Glucose-induced Toxicity in Insulin-producing Pituitary Cells That Coexpress GLUT2 and Glucokinase

IMPLICATIONS FOR METABOLIC ENGINEERING*

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We have shown that intermediate lobe (IL) pituitary cells can be engineered to produce sufficient amounts of insulin (ins) to cure diabetes in nonobese diabetic mice but, unlike transplanted islets, ILins cells evade immune attack. To confer glucose-sensing capabilities into these cells, they were further modified with recombinant adenoviruses to express high levels of GLUT2 and the β-cell isofrm of glucokinase (GK). Although expression of GLUT2 alone had negligible effects on glucose usage and lactate production, expression of GK alone resulted in ~2-fold increase in glycolytic flux within the physiological (3–20 mM) glucose range. GLUT2/GK coexpression further increased glycolytic flux at 20 mM glucose but disproportionately increased flux at 3 mM glucose. Despite enhanced glycolytic fluxes, GLUT2/GK-coexpressing cells showed glucose dose-dependent accumulation of hexose phosphates, depletion of intracellular ATP, and severe apoptotic cell death. These studies demonstrate that glucose-sensing properties can be introduced into non-islet cells by the single expression of GK and that glucose responsiveness can be augmented by the coexpression of GLUT2. However, in the metabolic engineering of surrogate β cells, it is critical that the levels of the components be closely optimized to ensure their physiological function and to avoid the deleterious consequences of glucose-induced toxicity.

Type 1 diabetes in humans and in nonobese diabetic (NOD)1

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1 The abbreviations used are: NOD, nonobese diabetic; POMC, proopiomelanocortin; IL, intermediate lobe; ins, insulin; ILins, insulin-producing intermediate lobe; GLUT2, glucose transporter isotype 2; GK, glucokinase; KATP, channels, ATP-sensitive potassium channels; GSIS, glucose-stimulated insulin secretion; ACh, acetylcholine; HK, hexokinase; PFK, phosphofructokinase; G6P, glucose 6-phosphate; GAP, glyceraldehyde 3-phosphate; F1,6BP, fructose 1,6-bisphosphate; ARMC, glucokinase promoter; CMV, cytomegalovirus.

Ad, adenovirus; GFP, green fluorescence protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; kb, kilobase(s); m.o.i., multiplicity of infection; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; TMR, tetramethylrhodamine; bp, base pair(s); AL, anterior lobe; hGH, human growth hormone; CMV, cytomegalovirus.
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The high capacity glucose transporter, GLUT2, is also unique in having a high \( K_m \) (~17 mM) for glucose (7) but is believed to play a more “permissive” role in glucose sensing by allowing the rapid equilibration of glucose across the plasma membrane (8). However, several studies have suggested that the expression of GLUT2 is required for conferring glucose-sensing capabilities in non-\( \beta \) cell lines (9, 10). In addition, it has been observed that GLUT2 and GK are coexpressed not only in \( \beta \) cells and hepatocytes but also in glucagon-responsive neurons in the hypothalamus and the gut (11), further suggesting that GLUT2 may be an important component of the glucose-sensing apparatus in non-islet cells.

It has been suggested that the simple “iterative engineering” of glucose-sensing components into cells or cell lines may simulate the performance of normal islet \( \beta \) cells (12). The goal of this study was to determine whether the expression of GK or GLUT2, alone or in combination, would confer glucose-sensing capabilities into \( \alpha \)-cells following the manufacturer’s instructions. Fluorescence was monitored in a minimum volume of RPMI 1640 medium with 10\% fetal bovine serum (Life Technologies, Inc.) and DNA strand breaks were detected with a commercially available kit (Roche Molecular Biochemicals).

**EXPERIMENTAL PROCEDURES**

**Construction of Recombinant Adenoviruses—**Recombinant adenoviruses were generated using the strategy of Cre-lox recombination (13). A 1.7-kb XbaI/Smal cDNA fragment encoding the rat GLUT2 protein (14) or a 1.45-kb KpnI/XhoI cDNA fragment encoding the islet isoform of GK (GK.B1 (15)) were inserted into the polylinker of the shuttle vector pAdMDM (13). To construct the GK-GFP fusion protein, a PstI/BamHI fragment containing part of the GK.B1 cDNA was first subcloned into the plasmid pEGFP-N3 (CLONTECH) resulting in pGK.B1/PstIUGFP. The remaining KpnI/PstI fragment of the GK.B1 cDNA was then ligated into pGK.B1/PstIUGFP resulting in pGK.B1/GFP. Fusion in-frame and orientation was confirmed by sequence analysis.

An XhoI/NotI fragment containing the GK.B1/GFP fusion protein was then ligated into pAdMDM.

**Cell Culture and Adenovirus Infection—**Pituitary tissues were excised from POMC-ins transgenic NOD mice (1), and single cell suspensions were prepared as described previously (16). Cells were cultured in RPMI 1640 medium with 10\% fetal bovine serum (Life Technologies, Inc.), 100 units/ml penicillin, and 100 \( \mu \)g/ml streptomycin. Cells were incubated at 37 \( \degree \)C, 5\% CO\(_2\) for 7–10 days prior to adenoviral infection. The multiplicity of infection (m.o.i.) was calculated based on estimates that the cell number doubled from the time of culture to the time of infection. An m.o.i. of 1 was used for all experiments unless otherwise indicated. Adenoviruses were added in a minimum volume of RPMI medium to cover the cells. After incubation at 37 \( \degree \)C for 2 h, the viruses were aspirated and the cells were cultured for 48 h.

**TUNEL Labeling and Double Immunofluorescence Microscopy—**Cells (2.5 \( \times \) 10\(^5\)/well) were grown in duplicate on SonicSeal chamber slides (NUNC) for 7–10 days. After adenovirus infection and 48-h culture in glucose-free RPMI containing various glucose concentrations (3, 10, or 20 mM glucose), cells were washed with phosphate-buffered saline and DNA strand breaks were detected with a commercially available kit (In Situ Cell Death Detection Kit, TRM red, Roche Molecular Biochemicals) following the manufacturer’s instructions. Fluorescence was monitored in a microplate reader. 0.5 ml/mouse of 0.5 mM ethidium homodimer in 0.01% methanol were added to the wells, and images were captured on an AxioCam charge-coupled device camera equipped with AxioVision 2.05 software (Zeiss). 

**Glucose Uptake—**Cells, cultured at 2 \( \times \) 10\(^6\) cells/well in 24-well plates, were incubated for 30 min with Hanks’ buffer containing 0.3, 3, or 20 mM glucose and 2 \( \mu \)Ci of \( D-[5-3H] \)glucose or \( D-[2-3H] \)glucose (PerkinElmer Life Sciences). Uptake was terminated by the addition of ice-cold phosphate-buffered saline containing 1 mM HgCl\(_2\) and measured as described previously (7).

**Glucose Phosphorylating Activity—**Cells were cultured at 1 \( \times \) 10\(^6\) cells/well in 6-well plates. After trypsinization and washing in glucose-free balanced buffer, glucose-buffered saline was incorporated on ice in buffer (6, 15). The supernatant fractions were assayed for glucose-phosphorylating activity in buffer containing 0.03–0.5 mM and 6–100 mM glucose by a fluorometric method (6, 15). The values obtained were extrapolated to a temperature of 37 \( \degree \)C assuming a Q_10 \(_{25}^2\) of 2. The \( K_m \) and \( V_{max} \) were calculated from a Hanes plot.

**Insulin Secretion Studies—**Cells, cultured in 96-well plates at 5 \( \times \) 10\(^5\) cells/well, were incubated in buffer containing 3 or 20 mM glucose for 15 min. Supernatants were centrifuged and assayed for rat insulin by radioimmunomassay (Linco Research). Cellular glucose content was measured after acid ethanol extraction. For perfusion studies, 2 \( \times \) 10\(^6\) \( \alpha \)-cells were cultured on a 10.5-mm polystyrene terephthalate membrane (Becton Dickinson) for 5–10 days. The membranes were then transferred into perfusion micro-chambers. Cells were perfused with modified balanced buffer (137 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM Mg\(_2\)Cl\(_2\), 0.31 mM Na\(_2\)PO\(_4\), 0.4 mM KHPO\(_4\), 5 mM Na\(_2\)HCO\(_3\), 20 mM Hepes, pH 7.4) with 0.5% (w/v) bovine serum albumin (Fraction V, Sigma Chemical Co.). At the end of the experiments, cells were solubilized in 0.1 N NaOH, and the protein content was determined with a Bio-Rad protein assay kit.

**Northern Blot Analysis—**Total RNA was extracted from mouse tissues and the MIN6 cell line (17) with TRIzol (Life Technologies, Inc.). Northern blot analysis was carried out as described previously (1). Blots were hybridized with random-primed [\( ^{32}P \)]dCTP radiolabeled probes encoding GLUT1, GLUT2, GLUT3, GK, insulin, and \( \alpha \)-actin, with stripping of the blots between hybridizations. The GLUT1 probe was generated by reverse transcription-polymerase chain reaction with mouse brain mRNA as a template with the forward primer 5'-ACCTGCAGAGATGAAAGAAGG-3' and the reverse primer 5'-TGGAGAGCTCCCTCAGGTC-3'. The probe, containing a sequence common to islet and liver GK mRNA (positions 587–1254), was generated from mouse liver RNA with the forward primer 5'-CTGTGAGCAGGAAGGAAACATC-3', corresponding to exon 5, and the reverse primer 5'-GCTTTCGCCATGGCATTGAC-3', corresponding to exon 9. The membranes were exposed to x-ray film at -80 \( \degree \)C for 2 to 5 days.
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RESULTS

Characterization of the Endogenous Metabolic Machinery in IL Pituitary Cells—Northern blot analysis (Fig. 1A) revealed that IL cells did not express detectable GLUT2 mRNA transcripts, but they expressed the low Km erythroid-brain glucose transporter, GLUT1, at levels similar to brain. Further stripping and re-probing this blot with a 660-bp DNA fragment containing part of the GK sequence that is common to both liver and islets showed that IL cells expressed a 2.8-kb transcript of similar size and abundance to that of anterior lobe (AL) pituitary and MIN6 cells, compared with the smaller transcripts, but they expressed the low Km form of glucokinase (AdGK) under the control of the CMV promoter. Western blot analysis showed that the transduction of primary pituitary cell cultures with either AdGK or AdGLUT2 resulted in extremely large increases in immunoreactive GK or GLUT2 proteins, respectively (Fig. 2A). Expression of Functional GLUT2 and GK Proteins—Because IL cells did not express GLUT2 or functional GK proteins, recombinant adenoviruses were constructed that contained the cDNA encoding GLUT2 (AdGLUT2) or the functional islet isoform of glucokinase (AdGK) under the control of the CMV promoter. Western blot analysis showed that the transduction of primary pituitary cell cultures with either AdGK or AdGLUT2 resulted in extremely large increases in immunoreactive GK or GLUT2 proteins, respectively (Fig. 2A).

Cells treated with AdGLUT2 showed a concentration-dependent increase in 3-O-methyl-D-glucose uptake, in contrast to cells treated with the control LacZ-containing adenovirus that only express GLUT1 (Fig. 2B). LacZ-infected IL cells showed low Km glucose phosphorylating activity (Km = 41 ± 3.5 m

Fig. 1. A, Northern blot analysis of liver, brain, lung, MIN6, anterior lobe pituitary (AL), and ILins cells. Ten micrograms of total cellular RNA was loaded for each sample, and the blot was sequentially hybridized to 32P-labeled GLUT1, GLUT2, glucokinase, insulin, and β-actin probes. B, representative perfusion of ILins cells demonstrating Ca2+-dependent insulin secretion. ILins cells were perfused at 0.5 ml/min with buffer containing 3 mM glucose and were sequentially stimulated with 10-min pulses (indicated by the bar) of buffer containing 50 mM KCl, 10 μM acetylcholine (ACh), and 100 μM ACh. Successive 1-min fractions were collected. C, recombinant adenoviruses infected ILins cells with high efficiency. Primary cultures of ILins cells were grown on chamber culture slides for 10 days and treated with adenovirus containing a hGH reporter gene. Indirect double-immunofluorescence staining with insulin (Ins) and human growth hormone (hGH) antibodies demonstrated colocalization of GH (green, right) and insulin (Cy3, left) staining in virtually all ILins cells.
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Fig. 2. A, immunoblots of lysates from pituitary cells infected with recombinant adenovirus. Note the different amounts of protein loaded. Lanes: 1, 4 = liver; 2, 5 = AdLacZ; 3 = AdGLUT2; 6 = AdGK. B, 3-O-methyl-D-glucose (3-OMG) uptake in pituitary cells infected with AdGLUT2 or control AdLacZ. Values represent the mean ± S.E. of three independent experiments performed in duplicates.

Fig. 3. Glucose usage in pituitary cells treated with AdLacZ, AdGLUT2, AdGK, or AdGLUT2 and AdGK. The data represent the mean ± S.E. of six independent experiments done in duplicates. *, p < 0.001 compared with LacZ-infected cells.

μM, V_max = 55 ± 5 nmol/min/mg of protein, n = 7 separate experiments), similar to non-infected parental IL cells. In contrast, IL cells infected with the GK-containing adenovirus exhibited high levels of functional GK activity (K_m = 8.5 ± 0.5 μM, V_max = 2.08 ± 0.13 nmol/min/mg of protein, n = 5 separate experiments), which comprised ~98% of the total glucose phosphorylating activity, comparing the phosphorylation rates at 100 and 0.5 mM glucose.

Glucose-sensing Capabilities in IL Cells Expressing GLUT2 and GK—To investigate the metabolic impact of expressing GLUT2 and GK individually and in combination, we measured the production of 3H2O from d-[5-3H]glucose at 0.3, 3, and 20 mM glucose. As seen in Fig. 3, cells expressing GLUT2 alone showed a small increase in glucose usage compared with LacZ-infected cells that was significant only at very low glucose concentrations (0.3 mM glucose; p < 0.001). In contrast, cells expressing GK alone showed a 2-fold increase in glucose usage from 3 to 20 mM glucose (p < 0.001), compared with LacZ-expressing cells in which usage in this physiological glucose concentration was unchanged. Glucose usage at 20 mM glucose was further augmented by the coexpression of GLUT2 and GK, compared with cells expressing GK alone (p < 0.001), but this was associated with a disproportionate increase in glycolytic flux at 3 mM glucose (p < 0.001), resulting in an apparent left shift in the glucose dose-response curve. Of note, the conversion of d-[2-3H]glucose to 3H2O, as a marker of in situ glucose phosphorylation activity, paralleled that of d-[5-3H]glucose. In particular, at 3 mM glucose, production of 3H2O from d-[5-3H]glucose was similar in cells expressing GLUT2, GK, or LacZ (3.03 ± 0.34, 2.39 ± 0.15, and 2.85 ± 0.14 nmol/min/mg of protein, respectively); whereas in GLUT2/GK-coexpressing cells 3H2O production was ~2-fold increased (5.19 ± 0.17 nmol/min/mg of protein, p < 0.001).

Impairment in Insulin Secretion and Loss of Cell Viability by Apoptosis in IL Cells Coexpressing GLUT2 and GK—To assess whether the increased glucose usage in metabolically engineered ILins cells was coupled to glucose-stimulated insulin secretion (GSIS), we examined insulin release in response to glucose challenge under static incubation conditions. As previously noted, IL cells lack K_ATP channels but secrete abundant amounts of insulin in response to agents that increase intracellular Ca^{2+} (Fig. 1B). In β cells, GSIS can occur in the absence of functional K_ATP channels (by the so-called “K_ATP-channel-independent pathway”) when intracellular Ca^{2+} is raised (25). However, ILins cells failed to respond to glucose even when these agents were provided. Instead, as seen in Fig. 4, the insulin secretion in cells that coexpressed GK and GLUT2 was severely reduced (45%) compared with control infected cells (p < 0.05). These findings raised the possibility that the coexpression of GLUT2 and GK might be having adverse effects on cell viability.

To visualize the fate of the virally infected cells, we constructed recombinant adenoviruses expressing green fluorescence protein (GFP) alone (AdGFP) or GFP fused in-frame to the carboxyl terminus of GK.B1 (AdGK-GFP). The activity of AdGK-GFP was identical to that of native AdGK virus by glucose phosphorylation and glucose usage assays (data not shown). To assess whether there was toxicity due to nonspecific effects of adenoviral infection or due to the specific combination of virus used, we infected cells with various combinations of viruses at equivalent m.o.i. As can be seen in Fig. 5, cells expressing GLUT2 alone showed a small increase in glucose usage compared with LacZ-infected cells that was significant only at very low glucose concentrations (0.3 mM glucose; p < 0.001). In contrast, cells expressing GK alone showed a 2-fold increase in glucose usage from 3 to 20 mM glucose (p < 0.001), compared with LacZ-expressing cells in which usage in this physiological glucose concentration was unchanged. Glucose usage at 20 mM glucose was further augmented by the coexpression of GLUT2 and GK, compared with cells expressing GK alone (p < 0.001), but this was associated with a disproportionate increase in glycolytic flux at 3 mM glucose (p < 0.001), resulting in an apparent left shift in the glucose dose-response curve. Of note, the conversion of d-[2-3H]glucose to 3H2O, as a marker of in situ glucose phosphorylation activity, paralleled that of d-[5-3H]glucose. In particular, at 3 mM glucose, production of 3H2O from d-[5-3H]glucose was similar in cells expressing GLUT2, GK, or LacZ (3.03 ± 0.34, 2.39 ± 0.15, and 2.85 ± 0.14 nmol/min/mg of protein, respectively); whereas in GLUT2/GK-coexpressing cells 3H2O production was ~2-fold increased (5.19 ± 0.17 nmol/min/mg of protein, p < 0.001).
that were coinfected with GFP/LacZ, GK-GFP/LacZ, or GFP/GLUT2-expressing adenoviruses showed equivalently bright fluorescence intensity with minimal evidence of cell damage. In marked contrast, cells that were coinfected with GLUT2/GK-GFP-containing adenoviruses exhibited severely reduced GFP fluorescence intensity, plasma membrane blebbing, and cell fragmentation, suggestive of apoptosis. These studies demonstrate that the cell toxicity observed was due to the specific coexpression of GLUT2 and GK.

In all the studies described so far, IL cells were cultured in RPMI 1640 medium (containing 11 mM glucose) for 48 h to allow for expression of the adenoviruses. To determine whether the loss of cell viability was glucose dose-dependent and to further characterize the mode of cell death, cells were infected and cultured for 48 h in glucose-free RPMI 1640 medium to which 3, 10, or 20 mM was added. Apoptosis was assessed by a TUNEL assay to identify DNA fragmentation. As shown in Fig. 5B, cells coexpressing control GFP/LacZ viruses were microscopically normal at all glucose concentrations, whereas cells coexpressing the GK-GFP/LacZ viruses showed some mild loss of cell viability, but this occurred only at high (20 mM) glucose concentrations. No significant TUNEL labeling was seen in GFP-positive cells with either of these virus combinations, even at high glucose concentrations. In contrast, the cells that were coinfected with GK-GFP/GLUT2-containing adenoviruses showed a glucose dose-dependent loss of cell viability by apoptosis. At 3 mM glucose, GK-GFP/GLUT2-expressing cells appeared relatively healthy with only occasional TUNEL-positive cells, but, at 10 mM glucose, there was severe loss of cell viability with decreased GFP fluorescence intensity and a dramatic increase in TUNEL-positive cells. These cells also appeared shrunken with plasma membrane blebbing and densely fragmented nuclei. The changes became further pronounced when cells were cultured at 20 mM glucose. In GK-GFP/GLUT2-expressing cells, the percentage of GFP/TUNEL-TMR double-labeled cells increased from 3.5% (3 mM glucose) to 33% (10 mM glucose) to 41% (20 mM glucose), compared with the GFP/LacZ and GK-GFP/LacZ cells that showed fewer than 1% double-labeled cells within this glucose range.

**Accumulation of Hexose Phosphates and Compromise of Cellular Energy Metabolism**—To examine the biochemical basis for the lack of GSIS and the loss of cell viability, cells were

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![Fig. 5. The coexpression of GLUT2 and GK in ILins cells results in severe glucose-dependent apoptotic cell loss. A, fluorescence micrographs of pituitary cells infected with various combinations of recombinant adenoviruses at equivalent m.o.i. and cultured for 48 h in RPMI 1640 containing 11 mM glucose. Note that only the GK-GFP/GLUT2-coexpressing cells showed evidence of significant toxicity. B, representative TUNEL labeling of pituitary cells expressing GFP/LacZ, GK-GFP/LacZ, or GK-GFP/GLUT2 that were cultured at different glucose concentrations for 48 h. Fluorescence micrographs of GFP (green) and TUNEL-TMR (red) imaged individually and merged (Merge) demonstrate glucose-induced apoptosis in the GK-GFP/GLUT2-infected cells. Because neither the GFP/LacZ- nor the GK-GFP/LacZ-infected cells showed detectable TUNEL labeling, only the merged image is presented. Arrows point to representative apoptotic cells.](http://www.jbc.org/)

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We demonstrate that ILins cells can be engineered to express extremely high levels of functional GK and GLUT2 proteins. The expression of GLUT2 alone enhanced glucose usage only at very low glucose concentrations (i.e., 0.3 mM glucose), similar to a previous report (26). In contrast, the single expression of GK in ILins cells increased glucose usage and lactate production in the physiological glucose range with a 2-fold increase from 3 to 20 mM glucose, relative to control cells (p < 0.001). These results are consistent with the known regulation of GK enzyme activity that occurs over the same glucose range. The coexpression of GLUT2 and GK further increased glucose usage at 20 mM glucose, but unexpectedly, this was accompanied by a leftward shift in the glucose dose-response curve with disproportionately greater glycolytic flux at 3 mM glucose. Despite the stimulation of glycolysis in these engineered cells, insulin secretion was reduced with a pronounced accumulation of hexose phosphates, depletion of ATP, and apoptotic cell death.

Why did glycolysis and energy production go awry in these metabolically engineered cells? One factor may relate to the inherent organization of the glycolytic pathway. The term “turbo design” has been coined to describe the organization of many catalytic pathways that begin with one or more ATP-consuming steps, after which further metabolism and ATP-generating reactions yield a net production of ATP across the pathway (27). Turbo design is exemplified in glycolysis, in which two molecules of ATP are initially invested at the hexokinase (HK) and phosphofructokinase (PFK) steps as a prelude to the net synthesis of four molecules of ATP further down the pathway. This design feature makes tight regulation of the enzymes involved in the initial ATP-consuming steps absolutely essential (27). In mammalian cells the activity of HK is inhibited by its product, glucose 6-phosphate (G6P). However, GK is not subject to such feedback inhibition. Therefore, when GK is expressed at high levels, there is the risk of uncontrolled accumulation of G6P, especially at high glucose levels. In addition, because G6P is in equilibrium with fructose 6-phosphate, the fructose 6-phosphate would be expected to rise and increase flux through PFK.

In these studies, the levels of F1,6BP were ~5-fold higher than those of G6P but otherwise showed similar patterns of accumulation. A number of factors may contribute to the preferential accumulation of F1,6BP. First, PFK would be expected to be activated allosterically by the consumption of its inhibitor ATP (and presumably, the corresponding rise in the PFK activator AMP) during glucose phosphorylation. Second, because the product of the PFK reaction, F1,6BP, is itself a potent allosteric activator of PFK, the rise in F1,6BP levels may be disproportionate to the increases in GAP and ATP.

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possible that these changes did not occur at a constant rate but that the rise in F1,6BP and loss of ATP occurred more acutely toward the end of the incubation period such that phosphate depletion did inhibit the flow through GAPDH (which uses P1 as a substrate) and the glycolytic ATP production. Regardless of the exact timing, it is likely that the sequestration of phosphate in G6P and F1,6BP accounts for the ATP depletion and loss of cell viability.

It has been suggested that the reason why GK is not hazardous to cell types where it is normally expressed, such as β cells and hepatocytes, is that in the former, GK activity is the lowest of the activities of all glycolytic enzymes and, as such, constitutes the “rate-limiting” step of glycolysis (29); whereas in hepatocytes, GK is subject to a number of regulatory influences, including the GK regulatory protein. Furthermore, hepatocytes have additional reactions (e.g., glucose cycling through glucose-6-phosphatase and glycogen synthesis) that may prevent the excessive accumulation of glycolytic intermediates (30).

A striking feature of the cells that coexpressed GLUT2 and GK was the leftward shift in their glucose-response threshold with a disproportionate increase in glycolytic flux at 3 mM glucose. These findings were unexpected in view of the high Km of GLUT2 and GK and could not be predicted from studying the metabolic impact of each gene individually. A simple explanation is that when GK is expressed alone, endogenous GLUT1 limits the flux of glucose through the cells; but when GLUT2 and GK are coexpressed, neither transport nor phosphorylation is rate-limiting. However, it seems unlikely that glucose transport is rate-limiting, because even in LacZ-infected cells uptake at 3 mM glucose was ~2-fold higher than the glucose usage in cells coexpressing GLUT2 and GK (10.2 ± 0.9 versus 5.6 ± 0.4 nmol/min/mg of protein, respectively). These data suggest that in this setting there may be a direct interaction between GLUT2 and GK (31) or, more likely, in combination they may affect the activities of other components involved in the upper part of the glycolytic pathway (32).

Similar metabolic perturbations, i.e., activation of the first steps of glycolysis with the accumulation of hexose phosphates and loss of ATP, have been reported in several other systems. These include Saccharomyces cerevisiae yeast mutants that are unable to synthesize the HK inhibitor trehalose-6-phosphate (27), trypanosomes in which glycolysis is not compartmentalized (33), healthy subjects who received high doses of parenteral fructose, which is phosphorylated by fructokinase that bypasses the regulated HK step (34), and the INS-1 β-cell line that was engineered to overexpress large amounts of GK (35, 36). In the latter setting, control of glycolysis at the GK step was also lost, with a marked increase in glucose usage at low (2.5 mM) glucose concentrations. These effects were attributed to high level GK expression alone (35), but our findings raise the possibility that the endogenous GLUT2 may have also adversely contributed to this phenotype.

Another remarkable feature of GLUT2- and GK-coexpressing cells was that, despite exhibiting an acute 80% drop in intracellular ATP levels at high glucose levels, these cells showed pronounced amounts of apoptotic cell death. These findings were unexpected, because apoptosis is an energy-requiring process and progression to necrotic or apoptotic cell death is thought to depend in part on the cellular ATP content, with rapid ATP depletion usually resulting in necrosis (37). These studies suggest that, although the ATP levels in GLUT2/GK-coexpressing cells are severely reduced, these ATP levels are still sufficient to complete the apoptotic program. Recent studies have suggested that glucose may induce apoptosis in pancreatic β cells (38, 39). Although caution must be used against extrapolating our data to islets, these findings suggest that GLUT2 and GK may be involved in a glucose signaling pathway that, when imbalanced, results in metabolic toxicity and apoptotic cell death.

It has been suggested that the simple introduction of glucose-sensing components into cells or cell lines may simulate the performance of normal islet β cells. These findings raise important caveats to this notion and demonstrate the deleterious effects that can result when the expression levels of the metabolic components are not closely optimized. We must emphasize that the metabolic toxicity described in this report was not due to the high level expression achieved with this particular viral gene delivery system. We have recently created a series of transgenic mouse lines that coexpress GLUT2 and GK in IL cells at levels that are markedly lower than those achieved with adenoviruses. Interestingly, the size of the IL tissues in these transgenic mice corresponded inversely to the levels of GK expression, with the most severe reduction in the size of the IL tissues in the lines that expressed the highest levels of GK.2 Although the mechanism by which glucose stimulates insulin secretion in β cells is complex and clearly requires more than just the expression of GLUT2 and GK, these transgenic tissues should provide an excellent starting material for determining the requirements for optimal glucose-sensing in surrogate β cells.

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