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Transport and Metabolism of Glucose in an Insulin-Secreting Cell Line, βTC-1†

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ABSTRACT: Kinetic characteristics of glucose transport and glucose phosphorylation were studied in the islet cell line βTC-1 to explore the roles of these processes in determining the dependence of glucose metabolism and insulin secretion on external glucose. The predominant glucose transporter present was the rat brain/erythrocyte type (Glut1), as determined by RNA and immunoblot analysis. The liver/islet glucose transporter (Glut2) RNA was not detected. The functional parameters of zero-trans glucose entry were K_m = 9.5 ± 2 mM and V_{max} = 15.2 ± 2 nmol min^{-1} (μL of cell water)$^{-1}$. Phosphorylation kinetics of two hexokinase activities were characterized in situ. A low-K_m (0.036 mM) hexokinase with a V_{max} of 0.40 nmol min^{-1} (μL of cell water)$^{-1}$ was present along with a high-K_m (10 mM) hexokinase, which appeared to conform to a cooperative model with a Hill coefficient of about 1.4 and a V_{max} of 0.3 nmol min^{-1} (μL of cell water)$^{-1}$. Intracellular glucose at steady state was about 80% of the extracellular glucose from 3 to 15 mM, and transport did not limit metabolism in this range. In this static (nonperifusion) system, 2-3 times more immunoreactive insulin was secreted into the medium at 15 mM glucose than at 3 mM. The dependence of insulin secretion on external glucose roughly paralleled the dependence of glucose metabolism on external glucose. Simulations with a model demonstrated the degree to which changes in transport activity would affect intracellular glucose levels and the rate of the high-K_m hexokinase (with the potential to affect insulin release).

Much evidence has been advanced that the signal for insulin secretion is generated by the metabolism in β cells of the fuels which act as secretagogues (Meglasson et al., 1986). In isolated islets, it appears that transport of glucose does not significantly limit glucose metabolism, so the responsiveness of insulin secretion to extracellular glucose concentration would reflect the kinetics of the hexokinases present, which include the high-K_m glucokinase. In HIT-T15 cells, the response of insulin secretion to external glucose was inhibited 90% by the glucose-transport inhibitor phloretin, suggesting that transport might be a limiting step influencing the response to external glucose (Ashcroft & Stubbs 1987). The existence of β-cell lines with various glucose transport characteristics and glucose phosphorylation characteristics affords further opportunity to test the hypothesis that metabolism of the secretagogue fuel generates the secretory signal and to investigate the roles of early steps as determinants of the response to external glucose.

We have been developing techniques for characterizing glucose transport, glucose phosphorylation, and the interaction between them in various cell preparations including cultures (Whitesell et al., 1989a,b, 1990). In the present studies, those techniques were applied to the βTC-1 cell line which is known to increase its insulin release in response to increased medium glucose.

Experimental Procedures

Cell Culture and Incubations. βTC-1 cells (obtained from Dr. Doug Hanahan; Efrat et al., 1988) were grown in 35-mm culture dishes in Dulbecco's minimum essential medium (4.5

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g/L glucose) (Difco) supplemented with 10% fetal calf serum, and with additions of penicillin and streptomycin. Cells were used in the assays at 75% confluence.

Hepes-buffered salt solution (medium A) was prepared with distilled–deionized water and contained 128 mM NaCl, 10 mM Hepes, 1.4 mM $MgSO_4$, 5.2 mM KCl, 1.03 mM NaH_2PO_4, and 1.4 mM $CaCl_2$ and was adjusted to pH 7.4 with NaOH. For incubation, medium A was supplemented with 0.5% bovine serum albumin and 0.2 mM glucose (medium B).

Transport Assays. All manipulations of cells prior to fixation were carried out in an Isolette infant incubator at 37 °C on a rotating shaker platform (Tekmar) at 17 cycles/s. Prior to the transport assay, growth medium was removed by aspiration from the dish and exchanged with 1 mL of medium B 3 times at 10-min intervals to deplete intracellular glucose and extracellular insulin. To initiate the glucose transport assay, the medium was aspirated, and isotopic hexose solution (600 μL) was added to the cells. Isotopic solutions were prepared with medium B and contained 2 μCi/mL L-[^{3}H]-glucose (NEN) and 0.5 μCi/mL D-[^{14}C]glucose (NEN), with the indicated concentrations of unlabeled glucose. The reaction was stopped by pipetting 2 mL of chilled stop solution (medium A containing 200 μM phloretin). For sampling times under 10 s, the additions of both isotope and stop solution were timed by metronome. The stop solution was aspirated immediately, and the dish was rinsed 2 additional times with 2 mL of stop solution. Immediately after the dish was rinsed, 1 mL of 0.1 M NaOH was added to fix and extract the cells. The contents of the dish were suspended, and a 700-μL aliquot was transferred to a 20-mL scintillation vial. To this was added 5 mL of aqueous scintillation fluid, and all samples (including counting standards and a 10-μL sample of the isotopic hexose solution) were counted under identical conditions for double-label counting. In a few parallel incubations, 3-*O*-[^{14}C]-methyl-D-glucose was present instead of D-[^{14}C]glucose, its equilibrium volume (after 1 h) being taken as a measure of sugar-accessible cell water.

The conversion of intracellular [^{14}C]glucose to ^{14}C-labeled metabolites (which must remain in the cell until they are converted via numerous steps to CO_2, lactate, or pyruvate) tended to linearize the uptake time course. These time courses were fitted by nonlinear regression to an exponential and a linear component (Whitesell et al., 1990), with appropriate weighting of several early points from 2 to 20 s and recognition of early curvature. Subtracting the extracellular radioactivity remaining after washing of the culture plates was accomplished by use of the extracellular marker L-[^{3}H]glucose and improved the precision of the early points. The correlation of the time courses to the models was greater than 0.95 in each case.

Metabolic Assays. Cells were incubated as for transport assays except that 1900 μL of medium B containing various concentrations of glucose was added after rinsing away the growth medium. A preincubation of 10 min was found to be adequate to attain a steady state of uptake. After the preincubation, a 100-μL aliquot containing 0.2 μCi of [5-^{3}H]- or [2-^{3}H]glucose was added. At times from 10 min to 3 h thereafter, 200-μL samples of medium were fixed in 400 μL of 2-propanol. Minicolumns containing 0.8 mL of Dowex converted to the borate form were prepared for separating ^3H_2O and [^{3}H]glucose with 99.5% efficiency (Hammerstedt, 1973). A 100-μL aliquot of the sample was applied to a column and eluted with 3 mL of water. To correct for the [^{3}H]glucose which ran through the column, 0.01 μCi of [^{14}C]glucose was added to the sample before it was placed on the column. Scintillant (10 mL) was added to the collected effluent for double-label counting. Linearity and rate of increase of ^3H_2O were determined by linear regression.

Measurement of Insulin Secretion. Aliquots (200 μL) for insulin measurement were taken from the same incubations where ^3H_2O was measured, to ensure identical conditions for the two types of assays. A solid-phase ^{125}I radioimmunoassay was used with rat insulin standards (Diagnostic Products Corp.). The assay did not distinguish between proinsulin and insulin.

RNA Analysis. Total RNA was isolated from βTC-1 cells or normal rat liver by homogenization in guanidinium isothiocyanate and centrifugation through a 5.7 M cesium chloride cushion. Poly(A) RNA was isolated from total RNA using the Fastrack Isolation kit from InVitrogen. RNA was quantitated by the absorbance at 260 nm.

RNA was size-fractionated on a formaldehyde–1.5% agarose gel and transferred to a nylon membrane (Nytran, Schleicher & Schuell). The membrane was prehybridized in 5× Denhardt's [0.1% poly(vinylpyrrolidone), 0.1% bovine serum albumin, and 0.1% Ficoll 400], 5× SSC (0.75 M NaCl and 0.075 M sodium citrate), 0.1% SDS, and 50% formamide at 42 °C for 2 h, and then the labeled glucose transporter probe was added. After hybridization for 18 h at 42 °C, the membrane was washed twice in 1× SSC/0.1% SDS at 60 °C for 30 min. The membrane was exposed for autoradiography for 1–5 days. To probe for the second glucose transporter species, the membrane was boiled twice for 5 min in 0.1× SSC/0.1% SDS. Repeat autoradiography following boiling demonstrated that none of the first probe remained. The membrane was then prehybridized as described above and then the second glucose transporter probe added. Hybridization, washing, and autoradiography were performed as described above.

Glucose transporter cDNAs were gifts: rat liver/islet glucose transporter (Glut2, Thorens et al., 1988) from Drs. Bernard Thorens and Harvey Lodish; and rat brain glucose transporter (Glut1; Birnbaum et al., 1988) from Dr. Ora Rosen. The Glut2 probe was a 1500 bp *Eco*RI fragment. The Glut1 probe was a 1500 bp *Bgl*II/*Xho*I fragment. DNA fragments were separated by agarose electrophoresis and random primer labeled with the BRL random priming kit (BRL, Bethesda, MD) and [α-^{32}P]dATP to a specific activity of (0.5–1.0) × 10^9 cpm/μg of DNA.

Immunoblotting. Total cellular membranes were isolated from βTC-1 cells following lysis in 5 mM Tris (pH 8.0) containing 0.1 mM diisopropyl fluorophosphate (DIFP). The residue was scraped from the plate and homogenized by hand with 30 strokes in a glass homogenizer. The homogenate was centrifuged at 600*g* for 10 min at 4 °C. The supernate was centrifuged at 100000*g* for 30 min at 4 °C. The membrane pellet was resuspended in 50 mM Tris (pH 8.0), 1 mM EDTA, and 0.1 mM DIFP. Protein concentration was quantified by the Bio-Rad assay. Total membrane protein was separated by SDS–polyacrylamide gel electrophoresis (10% gel) and electrotransferred (Transblot apparatus, Bio-Rad, 70 V × 5 h) to a poly(vinylidene difluoride) membrane (Immobilon, Millipore). The membrane was incubated in Tris-buffered saline plus 0.05% Tween-20 (TBST) containing 5% powdered milk. The membrane was washed in TBST and incubated for 1 h at room temperature with a polyclonal antiserum (1:200) to the C-terminus of Glut1 (gift of Dr. Samuel Cushman). The membrane was washed in TBST and incubated for 1 h at room temperature with protein A–alkaline phosphatase (1:1000 in TBST) from Boehringer-Mannheim. Following three washes in TBST, the membrane was incubated with BCIP (5-bromo-4-chloro-3-indolyl phosphate *phtoluidine* salt)

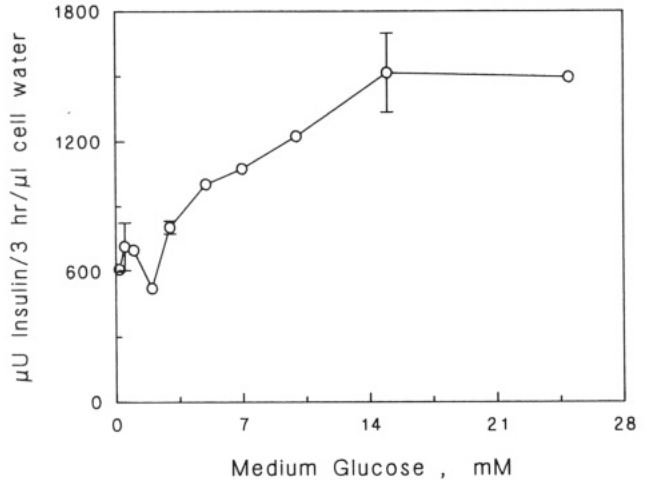

FIGURE 1: Dependence of insulin secretion on medium glucose concentration. The measurements shown are from incubations which include those for which metabolic data are shown in Table II. Samples for insulin were taken from duplicate incubations after the last sampling for ^3H_2O. Standard errors of the mean are given for values at 0.5, 3, and 15 mM glucose which represent five determinations at each concentration. Points without bars represent the means of duplicates.

and NBT (*p*-nitro blue tetrazolium chloride) in carbonate buffer (0.1 M, pH 9.8) to develop the alkaline phosphatase color. The color development was stopped by rinsing the membrane in distilled water.

RESULTS

Dependence of Insulin Secretion on Glucose Concentration. An insulin secretion assay was performed using cells which contained 0.5–1 μL of intracellular water per dish. Insulin was secreted continuously, and easily measurable increases in response to glucose above 3 mM occurred as early as 10 min after a change of medium. As seen in Figure 1, insulin secretion at maintenance glucose concentrations (0.2–2 mM) was about one-third of that with the highest concentrations (15–25 mM), the response between 3 and 15 mM being approximately linear. Glucose concentrations below 0.2 mM were not tested, since any effects observed might be associated with cell damage.

Identification of the βTC-1 Cell Glucose Transporter by Northern Blot and Immunoblotting. RNA [total and poly(A)] was probed with the Glut1 and Glut2 cDNAs. No Glut2 RNA was detected in either the total or the poly(A) fraction (Figure 2A, top). The βTC-1 cells contain Glut1 RNA, which was detected only with poly(A) RNA and was not seen in total RNA (Figure 2A, bottom). The hybridization conditions (see Experimental Procedures) were specific for distinguishing the two glucose transporter mRNA species.

The βTC-1 cells contain the Glut1 protein as shown by the presence of a 55-kDa protein that reacts with a polyclonal antiserum specific for this glucose transporter species (Figure 2B).

Kinetic Characterization of Glucose Transport. Glucose transport kinetics (zero-*trans*) of βTC-1 cells were determined with the native substrate D-glucose at 37 °C by measuring the initial rate of uptake (2–20-s samples) at various extracellular glucose concentrations using methodology presented in detail elsewhere (Whitesell et al., 1990). Nine experiments were analyzed, with five shown in Figure 3. The $[S]/v$ vs [S] plots exhibited a positive slope and no consistent bend, indicating entry only by a single saturable entry process (no unsaturable entry, no second saturable entry process). The mean parameters are given in Table I.

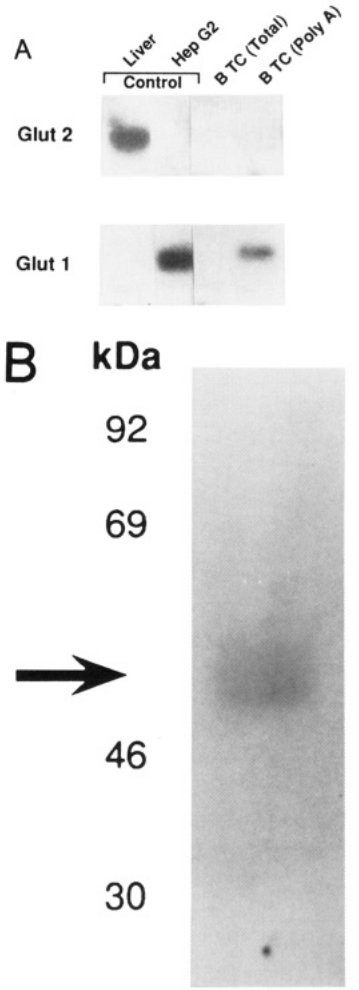

FIGURE 2: (A) Analysis of βTC-1 RNA with Glut2 and Glut1 probes. RNA was separated, transferred, and probed for Glut2 mRNA (top panel) as described under Experimental Procedures. Total RNA (20 μg) and poly(A) RNA (5 μg) from βTC-1 are shown. After being exposed for autoradiography, the first probe was removed from the membrane which was then probed for Glut1 mRNA (bottom panel). (B) Immunoblotting of βTC-1 cell membranes for Glut1. Total membranes were isolated from βTC-1 cells, and 100 μg of protein was fractionated for immunoblotting with a polyclonal antiserum specific for Glut1 as described under Experimental Procedures. The positions of molecular mass markers are shown in kilodaltons, and the position of the GT protein is marked with an arrow.

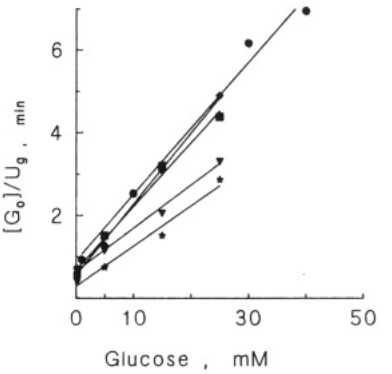

FIGURE 3: Dependence of glucose entry on external glucose concentration. Points from five separate experiments are identified by symbols. The experimental points represent reciprocals of initial rates of glucose clearance determined from time courses as described under Experimental Procedures. Individual experiments were analyzed for K_m and V_{max} by linear regression, and the results were included in Table I.

Transport activity in βTC-1 cells was tested after overnight incubation with glucose concentrations of 2, 12, and 25 mM

Table I: Kinetic Parameters of Glucose Transport and Metabolism

		hexokinases[b]	
	transport[a]	1	2
K_m (mM)	9.5 ± 2	0.036 (0.14)	10 (20)
V_{max} (nmol min^{-1} μL^{-1})	15.2 ± 2	0.40 (0.42)	0.29 (0.9)

[a] Transport parameters are given ± standard error of the mean for nine determinations. [b] Results from the curve-fitting shown in Figure 4A are shown in parentheses. Low and high K_ms are apparent half-saturation constants for glucose utilization which paralleled phosphorylation (Results). The values outside of parentheses were computed according to intracellular glucose concentration as demonstrated in Figure 4C. This characterization included a Hill coefficient of 1.4 according to eq 4.

(data not shown). This range of treatments led to no differences of the transport activity in βTC-1 cells, in contrast to previous results with 3T3-L1 cells where glucose transport activity was reduced 80% by preincubation in 25 mM glucose (Whitesell et al., 1990).

Glucose Phosphorylation and Glycolytic Rate. The exchange of ^3H with H_2O from [5-^{3}H]glucose is a sensitive assay for production of triose phosphates from glucose (Brown & Garratt, 1974). Metabolism of glucose to glycogen does not result in ^3H_2O from this isotope. The appearance of ^3H_2O from [2-^{3}H]glucose is a sensitive assay for glucose phosphorylation (Katz & Dunn, 1967) since tritium is transferred to H_2O during the rapid isomerization of glucose 6-phosphate and fructose 6-phosphate. Incomplete tritium release in this case would result in production of tritiated anions, which were not detected (data not shown). Significant metabolism of glucose to glycogen would be detected by an excess of ^3H_2O produced from [2-^{3}H]glucose over that from [5-^{3}H]glucose. Hydrolysis of glucose 6-phosphate (futile cycling) would also be detected by an excess of ^3H_2O from [2-^{3}H]glucose relative to [5-^{3}H]glucose. Metabolism of the two isotopes was measured at 1, 3, and 15 min (not shown). No significant differences in ^3H_2O production from [2-^{3}H]glucose and [5-^{3}H]glucose were detected, suggesting that glycogen deposition and futile cycling of glucose 6-phosphate were not important components of glucose metabolism in βTC-1 cells. A similar conclusion was also reached in studies of pancreatic islets (Ashcroft et al., 1972). These results confirmed that tritium release from [5-^{3}H]glucose faithfully reflected glucose metabolism.

Dependence of Glucose Metabolism on Extracellular Glucose Concentration. Figure 4A shows ^3H_2O production from [5-^{3}H]glucose over a wide range of medium glucose concentrations. The results were consistent with two parallel pathways at the rate-limiting step, one with a low K_m and the other with a high K_m. Since the highest rates of metabolism were less than 0.1 of the transport V_{max}, glucose phosphorylation is likely the rate-limiting step exhibiting the kinetic heterogeneity. The following paragraphs analyze the interaction between the transport and metabolism of glucose in more depth.

Calculation of Intracellular Glucose Concentration. The glucose transport rate law involves two or more constants and three variables (extracellular glucose concentration, $[G_o]$, intracellular glucose concentration, $[G_i]$, and the rate of glucose uptake at the steady state, U_g):

$$U_g = \frac{([G_o] - [G_i])F_g}{1 + [G_o]/K_{go} + [G_i]/K_{gi} + [G_i][G_o]/R_gB_g} \quad (1)$$

where F_g is the activity coefficient (V_{max}/K_m), K_{go} is the entry K_m, K_{gi} is the exit K_m, B_g is the equilibrilium exchange K_m, and R_g is the counterflow K_m. Only four of the five constants

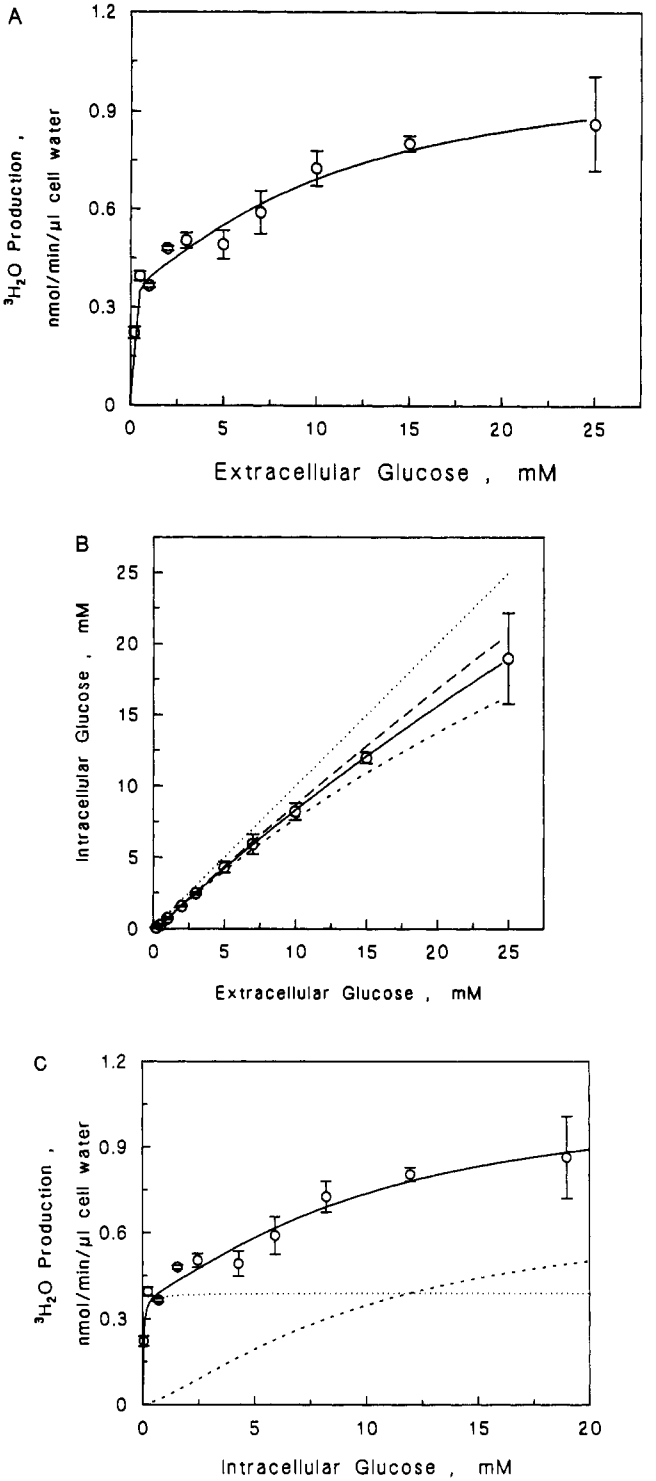

FIGURE 4: Coupling analysis of glucose transport and phosphorylation. (A) Rates of ^3H_2O released from [5-^{3}H]glucose as a function of extracellular glucose concentration. The line drawn through the points is the result of a two-component nonlinear regression to a modification of eq 4 lacking the exponent h. (B) Dependence of intracellular glucose concentration on extracellular glucose concentrations. Symbols and the solid line represent intracellular glucose calculated using eq 3 and assuming $B_g = R_g = K_{gi} = K_{go} = 9.5$ mM. The dashed lines were generated to evaluate the effect of the following assumptions: R_g and $K_{gi} = 19$ mM (long dashed line); R_g and $K_{gi} = 4.75$ (short dashed line). K_{go} and R_g were kept constant at 9.5 mM since this was experimentally demonstrated. Equality of intracellular and extracellular glucose concentrations is shown by the dotted line. (C) Rates of ^3H_2O release plotted as a function of intracellular glucose concentration. The data were fit by nonlinear regression to eq 4, including the exponent h. The fit to the data (solid line) reflects two component reactions: a hexokinase-type reaction (dotted line) and a glucokinase-type reaction (dashed line).

are independent, since those in the denominator of eq 1 are related:

$$1/K_{go} + 1/K_{gi} = 1/B_g + 1/R_g \quad (2)$$

Equation 1 was solved for intracellular glucose concentration so that it could be predicted from the known variables and constants:

$$[G_i] = \frac{F_g[G_o] - U_g - U_g[G_o]/K_{go}}{U_g/K_{gi} + U_g[G_o]/R_gB_g + F_g} \quad (3)$$

[This equation is related to one developed earlier (Kotyk & Janacek, 1975).]

For certain cell types, especially at lower temperatures, a significant quantity of a transported hexose on the inside of the cell will exert a stimulation of influx (trans effect). This occurs when outward reorientation of the occupied sugar binding site is quicker than that of the empty site. In the human and rat erythrocyte, this asymmetry is reflected in differences among the K_ms: $B_g > K_{go}$ and $K_{gi} > R_g$ (Lowe & Walmsley, 1986; Whitesell et al., 1989). A trans effect, when present, is greatly diminished at 37 °C, which was the temperature of all experiments done on βTC-1 cells. No trans effect was observed when transport was measured after the cells were preloaded with methylglucose. The absence of a trans effect implies that $B_g = K_{go}$, which was estimated to be 9.5 mM. From eq 2, it follows that $R_g = K_{gi}$. We have no measure of R_g and K_{gi}, but simulation showed this omission was not critical. We calculated $[G_i]$ with three values of K_{gi} and R_g (4.75, 9.5, and 19 mM). As seen in Figure 4B, the calculated $[G_i]$ was only about 10–20% less than $[G_o]$ and was essentially the same with each named value of K_{gi} and R_g. Thus, transport activity was in great excess as expected from the relative V_{max}s of transport and metabolism, and the entry of glucose did not significantly limit glucose metabolism at any concentration tested.

Characterization of Glucose Phosphorylation. Because $[G_i]$ in βTC-1 cells was only slightly lower than $[G_o]$, glucose phosphorylation characteristics are reflected rather faithfully in the plot of rate versus $[G_o]$ (Figure 4A). However they should be revealed more accurately in a plot of rate versus $[G_i]$, as shown in Figure 4C. The dependence of glucose phosphorylation on cell glucose concentration was modeled with the following two-component rate equation:

$$U_g = \frac{[G_i]V_1/K_1}{1 + [G_i]/K_1} + \frac{[G_i]^hV_2/K_2^h}{1 + [G_i]^h/K_2^h} \quad (4)$$

The solid line in the figure represents the best least-squares fit according to eq 4 with $[G_i]$ values from eq 3. The high-affinity pathway exhibited a V_{max} (V_1) of 0.40 nmol min^{-1} (μL of cell water)$^{-1}$ and a K_m (K_1) of 0.036 mM (dotted line). The low-affinity pathway exhibited a V_{max} (V_2) of 0.29 nmol min^{-1} (μL of cell water)$^{-1}$ and an apparent K_m (K_2) of 10 mM with a Hill coefficient (h) of 1.4 (short dashed line). These data are consistent with measurements of glucose phosphorylation in islet supernatants showing a high- and a low-affinity component with K_m's of 47 μM and 10.4 mM, respectively, and with cooperativity of the low-affinity component for glucose (Meglasson et al., 1982).

Sensitivity of $[G_i]$ and U_g to Transport Activity. Equations 1 and 4 were solved simultaneously to predict $[G_i]$ and U_g as functions of $[G_o]$ with various transport activities. A 40× increase of the transport V_{max} would increase $[G_i]$ and U_g only about 10% above observed values, illustrating that transport does not significantly limit utilization. A 50% reduction of transport activity would result in only a 10% reduction of total glucose utilization. Sensitivity is the fractional change of the dependent variable, utilization, relative to the fractional change of the independent variable, transport activity (Groen et al., 1982), so the sensitivity of glycolysis to transport is less than 0.2. However, the 50% reduction of transport activity would cause a 20% fall of cellular glucose and a similar fall in glucokinase rate, so sensitivities of cell glucose and glucokinase rate to transport activity are approximately twice that of glycolytic rate.

Discussion

Interaction of glucose transport and metabolism determines the level of intracellular glucose and is potentially of significance in glucose-sensing cells like the β-cells of the pancreas. The ability of pancreatic β cells to "sense" glucose and respond by appropriate secretion of insulin depends on the level of intracellular glucose and its phosphorylation by glucokinase (Meglasson et al., 1986; Magnuson, 1990). βTC-1 cells, derived from a transgenic insulinoma, appear to exhibit glucose sensitivity (Efrat et al., 1988) and were used in the present study as prototypes for analysis of the coupling of transport and metabolism of glucose and its relation to glucose sensitivity. Under the conditions used in our metabolic assays, insulin dependence on extracellular glucose was modest, but showed a 2–3-fold response between 3 and 15 mM glucose. The range of glucose concentrations over which insulin secretion was glucose-dependent resembled that of isolated islets in perifusion (Zawalich & Matschinsky, 1977), but the extent of the response was much smaller. βTC-1 cells in perifusion also exhibit a larger response to insulin secretion to a rise of glucose concentration than we observed in culture dishes, but the maximum response occurred at only 1 mM glucose (Efrat et al., 1988). These discrepancies may reflect the degree of glucose deprivation prior to raising the glucose concentration. Our cells were provided a maintenance concentration (0.2 mM) nearly saturating the low-K_m hexokinase while the cells of Efrat et al. were glucose-deprived during 40 min prior to addition of stimulatory concentrations.

Significance of the Native Substrate in Studies of Glucose Transport. Glucose rather than its analogues may be used as a transport substrate with the appropriate controls (Keller et al., 1981). This avoids the experimental bias introduced by differing substrate specificities among transporters. The problem with use of analogues is demonstrated by the rat white adipocyte, which provides an example of how the function of several transporters with different substrate specificities can complicate interpretation of regulatory effects on transport (Whitesell et al., 1989b). Glut1, Glut3, and Glut4 have been identified in adipose tissue (Kayano et al., 1990), but other species of glucose transporters may be present. One or all transporters are regulated acutely in ways which are poorly understood but include metabolic feedback and insulin stimulation. Because of these factors, the cells can exhibit one or more dominant kinetic characteristics depending on the conditions of prior incubation and hormonal stimulation. We tested transport in isolated adipocytes using methodology adapted for glucose as the substrate (Whitesell et al., 1989b). Under the specified conditions, a pattern of functional heterogeneity was found, probably from the activities of the distinct transporter species. Insulin under these conditions appears to change both the characteristics and the proportion of high- and low-affinity transporters. This leads to an overall increase in affinity of the system which has significance for intracellular glucose levels and metabolism. Much of this heterogeneity is obscured in experiments using methylglucose as the transport

substrate, possibly because the difference in affinity of the transporters toward methylglucose is not as great as the difference in affinity toward glucose. Use of glucose as the transport substrate eliminates these ambiguities and permits the correlation of transport and metabolic measurements. The present studies involved a cell with apparently homogeneous transport kinetics toward glucose, which simplifies the interpretation of metabolic studies.

Dependence of Intracellular Glucose Concentration and Metabolism on Transport Activity. Coordinate measurements of glucose transport and metabolism in the βTC-1 cell yielded the necessary parameters with which to model coupling of transporter to hexokinase and glucokinase. The information gained from the model was used to predict the dependence of glucose metabolism on extracellular glucose with various transport activities. A multifold increase of transport activity would not increase metabolism significantly at physiological glucose concentrations, an expression of the fact that transport is not a rate-limiting step in these cells. A 90% reduction of transport activity would reduce overall utilization only 30% at physiological glucose concentrations. Reductions in intracellular glucose and the high-K_m component of metabolism would be more significant than the reduction in overall glucose utilization.

In some cultured cells (Ullrey et al., 1982) including 3T3-L1 cells (Whitesell et al., 1990), overnight exposure to high glucose concentrations results in marked down-regulation of glucose transport which should affect sensitivity to external glucose. We were unable to detect this phenomenon in βTC-1 cells.

High-K_m Glucose Utilization. Glucokinase is thought to be the "glucose sensor" of pancreatic islets because it is not saturated at millimolar concentrations of intracellular substrate. Other studies have investigated the relative activities of high- and low-K_m hexokinases. In normal intact islets, metabolic characterizations assuming nonresistive transport showed that the low-K_m hexokinase activity is powerfully suppressed by product inhibition to less than a tenth of its potential activity so that the ratio of high- to low-K_m activity is 7 [from Trus et al. (1981)]. The full potential and kinetic properties of each hexokinase are seen in supernatants of cell extracts, where the enzymes are released from the constraints of transport and product inhibition (Shimizu et al., 1988). In the present studies, glucose phosphorylation kinetics were explored, using glucose transport characteristics to predict intracellular glucose concentrations associated with each external glucose concentration and phosphorylation rate. This allows definition of phosphorylation properties in situ and provides the basis for evaluating the importance of possible regulations of transporter activity when coupled to heterogeneous phosphorylation. Equation 4 fit the data of Table II and suggested some cooperativity of the high-K_m activity (Hill coefficient >1). Since low-K_m hexokinase is actually product-inhibited and glucokinase is relatively uninhibited, this analysis would underestimate the V_{max} and cooperativity of the high-K_m pathway. That is, the true V_{max} of the high-K_m hexokinase would be approximately the sum of the two estimated V_{max}s. These reservations do not affect the value of the model for describing the sensitivity of metabolism to possible transport modulations.

Cultured cell lines can provide a means to study the importance of both transporters and hexokinases to insulin secretion. For example, HIT cells secrete insulin in response to glucose, but low transport activity appears to limit the availability of intracellular glucose so that transport rather than glucokinase may determine insulin release (Meglasson et al., 1986; Ashcroft & Stubbs, 1987). Another cell line, RIN m5F, transports glucose very rapidly (Meglasson et al., 1986) but has negligible glucokinase, and its insulin secretion is unresponsive to glucose. The transporter type coupled to the hexokinase may be important as well. The dominant glucose transporter in βTC-1 cells was Glut1. Glut1 is characterized by a zero-trans entry K_m of 5–10 mM (at 37 °C) for glucose influx in human erythrocytes and various cultured cells. Glut2, found in pancreatic islets (Thorens et al., 1988; Orci et al., 1989; Johnson et al., 1990), has a higher K_m for glucose, 20–50 mM, and is most suitable for glucose-sensing tissues, since glucose influx is virtually proportional to extracellular glucose in the physiological range. A recently developed cell line, MIN6 (Miyazaki et al., 1990), expresses Glut2 almost exclusively, and its insulin secretion responds to glucose in the physiological range, as measured in a static assay similar to that employed here. A sister cell line, MIN7, expresses Glut1 and is unresponsive to glucose. The present results suggest that mere lack of Glut2 may not explain these differences, which may have more to do with the nature of the coupling of transport and glucokinase activities. βTC-1 cells possess more than enough glucose transport activity to accomplish efficient equilibration of extra- and intracellular glucose concentrations without Glut2, but the ratio of high- to low-K_m hexokinases was lower than it is in islets. Application of the analyses presented here in combination with techniques of molecular biology will present the opportunity to test whether the proportion of high- and low-K_m hexokinases and the coupling to the proper transporter are both essential to physiological glucose sensing by the β cell.

Conclusions. In βTC-1 cells, glucose transport occurred in excess, and the glucose concentration in the cell was close to that in the medium over a large range. Intracellular glucose would fall off rather steeply with reductions of transport activity below 20% of normal, and the dependence of glucokinase's activity on extracellular glucose would be shifted to the right. Insofar as insulin secretion is based on glucokinase activity, its dependence on extracellular glucose (lower curve in Figure 4C) would be shifted to the right by down-regulation of glucose transport. The extent to which transport would affect total glucose phosphorylation would be determined by the relative activities of high- versus low-K_m hexokinase as determined in situ. The ratio of high-K_m hexokinase activity to low-K_m activity was lower in βTC-1 cells and correlates with

Table II: Transport, Metabolism, and Intracellular Levels of Glucose with Increasing Medium Concentrations

extracellular glucose concn (mM)	intracellular[a] glucose concn (mM)	metabolism[b] (nmol min^{-1} μL^{-1})	influx (nmol min^{-1} μL^{-1})
0.2	0.056	0.22 ± 0.02	0.31
0.5	0.233	0.39 ± 0.01	0.75
1.0	0.722	0.37 ± 0.02	1.42
2.0	1.569	0.48 ± 0.01	2.6
3.0	2.469	0.50 ± 0.02	3.5
5.0	4.298	0.49 ± 0.04	5.1
7.0	5.932	0.59 ± 0.07	6.2
10.0	8.222	0.73 ± 0.05	7.5
15.0	11.98	0.80 ± 0.02	8.9
25.0	18.98	0.86 ± 0.14	10.4

[a] Intracellular glucose was calculated from the measured metabolic rate and transport coefficients using eq 3. [b] Metabolic rate was measured from the appearance of ^3H_2O from [5-^{3}H]glucose. Samples were taken at 20, 40, 60, and 180 min and corrected for the reductions of extracellular volume caused by sampling. They were normalized to intracellular water volume and analyzed by linear regression.

their less sensitive insulin secretion by comparison to normal islets.

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Registry No. Glucose, 50-99-7; insulin, 9004-10-8; hexokinase, 9001-51-8; glucokinase, 9001-36-9.

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