Expression and Function of GLUT-1 and GLUT-2 Glucose Transporter Isoforms in Cells of Cultured Rat Pancreatic Islets

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We have previously investigated glucose induction of glucokinase, glucose usage and insulin release in isolated cultured rat pancreatic islets (Liag, Y., Najafii, H., Smith, R. M., Zimmerman, E. C., Magnuson, M. A., Tal, M., and Mastchinsky, F. M. (1992) Diabetes (1992) 41, 792-806). Here we studied the expression and function of GLUT-1 and GLUT-2 glucose transporter isoforms, using the same system, i.e. isolated pancreatic rat islets immediately after isolation or cultured in the presence of 3 or 30 mM glucose for as long as 10 days. We found by immunofluorescence microscopy and Western and Northern blot analysis of islet extracts that GLUT-1 expression was induced in islet β-cells in tissue culture both with low or high glucose present. The induction of GLUT-1 was specific to β-cells but was not present in all β-cells and was not detected in α-cells. GLUT-2 expression was also specific for β-cells and was not observed in all β-cells. Some β-cells in culture coexpressed GLUT-1 and GLUT-2. The expression of the two glucose transporters was regulated in the opposite direction in response to glucose concentration in the culture medium. GLUT-1 was more effectively induced when glucose was low, and GLUT-2 expression was more pronounced when glucose was high in the culture media. Another difference between the two glucose transporters was that GLUT-2 expression was increased while GLUT-1 expression was decreased as culturing continued as long as 7 days. Thus, after 7 days of culture GLUT-2 expression in β-cells was nearly the same at low and high glucose, whereas GLUT-1 was practically absent no matter what the glucose level was. In attempts to correlate GLUT-1 and GLUT-2 expression to β-cell function glucose uptake and glucose-stimulated insulin release in fresh and cultured islets were measured. In freshly isolated islet glucose uptake was estimated to be 100-fold in excess of actual glucose use. Glucose uptake was reduced by 7-day culture to about one-third of that observed in freshly isolated islets no matter what the glucose concentration of the culture media. We conclude that in the present experimental system GLUT-1 and GLUT-2 expression and function are not closely associated with glucose usage rates or the secretory function of β-cells.

The endocrine β-cells of pancreatic islets secrete insulin in response to postprandial blood glucose levels of 5-10 mM. It appears that glucose has to be metabolized within the β-cells for triggering insulin release (1) and that this prerequisite for metabolism of the trigger glucose is to ensure that the insulin release is proportional to the predominant postprandial carbohydrate energy source of the organism. This design of signal recognition is unique and is different from that of the great majority of other molecular sensing systems in which the reversible binding of an agonist to a transmembrane receptor is the first important and sufficient step to engage the signal transmission chain. It is noteworthy in this context that other physiological and experimental fuel secretagogues for insulin such as amino acids (2), fatty acids (3), and ketone bodies also require the presence of and the metabolism of glucose to increase insulin secretion.

It has been known since 1968 (4) that the capacity of the pancreatic β-cell membrane to transport glucose into the cytosol is very high with a capacity comparable to that of liver cells. Glucose transport in the β-cells is accomplished via the GLUT-2 glucose transporter (5), which has a very high Vmax and a high Km for glucose (17 mM; 6), and glucose metabolism is governed by glucokinase, which has a relatively low Vmax but a similarly high Km (8-16 mM; 4, 7). It has been speculated that the two high Km systems might be physically associated in the form of a complex and that such a tandem system might control the glucose entry and metabolism of the β-cells (8). Therefore, a complex comprised of both proteins was hypothesized to serve the role of "glucose sensor" in β-cells (9, 10). Impairment of the GLUT-2 transport system was proposed to underlie the defective β-cell function in IDDM1 and NIDDM alike (9).

The present study and a closely related paper (11) address several observations and issues. First, isolated pancreatic islets cultured for 7 days in 30 mM glucose hypersecreted insulin when stimulated with glucose, whereas maintaining islets in 3 mM glucose resulted in glucose-insensitive islets (11). Second, overnight cultured islets newly expressed the low Km GLUT-1 isofrom (Km of 1–2 mM; 12) in addition to the GLUT-2 isofrom, which is present in freshly isolated islets (13). Third, glucokinase and glucose usage were induced manifold when high glucose was present in the culture media.

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1 The abbreviations used are: IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.
Glucose Transport in Cultured Islets

To understand fully the regulation of glucose metabolism and insulin release of pancreatic islets as influenced by the tissue culture condition, it was necessary to investigate the expression of the predominant glucose transporter GLUT-2 of islet cells cultured for extended periods in low or high glucose. It appeared also possible that cultured islet cells might continue to express the low \( K_a \) GLUT-1 glucose transporter in contrast to islet cells in situ, and this might influence the dose-response curve of glucose usage and of the insulin response to glucose. We therefore assessed GLUT-1 and GLUT-2 expression, actual glucose transport, and glucose-induced insulin release in response to time in tissue culture and to the level of glucose in the culture medium in this well controlled tissue culture system. We observed that the glucose level and the duration of culture influenced the expression of these two glucose transporter isoforms in an opposite way. We found that glucose transport in the freshly isolated or islets cultured in 3 or 30 mM glucose was far in excess of glucose usage (10–100 times) and had no clear correlation with glucose usage or glucose-induced insulin release. Therefore, under the present condition, marked alterations of GLUT-2 seem to have no significance for glucose usage and for glucose-induced insulin release from pancreatic islets.

MATERIALS AND METHODS

Islet Isolation and Culture—Islets were isolated from Wistar rats and processed either immediately after isolation or after maintaining them in tissue culture for various lengths of time in the presence of 3 or 30 mM glucose as described in detail previously (14).

Microscopic Immunofluorescence Study of Islet β-Cells—Freshly isolated islets or islets cultured for various time periods and at various glucose concentrations as indicated under “Results” were washed three times in phosphate-buffered saline (PBS) and fixed overnight at 4°C after immersing them in paraformaldehyde–lysolethanol fixative (15). Thereafter, 100 islets/tube were incubated overnight in PBS at 4°C, warmed to 37°C, and embedded in 300 μL of 2% low melting agarose/PBS at 37°C, pelleting the islets by brief centrifugation in a microcentrifuge. Agarose-embedded islets were equilibrated with PBS containing 0.6 mM sucrose and 0.02% sodium azide by rotating overnight at 4°C and were subsequently embedded in O.C.T. compound (Miles Laboratories Inc., Elkhart, IN), frozen in 2-methylbutane in liquid nitrogen for 8 min at maximum speed (Heat Systems-Ultrasonics, Inc., Farmingdale, NY), and kept at -70°C until staining. Glucose transporter isoforms GLUT-1 and GLUT-2 were detected by the indirect immunofluorescence staining method using the lithium chloride method as described by Auffray and Rougeon (19), and Northern blots were prepared according to Lehrach et al. (20). Briefly, the RNA (1–10 μg) was resolved on a 1% agarose/formaldehyde gel (narrow wells) and transferred to a nylon membrane filter (ICN; Irvine, CA). Filters were hybridized with random primed \(^{32}P\)-labeled full-length cDNA probes of both the rat GLUT-1 (gift from M. Birnbaum; 21) or the rat GLUT-2 (10). The hybridization solution contained 50% formamide, 5 × SSC (1 × SSC = 150 mM NaCl, 75 mM sodium citrate (pH 7)), 5 × Denhardt’s solution (1 × Denhardt = 2 g/liter each of polyvinylpyrrolidone, bovine serum albumin, and Ficoll 400), 5 mM EDTA, 1% sodium dodecyl sulfate (SDS), and 100 ng/ml poly(A). Hybridization was performed overnight at 42°C, and filters were washed twice in 2 × SSC at 42°C and twice in 0.2 × SSC at 65°C.

Islet Perfusion to Study Glucose Uptake and Insulin Release—Approximately 150 freshly isolated islets or islets cultured for 7 days in either 3 or 30 mM glucose were perfused in an open chamber system (22) for 40 min with Krebs-Ringer bicarbonate in the absence of glucose but containing 0.25 mM inulin as extracellular marker. At the 40-min time point, the glucose in the perfusion buffer was switched to 20 mM by adding 2 mM glucose stock solution into the perfusion buffer and then immediately such that the glucose concentration within 2–3s. Islets were perfused for another 20 min. The effluent was collected at different time points for insulin determination.

In some experiments, filters containing the islet samples were quickly frozen on dry ice at 23, 60, and 90 s after islets were exposed to high glucose and hypoxophenylethanolamine at 38°C. Frozen islets were dissected from the filters at low magnification (30×) and weighed with a quartz fiber fishpole balance (23). A fluorometric enzyme cycling method was used to determine the glucose and inulin contents of these islet samples (24). The total glucose space of the islet sample (extra plus intracellular glucose expressed of terms of milligrams of glucose) was calculated by dividing the glucose content of the islet sample (mmol/kg, dry weight) by the glucose concentration (mmol/liter). The extracellular space (liter/kg, dry weight) was calculated by the inulin content of the islet sample (μmol/kg, dry weight) divided by the blood inulin concentration (μmol/liter). The intracellular space of glucose resulted from the difference of the total glucose space and the inulin space. Perifused samples were obtained at frequent intervals and frozen until analyzed for glucose, inulin, or insulin.

RESULTS

Immunohistochemical Studies of Plasma Membrane Expression of GLUT-1 and GLUT-2 in Cells of Cultured Islets—Isolated pancreatic islets were cultured in medium with 3 or 30 mM glucose for the duration of 1, 2 and 7 days. Islets were then fixed and stained for GLUT-1 and GLUT-2 isoforms by indirect immunofluorescence with affinity-purified polyclonal antibodies specific for the carboxy terminal of both the two isotypes (10, 16). It is apparent from Fig. 1 that freshly isolated islets (here defined as day 0) expressed GLUT-2 and GLUT-2 isoforms that immunostainable GLUT-1 was not detectable. In culture conditions, there was a pattern of decreasing GLUT-2 expression during the first 2 days of culture and then recovery of precursor levels at 7 days. The same pattern was observed in islets cultured in presence of 30 mM glucose: initially GLUT-2 expression was markedly reduced during the first 2 days and then recovered to precursor levels at 7 days. The intensity of staining at each time point was higher in islets cultured at 30 mM glucose than in those cultured at 3 mM glucose.

GLUT-1 was induced by the tissue culture conditions using 3 mM glucose in at least half the number of the islet cells with a peak of expression after 2 days. In 30 mM glucose few cells expressing GLUT-1 were observed in the first 2 days, and no staining was observed after 7 days in tissue culture.
Thus GLUT-1 and GLUT-2 expression was regulated differently and in opposite directions in response to both the glucose concentration in the cultured islet and the time the islets were maintained in tissue culture. It is noteworthy that not all the islet β-cells expressed GLUT-1 or showed a reduced GLUT-2 fluorescence in response to tissue culture conditions, which implies heterogeneity of the β-cells within an islet.

To test whether GLUT-1 was expressed exclusively in β-cells, islets cultured for 1 day in 3 mM glucose were stained for GLUT-1 (Fig. 2, panel B), then double stained for GLUT-2 (panel A), and finally triple stained for glucagon (using the very intensive staining for glucagon, panel C). Staining for insulin was performed in an adjacent section within the same islet (panel D). The staining pattern showed that GLUT-1 and GLUT-2 were expressed on the plasma membrane of the same β-cells; adjacent sections stained individually for GLUT-1 and GLUT-2 showed that the same area was stained for both glucose transporters (not shown). By double staining, some β-cells were stained for GLUT-2 (arrows in panel A) and did not show staining for GLUT-1, but the reverse situation was not found. Clearly, cells that were stained for glucagon were not stained for either glucose transporter (open arrowhead).

Next, we tested the reversibility of the glucose-dependent induction/suppression of the glucose transporter expression by switching the glucose concentration in the culture medium. After 7 days in culture the medium was changed from 3 to 30 mM or from 30 to 3 mM glucose, and then the islets were maintained in the altered glucose concentration for additional 3 days. Islets were then fixed and examined by immunofluorescence for the expression of the different glucose transporters. Fig. 3 shows that GLUT-2 was induced in more cells when switching from 3 to 30 mM glucose and was reduced when switching from 30 to 3 mM glucose. The opposite happened to GLUT-1 expression, which was repressed when switching from low to 30 mM glucose and induced in a few cells upon switching from high to 3 mM glucose.

**GLUT-1 and GLUT-2 Protein Contents in Tissue Extracts**

<table>
<thead>
<tr>
<th>Glucose [mM]</th>
<th>GLUT-2</th>
<th>GLUT-1</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>30</td>
<td>↓</td>
<td>↑</td>
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</table>

Fig. 3. Immunofluorescence detection of GLUT-2, GLUT-1, glucagon, and insulin in islet β-cells cultured for 1 day in 3 mM glucose. Sections represent an islet cultured for 1 day in 3 mM glucose. The same section was stained first for GLUT-1 (B), then double stained for GLUT-2 (A), then triple stained for glucagon (C). An adjacent section within the same islet was stained for insulin (D).

**Fig. 1. Time course of immunohistochemical plasma membrane expression of GLUT-1 and GLUT-2 in pancreatic islets cultured in 3 or 30 mM glucose.** Sections were from freshly isolated pancreatic islets (0 time) or islets kept in tissue culture for 1, 2, and 7 days in the presence of 3 or 30 mM glucose as indicated. Sections were stained using an immunohistochemical technique (see “Materials and Methods”) for GLUT-2 (left two columns) or for GLUT-1 (right two columns). Bar represents 30 μM.
Glucose Transport in Cultured Islets

Fig. 3. Effect of glucose level on immunohistochemical plasma membrane expression of GLUT-1 and GLUT-2 in cultured pancreatic islets. Sections were from isolated pancreatic islets that were kept for 7 days (7; left side of panel) in 3 or 30 mM glucose (indicated above the figure) or from cultures in which the medium glucose concentration was switched from 3 to 30 mM (indicated below the figure) for an additional 3 days (7+3; left side of panel). Sections were stained using an immunochemical technique (see "Materials and Methods") for GLUT-2 (left two panels) or for GLUT-1 (right two panels). Bar represents 30 μm.

Fig. 4. Time course of expression of GLUT-1 and GLUT-2 proteins in pancreatic islets cultured in 3 or 30 mM as measured by Western blotting. Cell lysates (30 μg of protein/lane) were from: lanes 1–7, isolated pancreatic islets that were kept for the indicated length of time (0, 1, 2, and 7 days (d)) in tissue culture in 3 mM (3) or 30 mM (30) glucose; lanes 8 and 9, islets kept for 7 days in 3 or 30 mM glucose followed by culturing for an additional 3 days (7+3d) in medium that was switched from 30 to 3 mM (30/3) or from 3 to 30 mM (3/30); lane 10, from rat brain (B); and lane 11, from rat hepatoma H-4-II-E cell line (H). Lysates were then resolved by SDS-polyacrylamide gel electrophoresis (7.5% acrylamide) and transferred to a nitrocellulose filter. The filter was incubated for 1 h with a polyclonal serum specific for GLUT-1 and GLUT-2 proteins (see "Materials and Methods"), and bound antibody was detected with 125I-protein A. Exposure times with Kodak XAR-5 film at –70°C using an intensifying screen were 8 days for GLUT-1 and 3 days for GLUT-2. The lower band in freshly isolated islets probed for GLUT-2 is most likely a degradation product of GLUT-2 protein. Virtually identical results were obtained in a separate experiment.

point was obtained from 500 islets usually collected from two rats and represents therefore the average tissue response to the culture conditions, unlike the immunofluorescence studies in which selected single islets were explored. A 43-kDa band of GLUT-1 was recognized (Fig. 4, upper) which depended on the glucose concentration in the media. There was two to three times more GLUT-1 in 3 mM glucose than in 30 mM glucose. A peak of GLUT-1 expression in 3 mM glucose was seen at 1 and 2 days, whereas on day 7 GLUT-1 levels were much reduced. In 30 mM glucose GLUT-1 protein was also induced, however, to a significantly lower degree than in 3 mM glucose. The induction was similarly transient, with a peak of expression on days 1 and 2. The GLUT-1 protein was barely detected after switching the glucose concentrations in the medium, consistent with the histological immunofluorescence studies.

Identical blots of the same islet lysates were probed for GLUT-2 protein using polyclonal serum for the carboxyl terminus of GLUT-2. The resulting blot (Fig. 4, lower) showed high expression of GLUT-2 in fresh islet which repeatedly appeared as a 55-kDa band together with a second band at about 50 kDa. This latter band appeared only in freshly isolated islet preparations and was most probably a protease degradation product of GLUT-2 of a different size than the degradation product that had been observed before (25) with a size under 43 kDa. This result is possibly a result of using different collagenase preparations (Serva, Heidelberg versus Worthington; 25). GLUT-2 expression in cultured islets was as much as two times higher in high glucose at each time point when compared with low glucose. Maintaining islets in tissue culture decreased GLUT-2 expression initially, but then, with time, expression increased to a level which was even higher than that of freshly isolated islets. Importantly, after switching the glucose from 30 to 3 mM in the culture medium, GLUT-2 expression was reduced, whereas GLUT-2 expression was increased after switching from 3 to 30 mM glucose.

Thus GLUT-1 and GLUT-2 exhibited opposite behavior in response to the duration of culture and glucose concentration in the culture media. Considering the effect of time in culture, GLUT-2 protein suppression in culture was transient, and considering glucose concentration, glucose enhanced GLUT-2 while suppressing GLUT-1 expression.

RNA Levels of GLUT-1 and GLUT-2 in Cultured Islets—To test whether the regulation of the glucose transporters occurred at the transcriptional or the posttranscriptional level Norther blot analysis was performed with islets maintained in culture for different lengths of time, including a 3-h time point, in the presence of 3 or 30 mM glucose. Each time point was investigated with RNA from 2,000–3,000 islets. GLUT-2 mRNA levels (Fig. 5, upper panel) were markedly higher in islets cultured in 30 mM glucose than GLUT-2 mRNA levels in islets cultured in 3 mM glucose, except after 3 h in culture. In 30 mM glucose, with time in culture, GLUT-2 mRNA levels were first reduced and then increased again to a level that was significantly higher than the level detected in freshly isolated islets. In 3 mM glucose GLUT-2 mRNA was much reduced during the first 2 days, but on day 7 mRNA was increased again, reaching about half the level found in freshly isolated islets.

GLUT-1 mRNA (Fig. 5, lower panel) was not detected in freshly isolated islets, was induced within 3 h in tissue culture, and was somewhat higher in 3 mM glucose than in 30 mM glucose. As described above for the protein, after 7 days in culture the GLUT-1 mRNA was not demonstrable. Interestingly, after 7 days in culture higher molecular weight bands
The filters were hybridized under high stringency conditions with samples (1 pg/lane) from: isolated pancreatic islets that were tissue-cultured in 3 or 30 mM glucose, from rat liver (L), or from rat brain (B). RNAs were resolved by electrophoresis on a 1% agarose-formaldehyde gel and were transferred to nylon fibers. The filters were hybridized under high stringency conditions with GLUT-1 and GLUT-2 full-length cDNA randomly primed probes. Exposure times with Kodak XAR-5 film at -70 °C using an intensifying screen were 3 days for GLUT-2 and 7 days for GLUT-1. Virtually identical results were obtained in a separate experiment.

appeared in islet mRNA which might be unspliced GLUT-1 mRNA.

Thus, GLUT-1 and GLUT-2 mRNA in cultured islets were influenced by glucose concentration and by the duration of tissue culture and were comparable to the responses that were observed by immunoblotting and by histological immunofluorescence.

Glucose Uptake and Glucose-induced Insulin Release in Freshly Isolated or Cultured Islets—Using an “open chamber” system for islet perfusion, glucose-stimulated insulin release and glucose uptake were studied with fresh islets and islets cultured for 7 days in 3 or 30 mM glucose. When the glucose concentration in the perfusion medium was rapidly raised from 0 to 20 mM, onset and initial rate of insulin release were much faster in islets cultured in high glucose than with freshly isolated islets. Islets cultured in 3 mM glucose showed no insulin release response to glucose stimulation (Fig. 6, panels A and B). When islets were challenged with a low glucose stimulus by raising the perfusate glucose from zero to 5 mM a secretory response was totally lacking no matter what the pretreatment of the islets (not shown). The insulin release from freshly isolated islets suddenly exposed to 20 mM glucose increased 2-3-fold yet with considerable delay, and islets cultured in 3 mM showed no response to high glucose. Freshly isolated islets showed, however, a rapid manifold stimulation of insulin release when they were activated by a mixture of 20 mM glucose and 30 mM KCl (data not shown). This result indicated that islets which had been perfused for 40 min in the absence of glucose retained their secretory capacity. Altered glucose uptake by islet was considered as a possible cause for differences of the secretory responses of islets with different pretreatments. In freshly isolated islets, extra- and intracellular glucose were nearly equal after 23 s of islet exposure to 20 mM glucose. However, in islets cultured in 3 or 30 mM glucose, glucose uptake was delayed and approached equilibrium at 90 s (Table I). Using cultured islets and 6 or 9 min of glucose perfusion, an intracellular space of 1.52 ± 0.26 (n = 5) liters/kg, dry tissue was found. Using the earliest time point as a basis of calculation, glucose uptake rates at a concentration of 20 mM were 29, 10, and 10 mmol/liter/min in freshly isolated islets or islets cultured in either 3 or 30 mM glucose, respectively (Table II). Thus, islets cultured for 7 days in 30 mM glucose have a reduced rate of glucose uptake but a higher than control level of GLUT-2.

DISCUSSION

We demonstrated previously that pancreatic islets in culture adapted to high glucose levels in the medium by a concordant increase of glucokinase activity, glucose usage, and glucose-stimulated insulin release (11, 14). The adaptive responses were of impressive magnitude and were linearly related to the glucose level over a wide range from 3 to 30 mM. Since the secretory response of the β-cell to glucose depends on glucose uptake and metabolism, it seemed to be crucial to explore whether glucose transport might be affected similarly by the culture conditions. In particular, it was important to investigate whether the β-cell and hepatocyte-specific glucose transporter GLUT-2 might be influenced by the glucose level in the culture medium and to assess whether other known glucose transporters (e.g. GLUT-1) might be induced as well. The issue has gained a timely significance because recent reports have attributed a key regulatory role to β-cell GLUT-2 in the process of glucose recognition and explained the secretory β-cell defect in both IDDM and NIDDM by a reduction or loss of β-cell GLUT-2 (9).
Glucose Transport in Cultured Islets

Table I
Glucose uptake of freshly isolated islets or of islets cultured in 3 or 30 mM glucose for 7 days

The table contains the results of the actual glucose and insulin measurements in islets perfused for various lengths of time with 20 mM glucose but preloaded for 40 min with 0.25 mM inulin in the absence of glucose (first three columns). It also presents the results of calculations of the spaces of distribution of the two solutes expressed in terms of liters/kg dry tissue (fourth through sixth columns). A total glucose space of nearly 3 liters/kg, dry weight, as determined 90 s after exposure to glucose is consistent with the cellular composition of the islet and with the results of previous measurements of total and intracellular water spaces (26). The present glucose transport data allow a comparison with previous results of glucokinase and glucose usage determinations on the basis of DNA contents of about 20 ng/μg of dry tissue (26).

<table>
<thead>
<tr>
<th>Sampling time and no. perfusions</th>
<th>Glucose content/dry tissue</th>
<th>Inulin content/dry tissue</th>
<th>Total glucose space/dry tissue</th>
<th>Inulin space/dry tissue</th>
<th>Intracellular glucose space/dry tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 (9)</td>
<td>44.14 ± 6.48</td>
<td>163.28 ± 11.52</td>
<td>2.29 ± 0.25</td>
<td>0.88 ± 0.05</td>
<td>1.52 ± 0.23</td>
</tr>
<tr>
<td>60 (5)</td>
<td>57.64 ± 2.93</td>
<td>237.22 ± 28.62</td>
<td>2.88 ± 0.16</td>
<td>0.99 ± 0.12</td>
<td>1.89 ± 0.12</td>
</tr>
<tr>
<td>90 (4)</td>
<td>58.24 ± 5.35</td>
<td>173.21 ± 18.29</td>
<td>2.91 ± 0.21</td>
<td>0.72 ± 0.08</td>
<td>2.19 ± 0.25</td>
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<tr>
<th>Islets cultured in 3 mM glucose</th>
<th>Glucose content/dry tissue</th>
<th>Inulin content/dry tissue</th>
<th>Total glucose space/dry tissue</th>
<th>Inulin space/dry tissue</th>
<th>Intracellular glucose space/dry tissue</th>
</tr>
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<tbody>
<tr>
<td>23 (7)</td>
<td>21.49 ± 3.66</td>
<td>144.98 ± 19.42</td>
<td>1.15 ± 0.16</td>
<td>0.61 ± 0.08</td>
<td>0.56 ± 0.13</td>
</tr>
<tr>
<td>60 (4)</td>
<td>34.15 ± 2.18</td>
<td>136.02 ± 14.48</td>
<td>1.71 ± 0.11</td>
<td>0.57 ± 0.06</td>
<td>1.14 ± 0.14</td>
</tr>
<tr>
<td>90 (4)</td>
<td>57.53 ± 3.81</td>
<td>234.83 ± 36.36</td>
<td>2.88 ± 0.19</td>
<td>0.98 ± 0.15</td>
<td>1.59 ± 0.19</td>
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<tr>
<th>Islets cultured in 30 mM glucose</th>
<th>Glucose content/dry tissue</th>
<th>Inulin content/dry tissue</th>
<th>Total glucose space/dry tissue</th>
<th>Inulin space/dry tissue</th>
<th>Intracellular glucose space/dry tissue</th>
</tr>
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<tbody>
<tr>
<td>23 (7)</td>
<td>26.19 ± 3.97</td>
<td>196.42 ± 18.94</td>
<td>1.40 ± 0.17</td>
<td>0.82 ± 0.08</td>
<td>0.58 ± 0.15</td>
</tr>
<tr>
<td>60 (4)</td>
<td>42.13 ± 4.76</td>
<td>293.74 ± 15.09</td>
<td>2.11 ± 0.24</td>
<td>0.84 ± 0.06</td>
<td>1.36 ± 0.21</td>
</tr>
<tr>
<td>90 (4)</td>
<td>52.52 ± 4.17</td>
<td>210.54 ± 21.33</td>
<td>2.62 ± 0.21</td>
<td>0.98 ± 0.09</td>
<td>1.73 ± 0.15</td>
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*p < 0.05 compared with freshly isolated islets at the same time point.
*p < 0.01 compared with freshly isolated islets at the same time point.

Table II
A quantitative comparison of glucose uptake, glucokinase activity, and glucose usage of freshly isolated or cultured islets with 20 mM glucose concentration as the basis of comparison

Data shown here were obtained from a study of glucose induction of glucokinase, glucose usage, and glucose-induced insulin release in cultured pancreatic islets (11). The original data of glucose uptake, glucokinase activity, and glucose usage were expressed in terms of liters/kg, dry weight (this paper) or mol/kg of DNA/h (11). To facilitate the comparison with the results from various publications, the uniform expression of mmol/liter/min was used, and calculations are based on the following assumptions: (a) 20 ng of DNA/islet (27); (b) 3 nl of water volume/islet; 1 kg, wet weight, thus equals 0.75 liter of tissue water (28); and (c) 1 μg dry weight/islet (29).

Table II

<table>
<thead>
<tr>
<th>Glucose uptake activity usage</th>
<th>mmol/liter/min</th>
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<tr>
<td>Fresh islets</td>
<td>28.96 ± 0.45</td>
</tr>
<tr>
<td>Islets cultured in 3 mM glucose, 7 days</td>
<td>10.47 ± 0.22</td>
</tr>
<tr>
<td>Islets cultured in 30 mM glucose, 7 days</td>
<td>11.04 ± 0.98</td>
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It is obvious that the control strength of glucose transport in the regulation of glycolysis is very small in the normal freshly isolated islet.

After culturing islets for 7 days glucose transport was decreased to about one-third of that observed with freshly isolated islets no matter how much glucose was present in the medium (i.e. from 29 to 10 mmol/liter/min). Islets cultured for 7 days in 30 mM glucose had increased levels of GLUT-2, no GLUT-1 (Figs. 1 and 4), yet decreased levels of glucose transport. There are at least two possible explanations for this apparent dissociation of GLUT-2 expression and actual glucose transport. First, a fraction of GLUT-2 protein may be located at a site close to but not identical with the plasma membrane and may therefore be nonfunctional. High resolution electron microscopy could be helpful in testing this possibility (30). Second, GLUT-2 function might be altered for some other yet unknown reasons. An altered phosphorylation state is a possible explanation. For example, phorbol ester increased phosphorylation of the glucose transporter in cultured human fibroblasts, and this modification may have stimulated glucose transport (31).

The reduced rate of glucose uptake contrasts with a significant increase of glucokinase and glucose usage in the presence of high glucose (0.98 and 0.76 mmol/liter/min, respectively) but parallels the decrease of these two parameters in islets cultured in low glucose (0.22 and 0.18 mmol/liter/min, respectively) (Table II). Even though glucose transport was reduced in the islets cultured in high or low glucose its capacity exceeded by 10-fold the capacities of glucokinase and glycolysis of islets cultured in 30 mM glucose and by about 45-fold these two processes of islets cultured in 3 mM glucose. Similarly striking is a comparison of glucose-induced insulin release with glucose transport of freshly isolated islets or islets cultured in low or high glucose: glucose-stimulated insulin release which varies over a 50-fold range is not correlated with the capacities of islet tissue glucose transport or with the GLUT-2 level. It appears to be governed by other deter-
Glucose Transport in Cultured Islets

17247

minants. It needs to be appreciated that the first phase of glucose-stimulated insulin release, which is thought to depend on the rapid transport of glucose into the β-cell, is entirely preserved or even enhanced in islet cultured for 1 or 2 days in 30 mM glucose despite the apparent decrease of GLUT-2 in the β-cell membrane (11).

The data clearly demonstrate, however, that isolation and transfer of pancreatic islets to the organ culture condition and to low or high glucose concentration of the culture medium profoundly influence the expression of the glucose transporters GLUT-1 and GLUT-2. Three observations are remarkable: first, the rapid but transient appearance of GLUT-1 in the plasma membrane of islet β-cells; second, the transient drastic decline and rebound of GLUT-2 with duration of culture; and third, the opposite effect that high glucose has on these two phenomena, causing a lowering of GLUT-1 expression and an increase of GLUT-2 expression. It is noteworthy that immunohistochemical data and results of Western and Northern blot analysis provided an internally consistent picture.

The transient appearance of GLUT-1 in the β-cell membrane of pancreatic islets submitted to the organ culture condition, in particular when the glucose concentration was low, is very striking but puzzling. It seems that the stress of culturing rather than lowering of glucose initiated the induction of GLUT-1. The transient decline of GLUT-2 is similarly striking and also appeared to be caused by the isolation and transfer of pancreatic islets to extracorporal conditions. It seemed that high glucose merely blunted the impact of the insult. The GLUT-1 response seen here resembles the stress response observed in cultured cells by others (32). GLUT-1 and the glucose-regulated protein 78 were induced concomitantly in cultured L-8 skeletal muscle cells by low glucose and by various drugs, and they were suppressed by high glucose. The authors proposed that GLUT-1 belongs to the family of stress proteins and that its expression may serve a specific yet unknown purpose during cellular stress.

The present data are at variance with results of a recent publication which reported that GLUT-1 mRNA was present in freshly isolated islets and was induced by high glucose in islets cultured for 24–72 h (33). This same study also failed to notice the transient decline of GLUT-2 mRNA but concurred in showing that high glucose induced GLUT-2 mRNA. Available information is insufficient to explain the discrepancies.

The biological significance, if any, of the marked GLUT-1 and GLUT-2 changes of cultured pancreatic β-cells remains to be established. It is concluded, however, that the adaptive response of β-cells to high glucose, as manifested by greatly enhanced glucose metabolism and heightened glucose-induced insulin release, was not related to the effect of glucose on rapid glucose transport or on transporter expression. In fact the capacity of β-cell glucose transport was observed to be so far in excess of the rate of glucose metabolism that only very dramatic changes of the transporters could be expected to influence glucose metabolism and glucose-induced insulin release. It should be obvious that the islet culture system as used here is highly suitable for studying the regulation of GLUT-1 and GLUT-2 expression in pancreatic β-cells at the mRNA, protein, and immunohistochemical levels and for evaluating the functional significance of glucose transporter expression in a reliable manner not readily achieved in other experimental systems available to date.

REFERENCES


