GLUT-2 Gene Transfer into Insulinoma Cells Confers Both Low and High Affinity Glucose-stimulated Insulin Release

RELATIONSHIP TO GLUCOKINASE ACTIVITY*

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The rat insulinoma cell line RIN 1046–38 loses glucose-stimulated insulin secretion as a function of time in culture. We found that the loss of glucose sensing in these cells was correlated with the loss of expression of GLUT-2 and glucokinase. Stable transfection of RIN cells with a plasmid containing the GLUT-2 cDNA conferred glucose-stimulated insulin release in intermediate but not high passage cells, with the near-maximal 3-fold increase occurring at 50 μM glucose. GLUT-2 expressing cells also exhibited a larger response to the combination of 5 mM glucose + 1 μM forskolin than untransfected cells (7.9 versus 2.7-fold, respectively). GLUT-2 expressing intermediate passage, but not high passage, RIN cells exhibited a 4-fold increase in glucokinase enzymatic activity relative to nonexpressing controls. Glucokinase activity was also increased by transfer of the GLUT-2 gene into intermediate passage RIN cells via recombinant adenovirus. Precipitation of GLUT-2 expressing intermediate passage RIN cells with 2-deoxyglucose to inhibit low Km hexokinases resulted in a glucose-stimulated insulin secretion response that was shifted toward the physiologic range. These studies indicate that GLUT-2 expression confers both a high and low affinity glucose-stimulated insulin secretion response to intermediate passage RIN cells.

Loss of glucose-stimulated insulin secretion is an early event in the etiology of both major forms of diabetes. Major emphasis is currently being placed on understanding the molecular and biochemical mechanisms involved in the glucose response. While many aspects of this pathway remain unresolved, it is clear that glucose metabolism is required in order to induce insulin secretion (1, 2). β-Cell glucose metabolism is controlled mainly by the high Km glucose-phosphorylating enzyme glucokinase (2), but an important permissive role for the high Km facilitated glucose transporter of islets known as GLUT-2 has also been suggested (3–5). In fact, the decline in β-cell glucose responsiveness that is characteristic of a number of rodent models of non-insulin-dependent diabetes mellitus has been correlated with reduced expression of GLUT-2 in several laboratories (reviewed in Ref. 4).

Our recent work has focused on the effects of overexpression of glucose transporters in cell lines that are insulin secreting but lack glucose sensing (5–8). One model system that we have studied is the AtT-20ins cell line, which is derived from ACTH-secreting cells of the anterior pituitary and engineered for human insulin secretion by stable transfection with a viral promoter/human proinsulin cDNA construct (9). Stable transfection of AtT-20ins cells with GLUT-2 but not GLUT-1 confers glucose-stimulated insulin secretion, albeit with maximal effect at subphysiological glucose concentrations (7, 8). The left-shifted glucose dose-response curve may be explained by the markedly increased hexokinase:glucokinase ratio in these cells relative to normal islet β-cells.

While studies on non-islet cell lines can provide important mechanistic insights, a full appreciation of the impact of expression of glucose transporters and/or glucose phosphorylating enzymes on fuel-mediated insulin release requires studies of cell lines derived from β-cells. Various rodent insulinoma cell lines are available that are derived from β-cell tumors induced by x-irradiation of rats (10) or by insulin promoter-driven T-antigen expression in transgenic mice (11, 12). Such cell lines often have attenuated or absent glucose-stimulated insulin secretory responses, and when present, the glucose effect is generally maximal at subphysiological concentrations of the sugar. The loss of glucose response seems to be further exacerbated by time in culture. This phenomenon is clearly illustrated by the rat insulinoma cell line RIN 1046–38, which when studied after a short period of time in culture (passages 6–17) exhibits a 5-fold stimulation of insulin release in response to glucose, albeit with maximal effects observed at 0.5 mM glucose (13). At higher passage numbers (≥50), all glucose-stimulated insulin secretion is lost (13).

In the current study we show that the levels of both GLUT-2 and glucokinase are sharply reduced in RIN 1046–38 cells that have lost their glucose response as a function of time in culture. This observation led us to study the effects of restoring GLUT-2 and/or glucokinase expression to high levels in RIN cells. We show that expression of GLUT-2 at levels that approximate those found in normal islet β-cells confers glucose-stimulated...
insulin secretion at both physiologic and supphysiologic concentrations of the sugar and that this effect may be related to GLUT-2-mediated alterations in glucokinase enzymatic activity.

MATERIALS AND METHODS

Cell Culture—RIN 1046–38 cells were grown in Dulbecco's modified Eagle's medium containing 25 mM glucose, and supplemented with 10% fetal calf serum (Mediatech, Washington, D.C.), 100 milliliters of penicillin, and 100 μg of streptomycin/ml (Life Technologies, Inc.). Cells were grown in 10% CO₂ in a 37 °C incubator. Cells were detached weekly using 0.05% trypsin–EDTA solution (Life Technologies, Inc.) and kept under an atmosphere of 95% air and 5% CO₂ at 37 °C.

Stable Transfection of Cell Lines with GLUT2 or Glucokinase—At various passage numbers were transfected by electroporation with the pCB-7 vector containing the rat GLUT-2 cDNA (3, 6) or the rat islet glucokinase cDNA (3, 6). The transfection conditions were as previously described for AT-20ins cells (7, 8). Stable transfectants were selected by isolation of individual colonies in the presence of 150 μg/ml hygromycin (Calbiochem).

Transfer of the GLUT2 and Glucokinase Genes into RIN Cells with Recombinant Adenovirus—Recombinant adenoviruses were prepared by insertion of the rat GLUT-2 (14) or human glucokinase (Ref. 15; a gift from Dr. Graeme Bell, University of Chicago) cDNAs into the pACCMVP LpA vector adjacent to the cytomegalovirus early promoter. Viruses containing the GLUT-2 or islet glucokinase cDNAs (termed AdCMV-GLUT2 and AdCMV-GKI, respectively) were then delivered to transfection of the relevant pACCMVP LpA plasmids and the adenovirus vector pCMV17 as described in detail elsewhere (16, 17). RIN 1046–38 cells of intermediate passage number (passages 35–37) were plated in 6-well dishes at a density of approximately 4.0 × 10⁶ cells/well. Viral infection was carried out by exposing the cells to approximately 1 × 10⁷ particle-forming units of the AdCMV-GLUT2 or AdCMV-GKI viruses for 1 h in a final volume of 600 μl of medium. Cells were washed twice with phosphate-buffered saline (PBS) and then cultured in RPMI 1640 medium containing 11 mM glucose for 24–48 h. The medium was changed to RPMI 1640 containing 1 μM glucose 24 h prior to studies on glucokinase activity. The efficiency of gene transfer was estimated by incubating RIN cells with similar amounts of the AdCMV-βGal virus (18), which contains a nuclear localized variant of the Escherichia coli β-galactosidase gene. Expression of β-galactosidase was scored by fixing cells for 10 min in 0.5% glutaraldehyde in PBS, washing with PBS, and then incubating for 30 min in 35 mM K₄Fe(CN)₆/35 mM K₅Fe(CN)₆, 2 mM MgCl₂, and 1 mg/ml X-gal (5-bromo-4-chloro-3-indolyl β-D-galactosidaseonanase).

Blot Hybridization Analysis—Poly(A) RNA was prepared by the method of Ashley and MacDonald (19), resolved on formaldehyde/agarose gels, and transferred to nylon membranes (Micron Separations Inc.) as previously described (6–8). RNA blot hybridization analysis was carried out with [32P]-labeled antisense cDNA probes for GLUT-1, glucokinase, insulin, or β-actin probes (6–8). The membrane was prepared for each new hybridization by boiling in 0.1% SDS for 20 min.

GLUT2 Immunofluorescence—RIN cells were attached to coverslips, fixed, and permeabilized as previously described for AT-20ins cells (7, 8). Immunofluorescent staining of GLUT2 was carried out using a 1:2500 dilution of a commercial antibody preparation (East Acres Bio-Corp.) directed at the rat GLUT-2 C-terminal hexadecapeptide.

Glucose Transport Measurements—Cells were grown in DMEM supplemented with 10% fetal calf serum for 3 days prior to secretion experiments. Cells were washed in 6- or 12-well dishes with cells at 70–80% confluence. The cells were washed twice for 15 min in HEPES/bicarbonate-buffered salt solution with 0.1% bovine serum albumin that had been equilibrated at 37 °C with 95% O₂, 5% CO₂. The concentration of glucose in these washing solutions was 11.0 mM. RIN cells were incubated for 90 min with 60–90 min in HBSS supplemented with varying concentrations of glucose or glucose and forskolin as indicated in the figure legends. Insulin secretion was measured by radioimmun assay of the media, and data were normalized to the total cellular protein in each secretion well. In some experiments, the effects of 2-deoxyglucose on the dose-response characteristics of the adenovirus–transfected cells were assayed by preincubating cells for 30 min with 50 μM 2-deoxyglucose and 1 μM β-glucagon, followed by a brief washing of the cells with HEPES/bicarbonate and exposure to a range of glucose concentrations.

Glucokinase Enzymatic Activity—After removal of media and two washes with PBS, cells were extracted by homogenization in 300 μl of buffer/plate containing 50 mM HEPES pH 7.8, 40 mM KCl, 11 mM MgCl₂, 1 mM EDTA, and 1 mM dithiothreitol. The homogenate was cleared in a microcentrifuge, and the supernatant was used for enzymatic assays. Assays were performed essentially as described by Kuwajima et al. (21) with some modifications. The assay medium contained 50 mM unlabeled glucose, 5 μCi of [U-14C]glucose, and 30 μl of cell extract (about 60 μg of protein). Glucokinase activity was discriminated from hexokinase activity by performing the reactions in the presence of 10 mM 6-phosphate. Reactions were allowed to run for 90 min at 37 °C and terminated by the addition of 3% methanol in 95% ethanol. 30 μl of the mix were then applied to DEAE-cellulose paper discs (Schel- cher and Schellu), which after drying were washed 5 times for 20 min each and then overnight in distilled water. The radioactivity retained on the disc was detected by liquid scintillation counting after the addition of 10 ml of BioSafe II mixture (Research Products International Corp., Mount Prospect, IL). Results were expressed as micromoles/min/g of total protein.

Glucokinase Immunoblot—Subconfluent (approximately 70% confluence) cells from passage 30 (high passage) or passage 80 (low passage) RIN cells were infected with the AdCMV-βGal, AdCMV-GKI, or AdCMV-GLUT2 viruses as described above. Cells were collected and homogenized in the same buffer utilized for glucokinase enzymatic activity 72 h after viral infection. Aliquots containing 75 μg of protein were resolved on an 8% SDS-polyacrylamide gel. Samples were transferred to membrane and treated with immunopurified anti-glucokinase antibody U343, as previously described (22). The glucokinase-primary antibody complex was visualized on film with horseradish peroxidase goat anti-rabbit IgG and luminescent detection reagents (ECL kit, Amersham Corp.).

RESULTS

Expression of Insulin, Glucokinase, and GLUT2 mRNAs in RIN 1046–38 Cells as a Function of Time in Culture—Steady-state insulin, glucokinase, and GLUT2 mRNA levels were evaluated in RIN 1046–38 cells of low or high passage number (17 passages versus 30 passages, respectively). As seen in Fig. 1, insulin mRNA levels were substantially decreased in high passage RIN cells as compared with low passage RIN cells, while the mature glucokinase and GLUT2 transcripts were reduced to nearly undetectable levels. Levels of the GLUT1 erythroid/brian glucose transporter or actin mRNAs, in contrast, were unchanged within culture. The decreases observed in GLUT-2, glucokinase, and insulin transcripts were consistent with a previous report showing a lower insulin content and loss of glucose-stimulated insulin release in high passage RIN 1046–38 cells (13).

Expression of GLUT2 mRNA and Protein in Transfected and Adenovirus-infected RIN 1046–38 Cells—Steady-state GLUT2 mRNA levels were evaluated in 11 independent GLUT2-transfected RIN cell clones. Five of the 11 hygromycin-resistant clones contained substantial levels of GLUT2 mRNA derived from the transfected cDNA, which can be distinguished from the endogenous transcript by virtue of its smaller size (Refs. 7 and 8; data not shown). GLUT2 immunofluorescence was easily detectable in all of the cell lines in which GLUT2 mRNA was increased by transfection. Exposure of intermediate passage RIN cells to recombinant adenovirus containing the GLUT2 cDNA also resulted in high levels of expression of this transporter. Essentially 100% of the transfected or AdCMV/GLUT2-infected cells exhibited immunofluorescent staining at the plasma membrane (Fig. 2, A and B). No membrane-associated GLUT2 immunofluorescence was evident in untransfected intermediate passage RIN cells (Fig. 2C) or in RIN cells that had been infected
GLUT-2 Expression in Insulinoma Cells

**Fig. 1. Steady-state levels of various mRNA species in RIN 1046–38 cells as a function of time in culture.** Poly(A)"""" RNA was prepared from RIN 1046–38 cells after 17 passages (P17) or 69 passages (P69) and from normal rat liver. Ten μg of each of the insulinoma samples and 5 μg of the liver sample were electrophoretically resolved, blotted, and hybridized with the ^32P-labeled cDNA probes indicated to the right of each panel with stripping of the blot between hybridizations.

with recombinant adenovirus containing a nuclear localizing variant of the bacterial β-galactosidase gene (AdCMV-βGal), which served as a control for the effects of adenovirus infection per se. Fig. 2E underscores the efficiency of gene transfer with recombinant adenovirus since nearly all of the cells treated with the AdCMV-βGal virus express the β-galactosidase gene, as judged by the numbers of blue nuclei, while untreated cells exhibit no blue color in the presence of the β-galactosidase chromogenic substrate (Fig. 2F).

**Glucose Transport Activity**—Table I summarizes the kinetic constants for 3-O-methylglucose uptake in parental and GLUT-2-transfected RIN cells. Cells of intermediate passage number (RIN 30) had a K_m for glucose of 4 mM and a V_max of 6 mmol/min/liter of cell space. High passage cells (RIN 100) express a single concentration of 3-O-methylglucose (4 mM). As seen in Fig. 3, 3-O-methylglucose uptake was enhanced to a similar extent by stable transfection or by adenovirus-mediated gene transfer of the GLUT-2 cDNA into RIN cells relative to cells in which GLUT-2 expression was not manipulated. These data prove that the virus allows rapid and highly efficient transfer of GLUT-2 activity into the cell lines under study, thus allowing evaluation of the effects of GLUT-2 by an approach that is independent of clonal selection.

**The Effect of Stable Transfection of the GLUT-2 Gene on Glucose-stimulated Insulin Secretion and Glucokinase Activity in**

**RIN 1046–38 Cells**—Glucose-stimulated insulin secretion was evaluated in intermediate and high passage RIN cells with or without stable transfection with the GLUT-2 cDNA. As shown in Fig. 4A, neither intermediate nor high passage insulinoma cells exhibited any glucose-stimulated insulin secretory response. Restoration of GLUT-2 expression in intermediate passage insulinoma cells consistently conferred glucose-stimulated insulin release, with an average stimulated release that was 170% greater than basal insulin release observed in the absence of glucose (data are summarized for four independent GLUT-2 transfected lines). In contrast, GLUT-2 expression did not restore glucose-stimulated insulin release to high passage cells, indicating that these cells must lose components of the stimulus-secretion coupling machinery with time in culture that is not affected by GLUT-2 expression.

A number of recent studies have suggested that glucose at high concentrations causes increases in glucokinase enzymatic activity in isolated islets and parallel increases in the capacity for glucose-stimulated insulin release (23–25). In light of these data, we were interested in determining whether enhanced GLUT-2 expression might affect glucokinase activity in RIN cells. As can be seen in Fig. 4B, glucokinase activity correlates with the capacity for glucose-stimulated insulin release in the lines mentioned above. Thus, the glucose-responsive GLUT-2-transfected intermediate passage RIN cells exhibit a 4-fold increase in glucokinase activity compared with GLUT-2-transfected high passage cells or intermediate or high passage cells that do not express the GLUT-2 cDNA. As was the case for insulin secretion, enhanced glucokinase activity was consistently observed in four independent GLUT-2 transfected intermediate passage clones.

**The Effect of Adenovirus-mediated Transfer of the GLUT-2 Gene on Glucokinase Activity**—We have previously reported that GLUT-2 transfection of AtT-20ins cells confers a glucose-stimulated insulin secretion response (7, 8) but that such a maneuver has no significant effect upon glucokinase activity in these cells (7). In light of this difference between RIN and AtT-20ins cells and in order to be certain that the increased enzyme activity observed in RIN cells was not due to clonal selection, the AdCMV-GLUT2 recombinant adenovirus was used to transfer GLUT-2 into intermediate and high passage RIN cells. As shown in Fig. 5, glucokinase enzymatic activity was found to increase in intermediate passage insulinoma cells infected with the AdCMV-GLUT2 virus in a time-dependent manner, with a 37% increase in glucokinase activity 48 h after infection and a 72% increase at 72 h (the latter representing a statistically significant enhancement relative to the uninfected control cells (p < 0.01) and AdCMV-βGal-infected cells (p < 0.05)). The increase in glucokinase activity in cells infected with AdCMV-GLUT2 virus appeared to be accounted for by an increase in immunodetectable glucokinase protein, as indicated in the representative Western blot shown in Fig. 6. Densitometric scanning revealed an average 2.6-fold increase in glucokinase protein in AdCMV-GLUT2-infected cells relative to uninfected cells (three experiments). In contrast to the clear effect of AdCMV-GLUT2 treatment of intermediate passage cells, no change in glucokinase activity was observed in similarly infected high passage cells (passage 80, data not shown).

**GLUT-2 Expression Confers Both High and Low Affinity Glucose-stimulated Insulin Secretion in Insulinoma Cells of Intermediate Passage**—The fact that GLUT-2 expression increases glucokinase enzymatic activity in RIN cells suggested to us that the threshold for glucose-induced insulin release might also be affected. To test this possibility, we measured insulin release in response to glucose over the range of 0.01–20 mM. As shown in Fig. 7A, untransfected intermediate or high passage RIN cells failed to release insulin in response to any concen-
GLUT-2 Expression in Insulinoma Cells

**Fig. 2. GLUT-2 immunofluorescence in RIN 1046-38 cells.** Panel A, line 30/10, stably transfected with the pCB-7 plasmid containing the GLUT-2 cDNA; panel B, intermediate passage cells (passage 30) 48 h after infection with the AdCMV-GLUT2 recombinant adenovirus; panel C, parental (uninfected and untransfected) intermediate passage cells (passage 30); panel D, intermediate passage cells (passage 30) 48 h after infection with the AdCMV-βGal virus. Cells in panels A–D were treated with the anti-GLUT-2 antibody as described under “Materials and Methods.” Panel E, intermediate passage cells (passage 30) 48 h after infection with the AdCMV-βGal virus; panel F, uninfected intermediate passage cells (passage 30). Cells in panels E and F were incubated with the β-galactosidase chromogenic solution, as described under “Materials and Methods.”

**Table I**

<table>
<thead>
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<th>Cell line</th>
<th>$K_m$ for glucose uptake</th>
<th>$V_{max}$</th>
<th>mmol/min/liter</th>
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<td>6</td>
<td></td>
</tr>
<tr>
<td>RIN 30/7*</td>
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<td>25</td>
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<tr>
<td>RIN 30/10*</td>
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<td>35</td>
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<td>RIN 100/1*</td>
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<td>RIN 100/2*</td>
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<td>33</td>
<td></td>
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<tr>
<td>RIN 100/6*</td>
<td>25</td>
<td>37</td>
<td></td>
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<tr>
<td>Normal rat islets*</td>
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<td>24</td>
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</table>

Data for 3-O-methylglucose uptake into normal rat islets are shown for comparison and are taken from Ref. 17.

Table I

Measurements of 3-O-methylglucose uptake were performed as described previously (15). Kinetic parameters were calculated using double reciprocal plots of initial rates from two or three independent experiments. The asterisk indicates GLUT-2-transfected cell lines. Measurements of 3-O-methylglucose uptake were performed as described previously (15). Kinetic parameters were calculated using double reciprocal plots of initial rates from two or three independent experiments. The asterisk indicates GLUT-2-transfected cell lines. Measurements of 3-O-methylglucose uptake were performed as described previously (15). Kinetic parameters were calculated using double reciprocal plots of initial rates from two or three independent experiments. The asterisk indicates GLUT-2-transfected cell lines.

The relatively poor response of these cells to glucose and forskolin is not indicative of a generalized deterioration of the regulated secretory pathway in higher passage cells since larger responses were observed upon exposure of these cells to amino acids (arginine and glycine, 9-fold stimulation) or glyceraldehyde and glucose (3-fold) (data not shown). GLUT-2-transfected intermediate passage RIN lines exhibited a clear glucose-stimulated insulin secretory response, with the near maximal 2.7-fold increase in release observed at 50 μM glucose (Fig. 7B). There was an additional increase in insulin release observed at glucose concentrations of 5 mM or greater, but while this was seen in two independent cell lines, it was not statistically significant. The combination of glucose and forskolin induced a 7.9-fold increase in insulin release compared with cells incubated in the absence of either secretagogue. Thus, GLUT-2 transfection restored the glucose-stimulated secretory response characteristic of low passage insulinoma cells (13) and
conferred a glucose-potentiating effect on forskolin-induced insulin release.

We (5, 7, 8) and others (26–29) have postulated that the response of insulinoma or GLUT-2-expressing AtT-20ins cells to subphysiologic concentrations of glucose ("high affinity glucose response") is due to an imbalance in the hexokinase: glucokinase ratio in favor of hexokinase, such that glucose metabolism and generation of secretory signals are maximal at subphysiological glucose concentrations. Despite a marked elevation in glucokinase activity in GLUT-2-transfected RIN cells, maximal insulin release still occurs at glucose concentrations that are well below the threshold required for stimulation of the normal islet β-cell (≥5 mM). We reasoned that the concentration dependence of glucose-stimulated insulin release might be normalized to resemble that of the islet if hexokinase activity could be inhibited. In order to test this hypothesis, we preincubated GLUT-2-transfected intermediate passage RIN cells with 50 mM 2-deoxyglucose. Cells were exposed to the glucose analog for 30 min prior to analysis of their secretory response to glucose in order to allow 2-deoxyglucose 6-phosphate, an inhibitor of hexokinase but not glucokinase, to accumulate in the cells. As shown in Fig. 7C, pretreatment of cells with 2-deoxyglucose resulted in a glucose dose-response curve that more closely resembles the "low affinity" curve of the normal β-cell, with a shift in maximal response of 2 orders of magnitude (5 mM glucose rather than 50 μM glucose as observed in untreated cells). Thus, GLUT-2 expression confers both low and high affinity glucose-stimulated insulin secretion in intermediate passage RIN cells.

**DISCUSSION**

In this study we have investigated the role of GLUT-2 and glucokinase in glucose-stimulated insulin secretion in RIN 1046–38 cells. In addition to establishing that passage-dependent disappearance of glucose-stimulated insulin release is correlated with reduced expression of these two gene products, we show that restoration of GLUT-2 expression in intermediate
transfection or by recombinant adenovirus. Interestingly, the effects of GLUT-2 transfection on glucose-stimulated insulin secretion and glucokinase activity are confined to cells of low or intermediate passage number, since overexpression of GLUT-2 in high passage cells has no effect on either function.

Our data suggest that increases in glucokinase activity may mediate the glucose-sensing function that is conferred in GLUT-2-expressing RIN cells. It is as yet unclear whether increases in glucokinase activity alone are sufficient to impart enhanced glucose responsiveness. On the one hand, we have transfected intermediate passage RIN cells with a plasmid containing the cDNA encoding the islet isoform of glucokinase. One clone has been isolated to date (GK-8) that expresses high levels of glucokinase activity and that responds to glucose. The magnitude of the glucose effect in GK-8 cells, however, is less than that seen in GLUT-2-transfected clones (maximum of 2-fold versus 3.8-fold, respectively), and the GK-8 clone also lacks the glucose potentiation of forskolin-induced secretion that is found in GLUT-2-expressing lines (data not shown). On the other hand, transfer of the islet glucokinase cDNA into DNA cells via recombinant adenovirus causes a large increase in immunodetectable glucokinase protein (Fig. 6) and an 11-fold increase in glucokinase activity but does not confer a measurable enhancement in glucose-stimulated insulin secretion.\(^2\) In light of these observations, it seems that overexpression of glucokinase without GLUT-2 expression has little if any effect on glucose responsiveness in insulinoma cells. In a previous study, we have demonstrated that transfection of AtT-20ins cells with GLUT-2 but not GLUT-1 confers glucose-stimulated insulin secretion, despite equal rates of glucose usage in the GLUT-2- and GLUT-1-transfected cells (8). We interpreted this finding to mean that GLUT-2 may have particular structural features, which are not shared with GLUT-1, that mediate glucose sensing. The data of the current study are also consistent with an important facilitative role for GLUT-2 in glucose sensing. Further support is gained from work with insulinoma cell lines prepared from islet \(\beta\)-cell tumors induced by insulin promoter-directed T-antigen expression in transgenic mice. Lines that retained GLUT-2 expression in culture were found to be glucose-responsive, while other lines with predominant expression of GLUT-1 failed to respond to glucose as a secretagogue (12). It is important to point out that our data do not necessarily imply that overexpression of glucokinase will be without effect in normal islets, where GLUT-2 is naturally expressed and glucokinase is a rate-determining step for glycolysis (2).

Our study also provides the first insight into the relationship between GLUT-2 expression and glucokinase activity. Adenovirus-mediated transfer of GLUT-2 increases glucokinase activity by approximately 75%. Expression of GLUT-2 by stable transfection caused a more pronounced 4-fold increase in glucokinase activity. Because adenovirus integrates into genomic DNA with very limited efficiency (30), viral DNA is lost over time in rapidly replicating cells, thereby limiting the duration of experiments to 72 h or less after each infection. Thus, the more obviously enhanced expression of glucokinase in the stably transfected RIN cells could be due to the longer duration of expression of the GLUT-2 gene in these cells relative to the shorter adenovirus experiments, suggesting that GLUT-2 expression enhances glucokinase enzyme activity through a gradual process. At least part of the effect of GLUT-2 seems to reside in an increase in the amount of glucokinase protein, as shown in Fig. 6. A topic for future studies is to determine whether the full inductive effect also involves post-transla-

tional regulatory steps. Evidence for this type of regulation of islet glucokinase activity has been accumulating. Fasting and refeeding of rats, for example, results in significant changes in glucokinase enzymatic activity in islets (31) in the absence of any changes in glucokinase mRNA or protein levels (32). Furthermore, recent work has shown that exposure of isolated islets to varying glucose levels (23–25) or glucose infusion into intact animals (33) causes changes in glucokinase activity that are not fully accounted for by changes in glucokinase mRNA and/or immunodetectable glucokinase protein. The search for the mechanistic link between GLUT-2 expression and glucokinase activity in intact animals (33) causes changes in glucokinase activity that islet glucokinase enzymatic activity in islets (31) in the absence of any changes in glucokinase mRNA or protein levels (32).

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