18). A role for direct glucose sensing by L-cells as the trigger for early GLP-1 release remains controversial.

Glucose sensing by neuroendocrine cells has been studied extensively in pancreatic β -cells, which release insulin in response to physiological changes in glucose concentration. It is believed that glucose enters β -cells via the GLUT-2 transporter and is subsequently phosphorylated by glucokinase, the relatively low affinity of which enables it to regulate the glycolytic flux over a physiological range of glucose concentrations (19). The rates of glycolytic and mitochondrial ATP production are therefore dependent on the glucose concentration, and consequent changes in the ATP/ADP ratio are believed to be the principal signal to close ATP-sensitive K^+ channels (K_{ATP} channels) on the plasma membrane. The K_{ATP} channels are open at low glucose levels, allowing efflux of K^+ ions, which hyperpolarizes the membrane. As the glucose concentration rises, the ATP/ADP ratio increases, K_{ATP} channels close, and the membrane depolarizes, triggering the opening of voltage-gated calcium channels, the generation of Ca^{2+} -carrying action potentials, and stimulation of exocytosis (20–22). Secretion is further potentiated by agents that act through modulation of cAMP and protein kinases A and C (PKA,

PKC), which operate largely on steps distal to the entry of Ca^{2+} (23)

The electrophysiological properties underlying GLP-1 secretion have not previously been characterized because of the difficulties in purifying and identifying living L-cells. Secretion studies have been performed using partially purified L-cell preparations obtained by fetal rat intestinal cell culture or by elutriation (16,24–27). These preparations have a maximum L-cell content of ~30% and have been used to show that GLP-1 secretion is enhanced by activators of PKA and PKC. Such preparations have also been used to demonstrate that GLP-1 secretion is inhibited by the neurotransmitter galanin, acting through a disopyramide-sensitive pathway that might involve K_{ATP} channel opening (27).

PKA- and PKC-dependent GLP-1 secretion is also characteristic of the GLUTag cell line, which was derived from a colonic neuroendocrine tumor generated in a transgenic mouse expressing the SV40 T-antigen under the control of the proglucagon promoter (28,29). We have used GLUTag cells as a model to study the electrophysiological events underlying GLP-1 secretion. We show here that the electrical activity of GLUTag cells is modulated by glucose and by agents that affect K_{ATP} channel activity. The K_{ATP} channels exhibit similar pharmacological properties to those found in pancreatic β -cells, and have a similar subunit composition (Kir6.2/SUR1). Our results suggest that the stimulus-secretion pathways in L-cells and β -cells share common glucose-sensing machinery.

RESEARCH DESIGN AND METHODS

Cell culture. GLUTag cells (28) were cultured in Dulbecco's modified Eagle's medium (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 1:4 dilution when ~70% confluence was reached (approximately every 5 days).

GLP-1 secretion. For secretion experiments, cells were plated in 24-well culture plates and allowed to reach 60–80% confluence. On the day of the experiment, cells were washed twice with 500 μ l 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 μ l of Krebs-Ringer for 2 h at 37°C, 5% CO_2 . Tolbutamide, forskolin, and isobutylmethylxanthine (IBMX) were prepared as 1,000 \times stocks in DMSO, and the final DMSO concentration was adjusted to 0.2% (or 0.3% for the experiment with tolbutamide, forskolin, and IBMX simultaneously). At the end of this incubation period, medium was collected and centrifuged to remove any floating cells. GLP-1 was assayed in the supernatants using an enzyme-linked immunosorbent assay (ELISA) that is specific for GLP-1 (7-36) amide and GLP-1 (7-37) (GLP-1 active ELISA-kit; Linco). Each sample was analyzed in duplicate.

Electrophysiology. Cells were plated onto 35-mm dishes 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 melted beeswax. Electrodes were fire-polished using a microforge (Narishige) and had resistances of 2.5–3 M Ω 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 –100 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 NaH_2PO_4 , 1.2 NaH_2PO_4 , 2.6 $CaCl_2$, 1.2 $MgCl_2$, and 10 HEPES (pH 7.4). Glucose and drugs were added to the bath solution as indicated. 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 was added to a final concentration of 200 μ g/ml. The pipette solution for recording washout currents contained (in mmol/l) 107 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 EGTA, 10 HEPES, and 0.3 ATP (pH 7.2). Tolbutamide was prepared as a 100-mmol/l stock solution, and diazoxide was prepared as a 68-mmol/l stock solution, in KOH. **Statistical analysis.** Results are presented as mean \pm SE. Statistical significance was tested using Student's *t* test, using a threshold for significance of $P < 0.05$.

RT-PCR. Whole RNA was prepared from $\sim 2.5 \times 10^7$ GLUTag cells using Tri-reagent (Sigma). First-strand cDNA synthesis was performed for 1 h at 42°C in a total reaction volume of 25 μ l containing 1 μ g of RNA, 500 ng of random hexamer primers (Promega), 25 nmol of each dNTP (Promega), 40 units of RNasin (Promega), 25 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 2 mmol/l dithiothreitol, 5 mmol/l $MgCl_2$, and 21 units of RT (SuperRT; HT Biotechnology). One microliter of this reaction was used for subsequent PCR amplification using the following specific primers, which span introns wherever possible (from 5' to 3'): Kir6.1 (accession no. D88159) sense GTCT-TCTAGGAGGACGCGTG (position 126), antisense TATCATACAGGG GGCTACGC (position 786); Kir6.2 (accession no. D50581) sense AAGAA AGGCAACTGCAACGT (position 243), antisense CCCCATAGAATCTCGTCA GC (position 1066); SUR1 (accession no. L40624 [rat]) sense GTGAGTTCCT GTCCAGTGCA (position 2091), antisense GTGATGTTCTCCTCCACCGT (position 2622); glyceraldehyde-3-phosphate dehydrogenase (GAPDH; accession no. M17701 [rat]) sense CGATCCCCTAACATCAAAT (position 264), antisense GGATGCAGGGATGATGTTCT (position 654); glucokinase (accession nos. L41629 and L41631) sense GCTGAGGCGTGAGGGACAG (position 361 [exon-1 β -cell]), sense AGCCAGACAGTCTCACCC (position 9210 [exon-1 liver]), antisense TCTGAGCCTTCTGGGGTGG (position 13863). The reaction was performed in a volume of 50 μ l containing 50 pmol of each primer of one sense/antisense pair, 0.2 mmol/l of each dNTP (Promega), 1.8 mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 9.0), 0.1% Triton X-100, and 0.75 units of Super-Taq-polymerase (HT Biotechnology) in a PCR-express thermocycler (Hybaid) using the following cycling protocol: initial 3-min denaturation at 95°C (in which time the polymerase was added [hot start]) was followed by 35 cycles of 94°C for 60 s, 58°C for 60 s, and 72°C for 60 s and a final elongation at 72°C for 7 min. Twenty microliters of the reaction was used for subsequent analysis by agarose gel electrophoresis (2% gel), and products were visualized by ethidium bromide fluorescence. The predicted sizes (bp) of fragments were as follows: Kir6.1, 661; Kir6.2, 824; SUR1, 532; GAPDH, 391; glucokinase, β -cell promoter, 605; glucokinase, liver promoter, 331. The primer pairs for SUR1, Kir6.1, and Kir6.2 have been described previously (30). No bands were detected in control experiments using water in place of the RT product.

RESULTS

Action potentials in GLUTag cells are triggered by glucose. To investigate whether the membrane potential of GLUTag cells is modulated by glucose, we studied intact cells by current clamp using the perforated-patch method. GLUTag cells perfused at low glucose concentrations (0–2 mmol/l) were electrically silent or fired infrequent action potentials. The mean resting potential in the presence of 2 mmol/l glucose was -53 ± 1.3 mV ($n = 13$), measured during action potential-free intervals. Increasing the glucose concentration to 20 mmol/l resulted in membrane depolarization and the generation of spontaneous action potentials (Fig. 1A and B). The mean action potential frequency in 20 mmol/l glucose was 1.3 ± 0.3 Hz ($n = 14$; Fig. 1C). We also observed a few cells that fired rapidly in 2 mmol/l glucose and were not affected by perfusion with low or high glucose. As this behavior was frequently associated with subsequent loss of the patch, such cells were not included in the analysis.

Metabolic inhibition with 3 mmol/l Na azide abolished action potentials in the presence of 20 mmol/l glucose (Fig. 2A) and resulted in membrane hyperpolarization to a mean voltage of -61 ± 4 mV ($n = 3$). Similar results from pancreatic β -cells have been attributed to the opening of

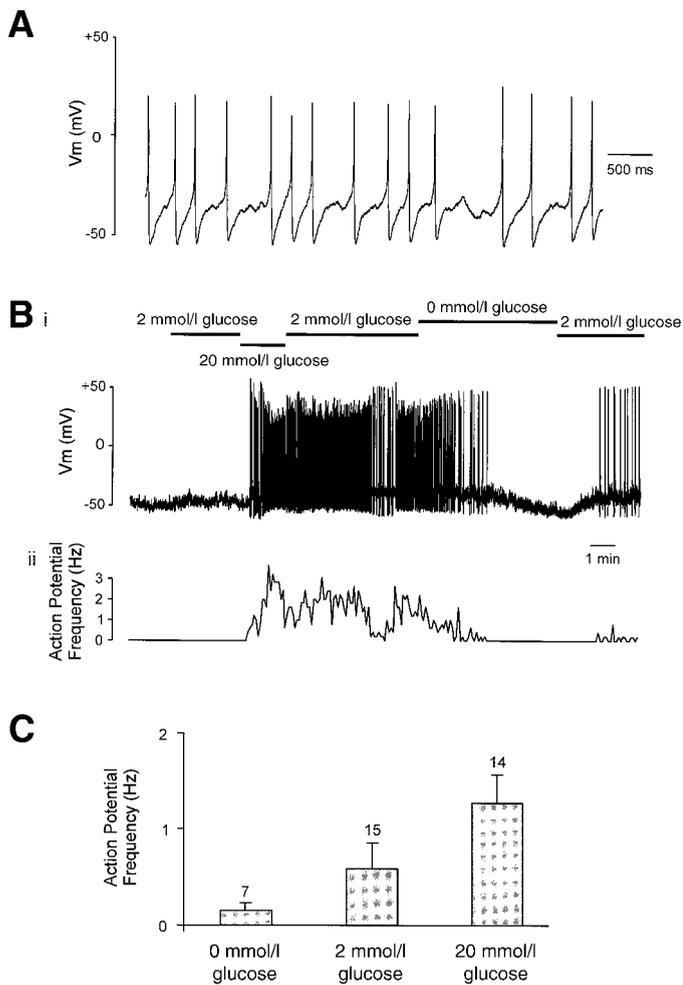


FIG. 1. Action potential frequency in GLUTag cells is modulated by glucose. **A:** Action potentials from an individual GLUTag cell in a current-clamp recording, using the perforated-patch whole-cell configuration of the patch clamp setup. The cell was perfused with 20 mmol/l glucose. **B:** Action potential frequency was modulated by perfusion with different glucose concentrations (indicated by the horizontal bars). A representative current clamp trace (i) and corresponding action potential frequency (ii) are shown. The action potential frequency was determined by counting events crossing above a threshold of -10 mV, with a bin size of 5 s. **C:** Mean action potential frequency at 0, 2, and 20 mmol/l glucose. The number of cells is given above each bar. Error bars represent 1 SE.

K_{ATP} channels in response to the falling concentration of ATP and rising concentration of ADP (21).

Glucose reduced the membrane conductance. As K_{ATP} channel closure would result in a reduced membrane conductance, we investigated the effect of glucose on the conductance measured in perforated-patch voltage-clamp experiments. Increasing the glucose concentration progressively reduced the slope conductance (Fig. 3), indicating that it causes net channel closure.

Washout currents develop in whole-cell experiments. We investigated the amplitude of washout currents in standard whole-cell voltage-clamp experiments. Current amplitudes were small immediately after establishment of the whole-cell configuration but increased after 2–5 min (Fig. 4A). The reversal potential of the washout current was -75 ± 1 mV ($n = 5$), consistent with the opening of K^+ selective ion channels. The currents were blocked by

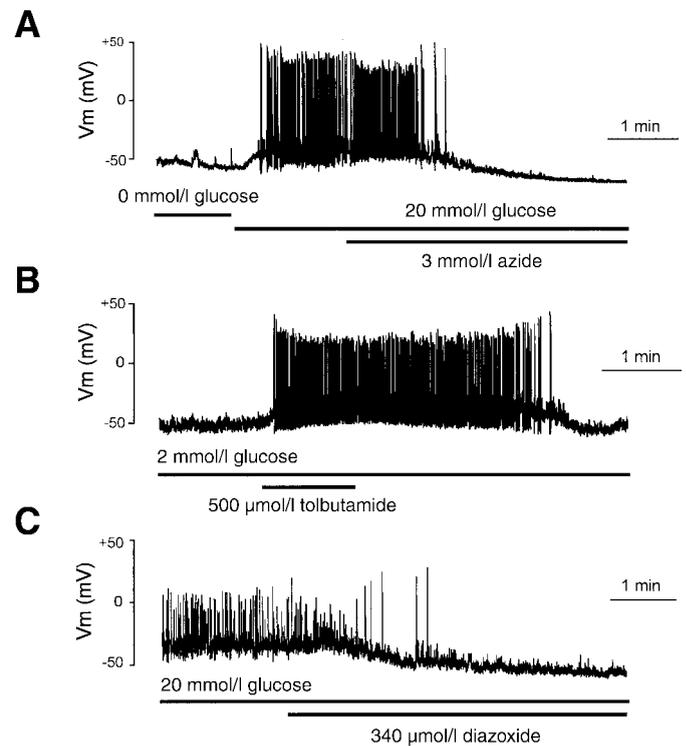


FIG. 2. Action potential frequency is modulated by agents that affect K_{ATP} channel activity. Effect of 3 mmol/l Na azide (A), 500 μ mol/l tolbutamide (B), or 340 μ mol/l diazoxide (C) on the action potential frequency in perforated patch current clamp recordings from different GLUTag cells. Test agents and glucose were applied as indicated by the bars.

500 μ mol/l tolbutamide (Fig. 4B), indicating that they flowed through open K_{ATP} channels.

Drugs that target K_{ATP} channels modify action potential frequency. Tolbutamide (500 μ mol/l) triggered the firing of action potentials in 2 mmol/l glucose (Fig. 2B). The mean action potential frequency in the presence of tolbutamide was similar to that in 20 mmol/l glucose (1.6 ± 0.6 Hz [$n = 6$] in tolbutamide vs 1.3 Hz in 20 mmol/l glucose). The K_{ATP} channel opener diazoxide (340 μ mol/l)

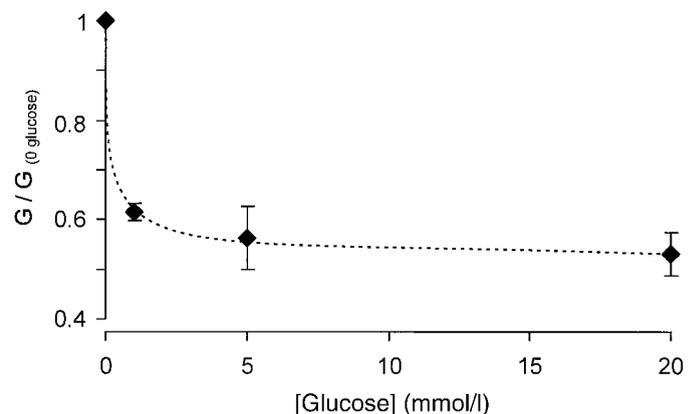


FIG. 3. The glucose dependence of the membrane conductance was measured in perforated-patch voltage-clamp experiments. The slope conductance was measured by applying a series of 1.2-s voltage ramps from -100 to -50 mV (holding potential -70 mV). The mean of 10 ramps was recorded at each glucose concentration. The conductance (G) in the presence of glucose was normalized to the conductance in the absence of glucose ($G_{0 \text{ glucose}}$). The number of cells was four in each case. Error bars represent 1 SE. The dotted line was fitted by eye.

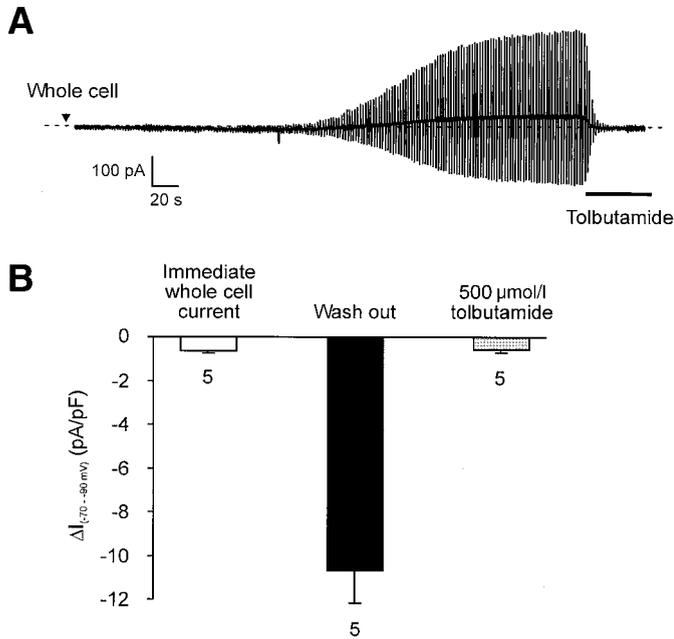


FIG. 4. Whole-cell washout currents in GLUTag cells. **A:** K_{ATP} current measured by standard whole-cell recording from a GLUTag cell. Pipette [ATP] = 0.3 mmol/l. Currents were recorded in response to ± 20 mV voltage pulses from a holding potential of -70 mV. The dashed line indicates the zero current level. Tolbutamide (500 μ mol/l) was added as indicated by the bar. The trace started immediately after establishment of the whole-cell configuration. **B:** Mean current amplitudes before washout of ATP, after washout, and after addition of tolbutamide. Currents were measured in response to a -20 -mV voltage step from a holding potential of -70 mV. Error bars represent 1 SE.

abolished action potentials in the presence of 20 mmol/l glucose and caused membrane hyperpolarization (Fig. 2C).

Detection of K_{ATP} channel subunits and glucokinase by RT-PCR. We used RT-PCR to investigate which K_{ATP} channel subunits are present in GLUTag cells and whether they also express glucokinase. Glucokinase primers were designed to distinguish between the upstream (β -cell) and downstream (liver) promoters. Figure 5 shows that GLUTag cells express Kir6.2 and SUR1 and glucokinase under the control of the upstream promoter. Similar

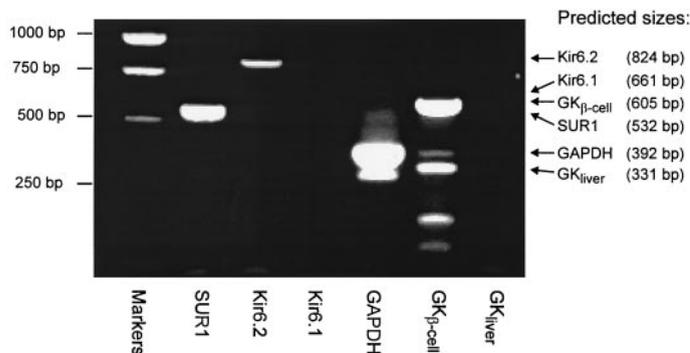


FIG. 5. Identification of K_{ATP} channel subunits and glucokinase by RT-PCR. The β -cell isoforms of the K_{ATP} channel (Kir6.2 and SUR1) and glucokinase were detected in GLUTag cells using RT-PCR. Glucokinase (GK) primers were designed to distinguish between the β -cell (upstream) and liver (downstream) promoters. The predicted positions and band sizes are indicated. Similar bands (including the additional bands in the GK β -cell lane) were detected in min6 cells.

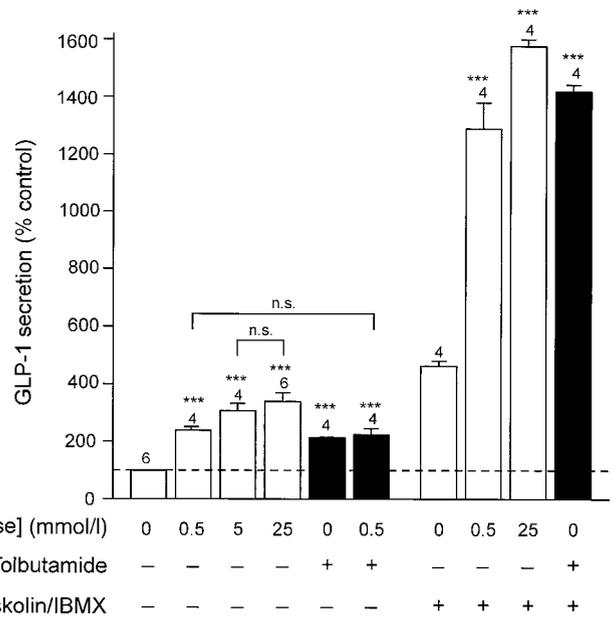


FIG. 6. Glucose stimulates GLP-1 secretion. GLP-1 secretion from GLUTag cells cultured for 2 h in Krebs Ringer buffer under the conditions indicated. Error bars represent 1 SE, and the number of dishes is indicated above each bar. Secretion was normalized to the baseline secretion in 0 mmol/l glucose measured in parallel on the same day. This baseline secretory rate in 0 mmol/l glucose was 26.6 ± 1.9 fmol per dish in 2 h ($n = 6$). $***P < 0.001$; n.s., not significant, compared with secretion in the absence of glucose and tolbutamide (experiments in the presence of forskolin/IBMX were compared with 0 mmol/l glucose in forskolin/IBMX).

results were obtained using RNA extracted from the insulinoma cell line min6 (data not shown).

Glucose and tolbutamide stimulate GLP-1 secretion from GLUTag cells. We next investigated whether glucose and tolbutamide stimulate GLP-1 secretion from GLUTag cells. Experiments were performed in Krebs Ringer solution to prevent interference by other possible nutrient secretagogues, such as fatty acids and amino acids. Under these conditions, 0.5, 5, and 25 mmol/l glucose enhanced GLP-1 secretion 2.4 ± 0.1 -, 3.1 ± 0.3 -, and 3.4 ± 0.3 -fold, respectively, relative to the secretion observed in the absence of glucose (Fig. 6). As described previously, strong enhancement of GLP-1 secretion was observed when cytoplasmic cAMP levels were elevated by the addition of forskolin and IBMX (10 μ mol/l each); however, under these conditions, secretion was still modulated by the glucose concentration. Tolbutamide (500 μ mol/l) also stimulated secretion in the absence of glucose (2.1 ± 0.02 -fold in the absence of forskolin/IBMX and 3.1 ± 0.05 -fold in the presence forskolin/IBMX, relative to secretion in 0 mmol/l glucose under the same conditions). By contrast, we detected no further stimulation of GLP-1 secretion by tolbutamide in the presence of 0.5 mmol/l glucose.

DISCUSSION

Despite the interest in GLP-1 as a novel treatment for type 2 diabetes, the electrophysiological events underlying GLP-1 secretion have not previously been addressed. Using the GLUTag cell line, we show that GLP-1 secretion and electrical activity are triggered by an increase in glucose over the concentration range 0–25 mmol/l.

GLUTag cells possess functional glucose-sensing machinery. Our results support the idea that regulation of K_{ATP} channel activity plays a critical role in glucose sensing by GLUTag cells. Thus, cells were hyperpolarized (to ~ -55 mV) at low glucose concentrations, consistent with a potassium conductance playing a major role in determining the resting membrane potential. Increasing the glucose concentration caused net channel closure, as shown by the reduced membrane conductance. Sulfonylurea-sensitive whole-cell washout currents confirmed the presence of K_{ATP} channels. Tolbutamide triggered action potentials in the presence of 2 mmol/l glucose and stimulated GLP-1 secretion in zero glucose, indicating that a significant proportion of the K_{ATP} channels are open at low glucose concentrations. The identification of Kir6.2 and SUR1 by RT-PCR is consistent with the electrophysiological finding that the channels were sensitive to tolbutamide and diazoxide.

K_{ATP} channels have been detected previously in the poorly differentiated and multipotential CCK-secreting cell line STC-1, which also releases GIP and GLP-1 (31–33). CCK secretion from STC-1 cells was stimulated by 5 and 25 mmol/l glucose and by the nonspecific channel blocker disopyramide (31).

In pancreatic β -cells, glucokinase plays a major role in controlling flux through glycolysis at physiological glucose concentrations (19). Using RT-PCR, we showed that GLUTag cells also express glucokinase and that, as in the pancreatic β -cell, this is under the control of the upstream promoter. It has been shown previously that the β -cell glucokinase promoter can direct expression in neuroendocrine cell types, including some containing GLP-1 immunoreactivity (34). The finding that glucokinase is expressed in GLUTag cells further supports the idea that glycolytic flux, ATP generation, and K_{ATP} channel activity may vary at physiological glucose concentrations.

The idea that low concentrations of glucose trigger GLP-1 release through metabolism and K_{ATP} channel closure seems contrary to results obtained using perfused rat ileum, which showed that some nonmetabolizable glucose analogues also stimulate GLP-1 release (35). The ileal perfusion experiments, however, tested substrates at much higher concentrations (~ 250 mmol/l) than those used in the current study, and might stimulate secretion by an alternative mechanism.

Correlation between electrical activity and secretion. The glucose dependence of the membrane conductance in GLUTag cells is similar to that reported previously for pancreatic β -cells (36,37). The rate of GLP-1 secretion closely mirrored the change in membrane conductance, rather than reflecting the action potential frequency. Thus, the greatest fall in membrane conductance and the greatest increase in GLP-1 secretion occurred when the glucose concentration was increased from 0 to 1 mmol/l or 0.5 mmol/l, respectively. Action potential frequency, by contrast, continued to increase significantly when the glucose concentration was raised from 2 to 20 mmol/l. The results suggest that GLP-1 release may be elicited by relatively infrequent action potentials but shows little augmentation when the action potential frequency is further enhanced. This idea is supported by the finding that tolbutamide increased the action potential frequency in 2 mmol/l

glucose but only stimulated secretion in the absence of glucose. The finding that GLUTag cells respond to glucose at very low concentrations (0–5 mmol/l) but are not sensitive to additional increments (5–25 mmol/l) is consistent with previous reports that L-cells in primary culture do not respond to changes in the glucose concentration from 5 to 25 mmol/l (16).

Physiological implications of glucose sensitivity. Fetal rat intestinal cells have been shown to secrete GLP-1 in response to glucose, but only in the additional presence of insulin (24). Here we provide—to our knowledge, for the first time—evidence for direct sensing of glucose in an L-cell in vitro system. The high glucose sensitivity (0–5 mmol/l) of GLUTag cells raises the question of whether glucose concentrations in the gut lumen or plasma are ever low enough to switch off GLP-1 secretion in vivo. Although glucose concentrations as low as 0.5 mmol/l are not normally reached in plasma, measurements from the distal small intestine of rats during normal feeding behavior revealed glucose concentrations in the range of 0–3 mmol/l (38). As L-cells in vivo are strongly polarized (1,10), they may detect glucose differentially at apical and basolateral surfaces. Low expression of glucose transporters on the basolateral membrane, for example, could render the cells largely blind to glucose in the plasma. It is also possible, however, that immortalization of L-cells to form the GLUTag cell line may be associated with altered glucose sensitivity, as has been reported in some insulinoma cell lines (39), or that cultured cells may lack a tonic K_{ATP} channel opener. There is evidence, for example, that the neurotransmitter galanin may activate K_{ATP} channels in the pluripotent intestinal neuroendocrine cell line STC-1 (33) and could thereby influence the glucose sensitivity in vivo.

The distribution of L-cells along the gut shows some species variation, although the cells are universally present in ileum and colon. In humans, L-cells are largely absent from the duodenum but are numerous in the jejunum, ileum, and colon (10). The high frequency of L-cells in the distal gut, taken together with the lack of glucose sensitivity of isolated cells when measured between 5 and 25 mmol/l, has led to the conclusion that the rapid rise in GLP-1 that follows meal ingestion is not due to direct glucose sensing by L-cells. If, however, the L-cells are capable of responding at low glucose concentrations, as our data suggest, then cells in the jejunum may experience stimulatory conditions very soon after a meal. Indeed, studies on human subjects showed that the rate of glucose entry into the duodenum exceeded the maximal duodenal absorptive capacity within 5 min after a glucose meal (40), which would result in the early delivery of small amounts of glucose to the jejunum.

There is considerable evidence supporting the hypothesis that luminal glucose is the trigger for the late phase of GLP-1 release. Infusion of glucose in rat, pig, and dog (13–15) or carbohydrate in human (41) directly into the ileal lumen triggers GLP-1 secretion. Retarding sugar absorption in humans using the α -glucosidase inhibitor acarbose results in earlier and larger increments in plasma GLP-1 levels, which has been attributed to the greater supply of sugar reaching distal populations of L-cells (42,43). The reactive hypoglycemia observed in dumping

syndrome, which is associated with the arrival of high concentrations of sugar in the distal small intestine and colon, has also been attributed to increased GLP-1 release (44,45).

Augmentation of GLP-1 secretion by forskolin and IBMX. Forskolin and IBMX enhanced GLP-1 secretion approximately fourfold in the presence of different glucose concentrations or tolbutamide. This is a similar magnitude of stimulation to that reported previously in the GLUTag cell line (28). Activation of PKA and PKC has also been shown to enhance GLP-1 release from cultured L-cells. One mechanism underlying the action of PKA may be increased transcription of the proglucagon gene (28,29,46). In addition, protein kinases and/or cAMP may directly stimulate exocytosis as in the pancreatic β -cell (4). Modulation of protein kinase activity may provide an important mechanism for the neural and hormonal regulation of GLP-1 release, e.g., by GRP and GIP (16–18).

The finding that glucose increments from 5 to 25 mmol/l or tolbutamide added in the presence of 0.5 mmol/l glucose failed to stimulate GLP-1 release contrasts with the effect of these agents on insulin release from pancreatic β -cells. Despite the similarity in the glucose sensitivity of the membrane conductances in GLUTag cells and β -cells, insulin release is largely stimulated at glucose concentrations between 3 and 20 mmol/l. In β -cells, there is evidence that both glucose and sulfonylureas have K_{ATP} channel-independent effects on exocytosis, although the underlying mechanisms and significance remain controversial (23,47). One explanation for our findings, therefore, is that these actions are absent or not significant in GLUTag cells.

Conclusions. Our results indicate that GLP-1 secretion from GLUTag cells is triggered by glucose through a mechanism involving K_{ATP} channel closure. The glucose-sensing machinery in GLUTag cells is similar to that found in pancreatic β -cells, although the glucose set point for GLP-1 release is set to the left of that for insulin release (37). Although it is possible that this is a feature of the GLUTag cell line and not of L-cells in vivo, it is also possible that the difference may reflect the normal physiology of the cells, as insulin release is regulated by the plasma glucose concentration, whereas L-cells may detect glucose in the gut lumen. Indeed, although intravenous glucose infusion potently stimulates insulin release in humans, it does not markedly affect GLP-1 release (3). The effect of sulfonylureas on GLP-1 secretion in vivo is not clear. Total GLP-1 levels, measured using nonspecific antibodies, were similar in sulfonylurea-treated and diet-treated subjects with type 2 diabetes (48). However, more recent studies using antibodies specific for active GLP-1 and GLP-1 have shown that meal-related GLP-1 secretion is impaired in type 1 and type 2 diabetes (49,50). An answer to whether sulfonylureas stimulate GLP-1 secretion in normal subjects is not apparent in the literature. Additional investigations into the mechanisms underlying GLP-1 secretion may provide novel drug targets for treating type 2 diabetes.

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