Glucose is thought to stimulate insulin release from islet β-cells through generation of metabolic signals. In the current study we have introduced the genes encoding the facilitated glucose transporters known as GLUT-1 and GLUT-2 into AtT-20ins cells to assess their impact on glucose-stimulated insulin release and glucose metabolism. We find that transfection of AtT-20ins cells with GLUT-2, but not GLUT-1, confers glucose-stimulated insulin release in both static incubation and perifusion studies. Cells transfected with GLUT-1 have a $K_m$ for 3-O-methyl glucose uptake of 4 mM and a $V_{max}$ of 5–6 mmol/min/liter cell space. These values are increased compared to untransfected AtT-20ins cells ($K_m = 2$ mm; $V_{max} = 0.5$ mmol/min/liter cell space), but are less than observed in GLUT-2-transfected lines ($K_m = 16–17$ mm; $V_{max} = 17–25$ mmol/min/liter cell space). Despite these dramatic differences in glucose transport affinity and capacity, the rates of [5-$^3$H]glucose usage are not different in the control and transfected lines over a range of glucose concentrations from 10 μM to 20 mM. We conclude that the specific effect of GLUT-2 on glucose-stimulated insulin release in AtT-20ins cells is not related to changes in the overall rate of glucose metabolism and may instead involve physical coupling of GLUT-2 with cellular proteins and/or structures involved in glucose signaling.

Glucose metabolism appears to be required for glucose-stimulated insulin release from pancreatic islet β-cells. The control of glucose metabolism in β-cells is thought to reside mainly at the level of glucokinase-catalyzed phosphorylation of glucose (reviewed in Ref. 1), but an important permissive role for the low affinity facilitated glucose transporter known as GLUT-2 has also been suggested (2–5). In recent studies, we have been investigating the utility of the non-islet cell line AtT-20ins for studies of the specific roles of GLUT-2 and glucokinase in the control of glucose-stimulated insulin release (reviewed in Ref. 5). These cells are derived from corticotropin-secreting cells of the anterior pituitary and have been engineered for constitutive expression of human insulin (6). We established that the islet isoform of glucokinase is naturally expressed in AtT-20ins cells and were able to detect low levels of its enzymatic activity (20–30% of the activity in normal islets), but also showed that the cells lack natural expression of GLUT-2 and fail to respond to glucose (7). Upon stable transfection with GLUT-2, AtT-20ins cells exhibit increased insulin content, glucose potentiation of non-glucose secretagogues, and a direct stimulation of insulin release by glucose, albeit with maximal effect at subphysiological concentrations of the sugar (8). We concluded that GLUT-2 expression in this endocrine cell line allowed glucose-regulated hormone release and that the response to subphysiological levels of glucose (maximal at 10–50 μM) was consistent with the relative predominance of low $K_m$, hexokinase activity in these cells.

The current work was directed at determining whether glucose-stimulated insulin secretion can be conferred in AtT-20ins cells by overexpression of GLUT-1, an alternate member of the family of facilitated glucose transporters. Glucose-stimulated insulin secretion was measured in multiple GLUT-1- and GLUT-2-transfected AtT-20ins cell lines both by static incubation and perifusion techniques, thereby allowing a detailed evaluation of the magnitude and dynamics of any glucose-induced responses. By measuring 5-$^3$H]glucose usage in GLUT-1- versus GLUT-2-transfected lines, we have also attempted to dissect the relative importance of glycolytic flux versus transporter isoform expression per se in mediating glucose sensing.

**MATERIALS AND METHODS**

**Stable Transfection of AtT-20ins Cells with GLUT-2**—A 1.8-kilobase Sulf fragment of the cDNA encoding human GLUT-1 (a gift from Dr. Graeme Bell, University of Chicago) was subcloned into the vector pCB-7 immediately downstream of its cytomegalovirus promoter. The pCB-7 vector also contains a hygromycin resistance marker. The cloning process removed 660 bp from the 3′-untranslated region and 84 bp from the 5′-untranslated region of the GLUT-1 cDNA. AtT-20ins cells were transfected with this construct using electroporation as described previously (8). 15 stable transfurants were isolated, grown for several passages in media containing 125 μg/ml of hygromycin B (Boehringer Mannheim), and analyzed for GLUT-1 expression. As a control, cells were also selected with hygromycin following transfection with the pCB-7 vector lacking a GLUT insert.

Analysis of GLUT-1 and GLUT-2 Expression—RNA blot hybridization analysis was carried out as described (9). Blots were hybridized with $^{32}$P-labeled antisense cRNA probes for GLUT-1 (7), GLUT-2 (8), or insulin (9), a cDNA probe for proopiomelanocortin (POMC) (10).

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Steven D. Hughes†, Christian Quaade‡, John H. Johnson§, Sarah Ferber‡, and Christopher B. Newgard†‡

From the SGifford Laboratories for Diabetes Research and the Departments of Biochemistry and Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75335 and the Veterans Affairs Medical Center, Dallas, Texas 75216

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† To whom correspondence should be addressed: Gifford Laboratories for Diabetes Research & Dept. of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235. Tel.: 214-688-2930; Fax: 214-688-8281.

‡ To whom correspondence should be addressed: Gifford Laboratories for Diabetes Research & Dept. of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235. Tel.: 214-688-2930; Fax: 214-688-8281.
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Transfection of At-T20i, cells with the cytomegalovirus promoter/human GLUT-1 cDNA construct yielded 15 hygromycin-resistant clones. These were evaluated for GLUT-1 expression by blot hybridization analysis using a radiolabeled GLUT-1 antisense RNA probe. Fig. 1A shows steady-state levels of GLUT-1 mRNA in five representative clones. Controls for the specific effects of GLUT-1 transfection included lanes containing RNA from the parental At-T20i, cell line, the previously described GLUT-2 expressing cell line CGT-6 (8), and a rat insulinoma cell line RIN1046-38 (15). These three control cell lines all contained low to moderate levels of a 2.8-kilobase transcript corresponding to GLUT-1; the

Results

Fig. 1. RNA blot hybridization analysis of GLUT-1- and GLUT-2-transfected At-T20i, cell lines. Panel A, total RNA was prepared from five GLUT-1-transfected lines (designated 1-2, 1-5, 1-8, 1-10, and 1-12), from GLUT-2-transfected CGT-6 cells, rat insulinoma (RIN) 1046-38 cells, and untransfected At-T20i, cells (AtT). 10 µg of total RNA was loaded/lane, and blot hybridization was carried out sequentially with the antisense RNA or oligonucleotide probes indicated to the left of the panel, as described under "Materials and Methods." Panel B, total RNA was prepared from six GLUT-2-transfected lines (designated 2-4, 2-8, 2-9, 2-12, 2-14, and 2-17), from the previously described GLUT-2-transfected line CGT-6, untransfected At-T20i, cells (AtT), 10 µg of total RNA was loaded per lane, and blot hybridization was carried out sequentially with the antisense RNA or oligonucleotide probes indicated to the left of the panel, as described under "Materials and Methods." Note, the increased 18 S rRNA signal in panel B relative to panel A is consistent with the fact that more RNA was loaded for this blot.

PROBES

GLUT-1

POMC

Insulin

18S rRNA

B.

GLUT-2

POMC

18S rRNA

A.

PROBES

CGT-6 RIN AtT 1-2 1-5 1-8 1-10 1-12

2-4 2-8 2-9 2-12 2-14 2-17 CGT-6 AtT 1-8

CGT-6 RIN AtT 1-2 1-5 1-8 1-10 1-12

2-4 2-8 2-9 2-12 2-14 2-17 CGT-6 AtT 1-8

Panel A, total RNA was prepared from five GLUT-1-transfected lines (designated 1-2, 1-5, 1-8, 1-10, and 1-12), from GLUT-2-transfected CGT-6 cells, rat insulinoma (RIN) 1046-38 cells, and untransfected At-T20i, cells (AtT). 10 µg of total RNA was loaded/lane, and blot hybridization was carried out sequentially with the antisense RNA or oligonucleotide probes indicated to the left of the panel, as described under "Materials and Methods." Panel B, total RNA was prepared from six GLUT-2-transfected lines (designated 2-4, 2-8, 2-9, 2-12, 2-14, and 2-17), from the previously described GLUT-2-transfected line CGT-6, untransfected At-T20i, cells (AtT), 10 µg of total RNA was loaded per lane, and blot hybridization was carried out sequentially with the antisense RNA or oligonucleotide probes indicated to the left of the panel, as described under "Materials and Methods." Note, the increased 18 S rRNA signal in panel B relative to panel A is consistent with the fact that more RNA was loaded for this blot.

(dpm sample - dpm zero time)

(specific activity dpm/pmol glucose) × EQC × time (min) × protein (mg)
GLUT-1 probe also appeared to cross-react weakly with the highly expressed GLUT-2 transcript in the CGT-6 cell line. Four of the five GLUT-1-transfected lines (GT1-5, 8, 10, and 12) showed profound increases (50-100-fold) in levels of GLUT-1 mRNA, while a lesser increase (5-fold) was observed in the fifth line studied (GT1-2). The GLUT-1 mRNA detected in the transfected cells is slightly smaller in size than that of native AtT-20transfected cells due to partial deletion of the 3'-untranslated region in the process of subcloning into the pCB-7 vector. In addition to the previously characterized GLUT-2-expressing AtT-20transfected cell lines CGT-5 and CGT-6 (8), seven newly isolated GLUT-2-expressing clones were analyzed in this study. Five representative clones in Fig. 1B (GT2-4, 8, 9, 12, 14) have GLUT-2 levels equal to or greater than the level in the CGT-6 cell line. For both panels of Fig. 1, 18 S rRNA probe hybridization is shown as a control for gel loading, and POMC mRNA levels were measured as a marker for the differentiated AtT-20 cell phenotype. All lines retained abundant levels of POMC mRNA. The blot in panel A was also probed with the labeled antisense insulin cRNA. Insulin mRNA was markedly elevated in CGT-6 cells relative to GLUT-1-transfected or parental AtT-20transfected cells, consistent with our previous report of increased insulin content in GLUT-2 expressing AtT-20transfected cells (8). Note that twice as much RNA was loaded in each lane for the blot of panel B compared to the blot in panel A. RIN1046–38 cells lacked POMC expression and had higher levels of insulin mRNA than any of the AtT-20transfected lines.

Two of the GLUT-1-expressing clones that contained high levels of GLUT-1 mRNA (GT1-10 and GT1-15) were used for immunocytochemical staining of GLUT-1 protein, using an antibody directed against the C terminus of GLUT-1. As shown in Fig. 2, line GT1-10 showed plasma membrane-associated GLUT-1 signal of much greater intensity than that seen in the parental cell line (data are shown for line GT1-10 only; expression in line GT1-15 was qualitatively identical). In addition to the strong membrane-associated staining, there was significant intracellular signal that was polarized toward regions of cell-cell contact.

Table I summarizes the kinetic constants obtained by Lineweaver-Burke analysis of 3-O-methyl glucose uptake measurements in lines GT1-10 and GT1-15. These data are compared to previously published values obtained for GLUT-2-transfected and parental AtT-20transfected cell lines (8) or normal rat islets (16). The two GLUT-1-overexpressing cell lines exhibited a \( K_m \) for 3-O-methyl glucose of 4 mM and \( V_{max} \) values of 5 mmol/min/liter (GT1-10) and 6 mmol/min/liter (GT1-15). This represents only a slight increase in \( K_m \), but a 10-fold increase in \( V_{max} \), relative to untransfected AtT-20transfected cells (\( K_m = 2, V_{max} = 0.5 \)). Despite these changes, the \( K_m \) and \( V_{max} \) values for 3-O-methyl glucose uptake into GLUT-1-expressing cells are significantly lower than the GLUT-2-expressing lines CGT-5 and CGT-6 (\( K_m = 17-18 \) and \( V_{max} = 18-25 \)) or normal rat islets (\( K_m = 18, V_{max} = 24 \)). We have not measured the number of glucose transporters present in GLUT-1-versus GLUT-2-expressing cells, so no firm conclusions about differences in intrinsic activities or turnover numbers can be made at this time. It should be noted, however, that the RNA blot hybridization analysis shown in Fig. 1 was performed with GLUT-1 and GLUT-2 probes of similar specific activities, with a clearly enhanced signal obtained for the GLUT-1 probe.

As an initial screen, we measured insulin secretion in response to glucose, forskolin, and the combination of these secretagogues in the GLUT-1- and GLUT-2-transfected lines using the previously described static incubation protocol (8).
Glucose Transporter Effects in AtT-20ins Cells

TABLE II
Insulin secretion from AtT-20ins cells transfected with GLUT-1 or GLUT-2

Cells were grown in DMEM media supplemented with 1 mM glucose for 3 days prior to an experiment. Cells were then washed twice with HBSS lacking glucose for 10 min each, and incubated with HBSS alone (no secretagogues), HBSS + glucose (range of 0.1–10 mM) or HBSS + 5 mM glucose + 0.5 μM forskolin for 3 h. Insulin accumulation over the experimental period was measured by radioimmunoassay and expressed in terms of the total cellular protein. Values represent the mean ± S.E. for four independent cell samples.

| Cell lines | Insulin release, no secretagogues | Maximal glucose stimulated release | Insulin release, glucose + forskolin |
|------------|----------------------------------|-----------------------------------|-----------------------------------|
| GLUT-1 lines |                                  |                                   |                                   |
| GT-1-2     | 96.1 ± 0.5                       | 136.8 ± 2.3                       | 183.0 ± 3.8                       |
| GT-1-5     | 61.0 ± 6.0                       | 96.1 ± 1.1                        | 119.5 ± 0.8                       |
| GT-1-8     | 59.6 ± 9.5                       | 81.8 ± 18.3                       | 69.6 ± 19.4                       |
| GT-1-10    | 71.3 ± 0.4                       | 89.8 ± 1.5                        | 99.2 ± 0.2                        |
| GT-1-12    | 80.1 ± 4.4                       | 86.2 ± 3.1                        | 111.1 ± 2.3                       |
| GT-1-15    | 59.2 ± 0.3                       | 74.5 ± 1.9                        | 82.5 ± 1.3                        |
| GLUT-2 lines |                                  |                                   |                                   |
| GT2-1      | 136.1 ± 5.2                      | 254.3 ± 17.4                      | 689.2 ± 53.6                      |
| GT2-2      | 50.0 ± 1.6                       | 97.6 ± 3.0                        | 186.3 ± 4.4                       |
| GT2-4      | 60.4 ± 7.2                       | 118.4 ± 11.7                      | 208.7 ± 4.6                       |
| GT2-8      | 76.3 ± 1.6                       | 201.9 ± 7.9                       | 461.8 ± 8.0                       |
| GT2-9      | 65.3 ± 3.2                       | 201.4 ± 7.7                       | 378.2 ± 19.0                      |
| GT2-12     | 86.8 ± 4.8                       | 315.6 ± 12.3                      | 677.4 ± 26.1                      |
| GT2-14     | 62.0 ± 2.1                       | 144.9 ± 13.9                      | 249.0 ± 12.6                      |
| GT5-7      | 98.9 ± 7.0                       | 284.8 ± 1.7                       | 346.3 ± 14.3                      |
| GT5-6      | 134.9 ± 6.4                      | 234.1 ± 9.8                       | 418.4 ± 38.9                      |
| Control    |                                  |                                   |                                   |
| CTC-P      | 56.5 ± 0.6                       | 55.2 ± 4.2                        | 89.9 ± 7.5                        |

As shown in Table II, all of the GLUT-2-transfected AtT-20ins lines exhibited a glucose-stimulated insulin secretion response (range of 70–280%) increases relative to cells incubated in the absence of secretagogues) as well as a potent response in the presence of glucose + forskolin (range of increases of 240–680%), while all of the GLUT-1-transfected lines or cells transfected with the expression vector lacking a GLUT insert (CTC-P) responded slightly to glucose (range of 5–60%) or glucose + forskolin (17–95%), or not at all. The small response to glucose + forskolin in CTC-P or GLUT-1-transfected cells is not due to a generally diminished regulated secretory pathway, since the combination of 5 μM forskolin and 1 mM isobutylmethylxanthine exerted a 4–5-fold stimulatory effect on insulin release in all three cell types (data not shown). The data for the different types of cell lines (transfected with GLUT-2, GLUT-1, or vector lacking a GLUT insert) are summarized in Fig. 3 and expressed as the percent increase in insulin release induced by glucose or glucose + forskolin relative to cells incubated in the absence of either secretagogue. Poooling of the data in this manner highlights the significant insulin secretion response to glucose (p ≤ 0.02) or glucose + forskolin (p = 0.002) in GLUT-2-transfected cells relative to GLUT-1-expressing cells. Fig. 3 also shows the insulin secretory responses over a range of glucose concentrations. The finding of maximal insulin secretion at a glucose concentration of ≤100 μM in the GLUT-2-transfected cells is consistent with our previous studies in which CGT-5 and CGT-6 cells were found to be maximally responsive at 10–50 μM glucose (8).

Perifusion experiments were carried out to study the dynamics of insulin release from the various AtT-20ins cell lines and to evaluate whether glucose-stimulated insulin secretion from GLUT-2-expressing AtT-20ins cells occurs in a time frame that resembles the rapid islet β-cell response. In the representative experiment shown in Fig. 4A, lines CGT-6 (GLUT-2 transfected), GT1-15 (GLUT-1 transfected), and the parental AtT-20ins cells are compared. During the first 25-min-perifusion period with HBSS lacking glucose, there was a gradual decline in insulin release from all three cell lines. Switching to HBSS buffer containing 5 mM glucose resulted in a 10-fold increase in insulin release from CGT-6 cells. This increase was sustained in two samples (representing a total of 5 min), after which insulin secretion declined to a second plateau that was 3-fold above the preglycose level. Only small changes in insulin release were observed during this period for both the parental AtT-20ins cells and the GLUT-1-transfected GT1-15 line. Upon removal of glucose from the perfusate, insulin secretion from the GLUT-6 cells persisted at the glycerol-stimulated level for approximately 10 min, but then declined rapidly. The low level of insulin release from parental AtT-20ins cells and GT1-15 cells was further reduced during perifusion with glucose-free media. In the next phase of glucose stimulation, the CGT-6 cells again showed a clear secretory response to glucose, but the response was less rapid (requiring 15 min to reach maximum), and was without an obvious first and second phase. Switching back to buffer lacking glucose again resulted in a dramatic, albeit delayed reduction in insulin release from CGT-6 cells. At the end of the experiment, cells were perfused with HBSS containing the combination of 5 mM glucose and 0.5 μM forskolin. In keeping with the static incubation data presented here and elsewhere (8), GLUT-2-expressing CGT-6 cells exhibited a much stronger insulin secretory response to glucose + forskolin than either the parental cells or the GLUT-1-transfected line. The response of line CGT-6 to glucose + forskolin was sustained until the end of the experiment, indicating that the cells were not depleted of insulin during the perifusion. Consistent with this interpretation, only slight changes in insulin content were noticed in cells (all lines) when measurements were made before and after the perifusion (data not shown).

In order to establish the consistency of the glucose response in GLUT-2 expressing cells, we performed abbreviated versions of the perifusion experiment in Fig. 4A on several of our
Glucose Transporter Effects in AtT-20\text sub{i,} Cells

**FIG. 4.** Perifusion of AtT-20\text sub{i,} cell lines. For all panels, cells were grown in liquid culture on Matrigel-coated beads, and 50–100 × 10\textsuperscript{5} cells were loaded into a Pharmacia P10/10 column for perifusion studies, as described under "Materials and Methods." Panel A, insulin secretion from parental AtT-20\text sub{i,} cells, the GLUT-1-transfected line GT1-15, and the GLUT-2-transfected line CGT-6. Cells were perfused with the following: period I, HBSS with no supplements; period II, HBSS + 5 mM glucose; period III, HBSS with no supplements; period IV, HBSS + 5 mM glucose; period V, HBSS with no supplements; period VI, HBSS + 5 mM glucose and 0.5 mM forskolin. Panel B, insulin secretion from AtT-20\text sub{i,} cells transfected with CB-7 vector lacking a GLUT insert (CTC-P) and from the GLUT-2-transfected lines CGT-6, GT2-9, and GT2-12. Panel C, insulin secretion from AtT-20\text sub{i,} cells transfected with CB-7 vector lacking a GLUT insert (CTC-P; same data as panel B, shown for reference), the GLUT-2-transfected line GT2-6 (an independent experiment from those shown in panels A and B) and the GLUT-1-transfected lines GT1-5 and GT1-8. For panels B and C, cells were perfused with the following: period I, HBSS with no supplements; period II, HBSS + 5 mM glucose; period III, HBSS with no supplements; period IV, HBSS + 5 mM glucose and 0.5 mM forskolin; period V, HBSS with no supplements.

**FIG. 5.** Glucose usage in AtT-20\text sub{i,} cell lines. Glucose usage was determined by measuring the rate of generation of \textsuperscript{3}H\textsubscript{2}O from [5-\textsuperscript{3}H]glucose, as described under "Materials and Methods." The AtT-20\text sub{i,} cell lines assayed were as follows: untransfected, parental AtT-20\text sub{i,} cells (AtT-20\text sub{i,}); GLUT-2-transfected lines CGT-6 and GT2-12; GLUT-1-transfected lines GT1-5 and GT1-8. The symbol legend is provided at the right of the figure. Each data point represents the mean ± S.E. for four independent determinations.
Glucose Transporter Effects in AtT-20\textsubscript{ox} Cells

**DISCUSSION**

We have previously shown that overexpression of GLUT-2 in AtT-20\textsubscript{ox} cells confers glucose transport kinetics that are indistinguishable from normal rat islets. In addition, GLUT-2-expressing AtT-20\textsubscript{ox} cell lines exhibit glucose-stimulated insulin secretion, glucose potentiation of non-glucose secretagogues, and an increase in insulin content (8). The glucose-stimulated insulin secretion response in engineered AtT-20\textsubscript{ox} cells was found to be maximal at 10–50 μM glucose in our previous studies. This finding was consistent with the fact that glucokinase activity is reduced and hexokinase activity is increased in AtT-20\textsubscript{ox} cells relative to normal islets (8). Our interpretation of this data is that GLUT-2 transfection increases glucose entry into engineered AtT-20\textsubscript{ox} cells, but that in the face of dominant hexokinase activity, glucose metabolism and insulin release are maximal at subphysiological glucose concentrations. Further support for such a model is gained from experiments in which hexokinase activity has been increased by molecular manipulations in fetal islets (17) or increases naturally as a function of passage number in insulinoma cell lines (18). In both instances the dose dependence of glucose-induced insulin production or secretion is shifted to the left.

Based on the model advanced above, one might predict that any glucose transporter that is capable of substantially increasing glucose entry into transfected AtT-20\textsubscript{ox} cells should serve to activate glucose-stimulated insulin release. In the current work, we have tested this hypothesis by overexpression of the cDNA encoding GLUT-1. This transporter is expressed in a large number of tissues, most notably brain and erythrocytes, and is 55% identical in amino acid sequence to GLUT-2 (19, 20). It has been shown by a number of investigators to have a lower $K_m$ for glucose than GLUT-2 (16, 21–23), but since its $K_m$ is in the low millimolar range, it should provide a capacity for glucose transport that is not rate limiting for a metabolic pathway controlled by hexokinase, an enzyme with a $K_m$ for glucose in the range of 50 μM (24).

The strategy for the creation of stable, GLUT-1-overexpressing AtT-20\textsubscript{ox} lines was identical to that employed for GLUT-2 expression in these cells. We found that transfection with the pCB-7 plasmid in which the GLUT-1 cDNA is inserted next to the cytomegalovirus promoter resulted in as much as a 100-fold increase in GLUT-1 mRNA relative to the modest endogenous expression of this transporter in untransfected AtT-20\textsubscript{ox} cells. GLUT-1 immunofluorescence at the plasma membrane of transfected AtT-20\textsubscript{ox} cells was also dramatically increased. The increased expression of GLUT-1 translated into a small increment in the apparent $K_m$ for 3-O-methyl glucose from 2 to 4 mM and a more impressive 10-fold increase in maximal velocity from 0.5 to 5-6 mmol/min/liter cell space. Interestingly, the increase in maximal velocity observed in two independent GLUT-1-transfected clonal cell lines was only about one-third as great as the increase observed in two independent GLUT-2-transfected lines (Table 1). Previous studies have indicated that kinetic constants calculated for 3-O-methyl glucose uptake closely reflect the kinetics of D-glucose uptake for both GLUT-1- and GLUT-2-dominated systems (23).

Glucose-stimulated insulin secretion was studied in six GLUT-1 and nine GLUT-2-transfected AtT-20\textsubscript{ox} cell lines. Static incubation experiments clearly show that GLUT-2-transfected cells consistently respond to glucose as an insulin secretagogue while GLUT-1-transfected cells have only a very modest response. In keeping with our previous work (8), glucose-stimulated insulin secretion was found to be maximal at glucose concentrations in the range of 50 μM in GLUT-2-transfected cells. Furthermore, administration of the combination of glucose + forskolin resulted in a dramatically enhanced insulin secretory response in GLUT-2-transfected cells.

Perfusion experiments were performed in an effort to confirm these results and to simultaneously learn more about the dynamics of insulin release. Strikingly, GLUT-2-transfected cells, but not GLUT-1-transfected or vector-transfected cells, dramatically increased their insulin output within minutes of a change from HBSS buffer lacking glucose to the same buffer containing 5 mM glucose. Furthermore, return to HBSS lacking glucose resulted in reduction of insulin release from the GLUT-2-expressing cells. The finding of generally consistent effects of GLUT-2 versus GLUT-1 transfection in a large number of independent clonal lines in this study strongly supports the idea that the effects observed are a consequence of the expression of the particular glucose transporter isoform, and are not due to clonal variability.

We have considered three models that might potentially explain the divergent effects of GLUT-1 and GLUT-2 expression in AtT-20\textsubscript{ox} cells, as summarized in Fig. 7. In the first of these, we assume that the number of functional GLUT-2 molecules exceeds the number of functional GLUT-1 molecules at the plasma membrane surface for the respective transfection experiments. The second, related model would hold that the number of glucose transporter molecules is similar for the two transfection experiments, but that GLUT-2
Glucose Transporter Effects in AtT-20\textsubscript{ins} Cells

2 has a higher intrinsic specific activity than GLUT-1. For either of these models, the specific effects of GLUT-2 in mediating glucose-stimulated insulin release would be explained by enhanced glucose entry.

Direct measurement of glucose uptake in the transfected lines revealed that maximal velocity of glucose uptake in GLUT-1-transfected cells was indeed only one third of that in GLUT-2-transfected cells. Despite the large differences in glucose uptake capacity among cell lines, the rate of glucose usage was found to be similarly affected by the glucose concentration in GLUT-2- and GLUT-1-transfected or control cells, with an $S_{0.5}$ for glucose metabolism in the range of 2 mM in all cases. These observations suggest that glucose uptake is not rate limiting for glucose metabolism in AtT-20\textsubscript{ins} cells, and that the rate of metabolism is instead limited by a low $K_c$ component (possibly hexokinase). Together, the foregoing observations seem to provide support for the third of the proposed mechanisms in Fig. 7, namely that GLUT-2, but not GLUT-1, is capable of forming productive interactions with other components of insulin-secreting cells, and that this putative interaction is critical for functional glucose signaling. Potential partners for GLUT-2 coupling might include signal transduction molecules such as GTP-binding proteins. Alternatively, GLUT-2 might serve to nucleate complexes of metabolic proteins or enzymes capable of substrate "channeling" in a manner analogous to complexes or "metabolons" that occur among enzymes of the citric acid cycle (23).

While there is no direct evidence for participation of GLUT-2 in metabolic channeling of glucose, other reactions of the glycolytic sequence may be tightly coupled. Recently, Malaisse and Bodur (26) have postulated that coupling occurs within the enzyme sequence hexokinase/glucokinase-phosphoglucomutase-phosphofructokinase in order to account for generation of $^3$H$_2$O from [2-$^3$H]glucose in intact islets at a rate lower than that predicted by activities of these enzymes measured in vitro. It has also been proposed that the islet isoform of glucokinase, which is different from liver glucokinase in that it contains a highly charged N terminus (2, 7, 27), might be equipped for physical coupling with the GLUT-2 transporter and more efficient glucose utilization (2). The presence of GLUT-2 may promote the formation of an enzyme complex, such as the one described by Malaisse, in close proximity to the plasma membrane, enhancing glycolytic flux within this compartment and leading to changes in membrane potential. Such a model has the added benefit of potentially explaining a long-standing paradox of glucose signaling in β-cells, which is that ATP-sensitive K$^+$ channels become inhibited upon exposure of islet cells to glucose (28, 29), despite a very limited alteration in whole cell ATP/ADP ratio (30). Localized metabolism of glucose mediated by a GLUT-2 nucleated metabolon might allow effective local production of ATP in close proximity to adjacent K$^+$ channels. Precedent for such a model has in fact been advanced for myocytes, in which production of ATP in response to administration of glycolytic substrates has been shown to be more effective in closing K$^+$ channels than ATP produced by addition of Krebs cycle substrates (31).

We must also consider the possibility that the specific capacity for glucose signaling conferred by GLUT-2 transfection is only relevant to the non-islet AtT-20\textsubscript{ins} cell lines, and that glucose signaling in such cells occurs by a mechanism distinct from that operative in islet-derived cells. Although fuel-mediated signaling pathways of AtT-20\textsubscript{ins} cells may ultimately prove to differ from islet-derived cells in some aspects, a fundamental similarity of the two cell types is the apparent requirement for glucose metabolism for stimulated insulin release, as illustrated by the absence of a direct or potentiating response to non-metabolizable analogs of glucose. Since the overall rate of glucose metabolism is similar in GLUT-1- and GLUT-2-transfected cells, the glucose analog data suggest that either a portion of glucose flux is localized or channeled in GLUT-2-transfected cells, or alternatively, that the GLUT-2 protein provides a necessary but not sufficient signal that requires glucose metabolism for transmission. It should also be noted that the time frame of the response in perfusion experiments is extremely rapid, indicating that glucose is generating a specific signal, as opposed to having a generalized effect on cell viability. Finally, our recent studies show that transfection of glucose-unresponsive rat insulinoma cells greatly enhances their capacity for glucose-stimulated insulin release. In sum, the AtT-20\textsubscript{ins} cell system may ultimately prove to be relevant to understanding of the glucose-sensing pathway in normal islet cells.

It is of interest to compare the results reported herein, obtained by engineered expression of glucose transporter isoforms in insulin-secreting cells with other recent studies in which various experimental factors have been used to alter the relative levels of expression of these transporters. In agreement with our data, Miyazaki et al. (32) isolated a number of clonal cell lines from transgenic animals in which T-antigen expression directed by the insulin promoter was used to cause β-cell tumors. The MIN-6 cell line expressed GLUT-2 abundantly and exhibited a significant glucose-stimulated insulin secretion response. In contrast, the MIN-7 line, isolated in parallel with MIN-6, was found to express GLUT-1 predominantly and very little GLUT-2; these cells failed to respond to glucose (32).

A somewhat different picture emerges from studies with isolated islets. It has recently become appreciated that transfer of isolated islets to a tissue-culture environment causes increased expression of GLUT-1 and some reduction, but not a loss of GLUT-2 expression; this trend is most prominent when the islets are cultured at low glucose concentration (5 mM glucose) (33, 34). Interestingly, partial loss of GLUT-2 expression is correlated with complete absence of glucose-stimulated insulin release not only in isolated islets cultured

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at low glucose, but also in animal models of non-insulin-dependent diabetes mellitus (4). The partial loss of immunocytochemically detectable GLUT-2 that occurs upon transfer of islets to tissue culture can be largely overcome by culturing the islets at high glucose concentrations (30 mM) for 7 days (34). Culturing of islets at high glucose also causes a return of the glucose-stimulated insulin secretion response. Despite the return of immunologically detectable GLUT-2 after 7 days of culture at high glucose, glucose uptake was found to be decreased relative to freshly isolated islets. These data were used by Tal et al. (34) to argue that regulation of GLUT-2 activity is secondary to other factors, in particular the regulation of glucokinase enzymatic activity in the control of glucose-stimulated insulin release. This argument is based on the knowledge that the level of glucokinase activity is closely correlated with the magnitude of the insulin secretory response in islets cultured at low and high glucose concentrations (35, 36). While aspects of this formulation are likely to be correct, the data are also consistent with the new model advanced herein, in which GLUT-2 protein is required for effective signal transduction through physical coupling with other islet components. The loss of glucose-stimulated insulin release that occurs with only a partial loss of GLUT-2 protein in both the in vivo and in vitro models may fit with our model if a minimum level of GLUT-2 expression is required for effective interactive signaling, or alternatively, if loss of GLUT-2 expression is specific for a subpopulation of β-cells that are particularly competent for glucose sensing. Indeed, such functional heterogeneity in β-cells is now well accepted (37, 38) and may fit with recent observations suggesting that glucokinas is expressed in only a subset of β-cells (39).

In conclusion, elucidation of the mechanism for glucose-stimulated insulin release in the AtT-20i, cell model will require more extensive studies of the effect of GLUT-2 expression on glucose metabolism and signal transduction in these cells. Specific approaches that may be informative include structure-function studies of GLUT-2 and modulation of the hexokinase/glucokinase ratio within the model system described in this study.

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