Human and Rat Beta Cells Differ in Glucose Transporter but Not in Glucokinase Gene Expression

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Abstract

Glucose homeostasis is controlled by a glucose sensor in pancreatic β-cells. Studies on rodent β-cells have suggested a role for GLUT2 and glucokinase in this control function and in mechanisms leading to diabetes. Little direct evidence exists so far to implicate these two proteins in glucose recognition by human β-cells. The present in vitro study investigates the role of glucose transport and phosphorylation in β-cell preparations from nondiabetic human pancreata. Human β-cells differ from rodent β-cells in glucose transporter gene expression (predominantly GLUT1 instead of GLUT2), explaining their low K_m (3 mmol/liter) and low V_{MAX} (3 mmol/min per liter) for 3-*O*-methyl glucose transport. The 100-fold lower GLUT2 abundance in human versus rat β-cells is associated with a 10-fold slower uptake of alloxan, explaining their resistance to this rodent diabetogenic agent. Human and rat β-cells exhibit comparable glucokinase expression with similar flux-generating influence on total glucose utilization. These data underline the importance of glucokinase but not of GLUT2 in the glucose sensor of human β-cells. (*J. Clin. Invest.* 96:2489–2495.) Key words: glucokinase • GLUT2 • beta cells • insulin • diabetes

Introduction

In some patients with noninsulin-dependent diabetes mellitus (NIDDM)[1] the loss of glucose-induced insulin release is attributed to a defect in glucose sensing by pancreatic β-cells (1). The molecular nature of this defect has not yet been identified. In the absence of sufficient data on isolated human β-cells, current hypotheses have been derived from rodent insulin-producing β-cells. In rat β-cells the mechanism of glucose regulation requires intracellular catabolism of the sugar (2), with possible critical steps at the level of GLUT2, the liver-type glucose transporter (3), and glucokinase, the liver-type hexokinase isoform (4). Both proteins operate at proximal glycolysis and exhibit a high K_m for glucose. GLUT2 gene transfection confers glucose responsiveness to rat cell lines (5–7) while transgenic mice with down-regulated GLUT2 or glucokinase in β-cells exhibit disturbed glucose tolerance (8, 9). Parallel reduction of β-cell GLUT2 and loss of glucose-induced insulin release has been described in several rat and mouse models with spontaneous diabetes (10–12). Glucokinase gene mutations have been identified in families with maturity onset diabetes of the young (13) and in a subset of American blacks with NIDDM (14). On the contrary, no GLUT2 mutations have so far been observed in NIDDM (15), except for a single case in which heterozygosity was noticed for a conservative Val197 → Ile missense mutation (16). The present study on isolated human β-cells provides direct evidence for a role of glucokinase in the human glucose sensor, while the participation of GLUT2 could not be demonstrated.

Methods

Preparation of human islet beta cells. Human pancreatic tissue and islet cells were obtained as samples from a multicenter program on β-cell transplantation in diabetes (17) that has been approved by central and local ethical committees. The islets were isolated after collagenase perfusion and Ficoll gradient centrifugation (18). The cold preservation time of the organs was 10±3 h (mean±SD), while the islet isolation procedure took on average 5 h. The number of pancreata processed for islet or islet cell preparations used in this study was 23. The age of the donors (all nondiabetic) was 39±4 yr (mean±SE, range, 17–62 yr). The most frequent causes of death of the donors were intracranial hemorrhage (15:23) and severe head trauma (6:23). The dissociated islet cells (67±5% endocrine) contained 52±4% insulin-positive cells and 10±1% glucagon-positive cells. Viability of the cell preparations was 95±1%. Islet cells were either used freshly after isolation (Western blots) or after 2–5 d of culture in Ham's F10 medium, containing 6.1 mmol/liter glucose, supplemented with 0.5% BSA, 0.08 mg/ml penicillin, and 0.1 mg/ml streptomycin.

Glucose uptake, utilization, and phosphorylation. Initial rates of zero-*trans* [^{3}H]3-*O*-methyl glucose (3-OMG) uptake were measured over 30 s at 37°C using [^{14}C]urea as intracellular space marker and L-[^{3}H]glucose to correct for extracellular volume (19). Glucose metabolism was measured over 2 h incubations at 37°C (20). Cells were incubated in 100 μl Earle's-Hepes buffer containing the indicated concentrations of D-[5-^{3}H]glucose (25 μCi/ml) and D-[U-^{14}C]glucose (25 μCi/ml). The conversion of D-[5-^{3}H]glucose to ^3H_2O was measured simultaneously with the production of $^{14}CO_2$ from D-[U-^{14}C]glucose

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1. *Abbreviations used in this paper:* 3-OMG, 3-*O*-methyl glucose; NIDDM, noninsulin-dependent diabetes mellitus.

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(20). Cellular metabolism was stopped by the addition of 20 μl of 0.4 mol/liter citrate buffer, pH 4.9, containing 5 mmol/liter KCN, 10 μmol/liter antimycin A, and 10 μmol/liter rotenone. Hydroxy hyamine (Hewlett-Packard, Palo Alto, CA) was used to capture the produced $^{14}CO_2$. Tritiated water and $^{14}CO_2$ were measured via liquid scintillation counting. The radioactively labeled compounds were purchased from Amersham International, Little Chalfont, UK [D-[5-^{3}H]glucose (17 Ci/mmol), D-[U-^{14}C]glucose (298 mCi/mmol)] or from New England Nuclear, Boston, MA [L-[1-^{3}H]glucose [16 Ci/mmol], [^{14}C]urea [56 mCi/mmol] and 3-*O*-[^{3}H]methyl-D-glucose [87 Ci/mmol]). Glucose phosphorylation was determined fluorimetrically as described by Trus et al. (21). Enzyme activity was measured during 90 min incubations at 37°C both at ≤ 0.5 mmol/liter and at 1–40 mmol/liter glucose to distinguish low-K_m hexokinases from high-K_m glucokinase. *Leuconostoc mesenteroides* glucose 6-phosphate dehydrogenase was obtained from Boehringer Mannheim, Mannheim, Germany.

mRNA and protein analysis. Northern blot hybridization was performed as described before (19, 20). Autoradiographic exposure times required to generate the signals shown were 4 d (GLUT1), 6 d (GLUT2), 6 d (GLUT3), 42 d (glucokinase) and 8 h (β-actin). Liver glucokinase and β-actin mRNA signals were faint or absent with 2.5 μg total RNA but clearly present after blotting 10 μg RNA (not shown). The obtained radioactive signals were quantified via PhosphorImaging (Molecular Dynamics Inc., Sunnyvale, CA).

For the qualitative analysis of glucose transporter transcripts in human islet β-cells, 0.5 μg of total RNA was reverse transcribed and amplified using GeneAmp RNA-PCR kit (Perkin-Elmer Cetus Instruments, Emeryville, CA). Primers specific for the four glucose transporter isoforms were designed to anneal to specific sequences from GLUT1-5′ (codon 49–55), GLUT1-3′ (codon 263–269), GLUT2-5′ (codon 81–87), GLUT2-3′ (codon 295–301), GLUT3-5′ (codon 47–52), GLUT3-3′ (codon 262–267), GLUT4-5′ (codon 65–71), and GLUT4-3′ (codon 279–285), yielding PCR fragments of 663 bp.

Proteins were separated on 10% SDS-polyacrylamide gels and electroblotted to nitrocellulose membranes. The blotted filters were incubated with anti-GLUT1 (22) (1:1,000), anti-GLUT2 (23) (1:1,000), anti–rat glucokinase (24) (1:10,000), which recognizes both liver and islet cell glucokinase from rat and man, and anti-β-actin (1:1,000) for 60 min at room temperature (19). The ECL kit (Amersham International) was used for immunodetection. The signal intensities of the immunoblots were quantified via laser densitometry.

Immunohistochemical analysis of human tissue sections. Paraffin sections from formaldehyde-fixed human tissues were immunostained with anti-GLUT1 (1:200), anti-GLUT2 (1:50), anti-GLUT3 (1:100) or antiinsulin (1:1,000 mAb; BioGenex Laboratories, San Ramon, CA) and detection was done using the streptavidin-biotin-immunoperoxidase (ABC) technique. Pancreatic tissue blocks for immunocytochemistry were obtained from cold-preserved donor organs; furthermore, a partial pancreatic resection was obtained from a patient undergoing abdominal surgery for stomach cancer. Liver tissue from the same organ donors or liver biopsies were taken as positive control for GLUT2 immunostaining.

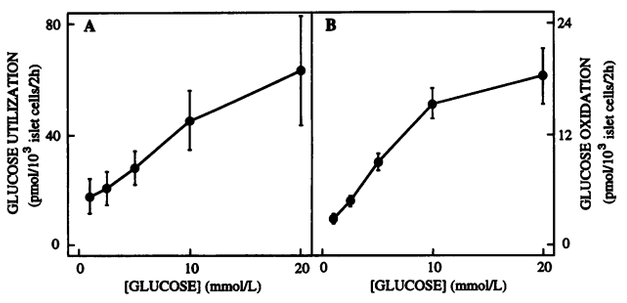


Figure 1. Glucose utilization (*A*) and oxidation (*B*) by human islet cells. Glucose utilization was measured as ^3H_2O production from [5-^{3}H] D-glucose and oxidation as $^{14}CO_2$ output from [U-^{14}C] D-glucose. Results are expressed as picomoles of glucose used or oxidized per 10^3 islet cells; data represent mean values±SE (n = 7).

Table I. Glucose Uptake, Phosphorylation, and Glycolysis in Human Islet Cells

[Substrate]	3-OMG uptake	Glucokinase activity*	Glycolysis
mM	*mmol/min per ltier*	*mmol/min per liter*	*mmol/min per liter*
5	2.2±0.2 (8)	0.38±0.15 (4)	0.33±0.07 (7)
10	2.9±0.3 (8)	0.60±0.20 (4)	0.52±0.11 (7)

All parameters were determined at 37°C. Mean values ±SE of (n) experiments are expressed per liter intracellular space (0.7±0.1 μl/10^6 cells; n = 10). * Glucokinase activity was calculated by subtracting hexokinase activity measured at 0.5 mmol/liter glucose (1.1±0.8 mmol/min per liter) from total glucose phosphorylation.

Alloxan uptake. Uptake of ^{14}C-labeled alloxan (2 mCi/mmol; Amersham International) was measured at 23°C, using [^{14}C]urea as intracellular space marker and L-[^{3}H]glucose as marker for extracellular space. The oxidation of ^{14}C-labeled alloxan to alloxanic acid was studied by TLC on 0.25 mm silica gel plates using 7:5:3 chloroform/acetic acid/ethanol (25) followed by autoradiography and laser scan densitometry of the spots. After 3 min incubation at 23°C in water 50% of radiolabeled alloxan had been oxidized.

Results

Metabolic properties of human islet cells. Human islet cells obtained from cultured isolated islets were incubated with various glucose concentrations to test their metabolic properties. Under these conditions, glucose induces a dose-dependent release of insulin (26). Cellular glucose utilization and glucose oxidation increased 4- to 6-fold when substrate levels were raised from 1 to 20 mmol/liter (Fig. 1). Compatible with their physiological role as glucose sensors, human islet cells exhibited glucose metabolism which was proportional to extracellular glucose levels between 5 and 10 mmol/liter (Fig. 1). Zero-*trans* uptake of the nonmetabolizable analogue 3-OMG was saturated half-maximally and maximally at 3 and 10 mmol/liter substrate, respectively, so that a 30% increase in transport was observed between 5 and 10 mmol/liter 3-OMG (Table I). The calculated uptake rates were ~ 10 times lower than those observed in rat islet cells (3, 20), yet exceeded metabolic flux through glycolysis by a factor of five, suggesting that transport is not rate limiting for overall glucose metabolism. On the other hand, high-K_m glucose phosphorylation via glucokinase proceeded at rates which were comparable to overall glycolysis in intact human islet cells (Table I); this glucokinase activity was similar to that in rat β-cells (20). Extracts of human islet cells were found to contain low-K_m hexokinases, which catalyzed more than 50% of total glucose phosphorylation at 20 mmol/liter glucose. This low-K_m phosphorylation was 80±5% suppressed by 2.5 mmol/liter glucose 6-phosphate (mean±SE; n = 4). When purified chromatographically using DEAE Sepharose, low-K_m hexokinase activity was 90% saturated at 1 mmol/liter substrate (data not shown). Fluorescence-activated sorting of endocrine and nonendocrine cells further showed that the low-

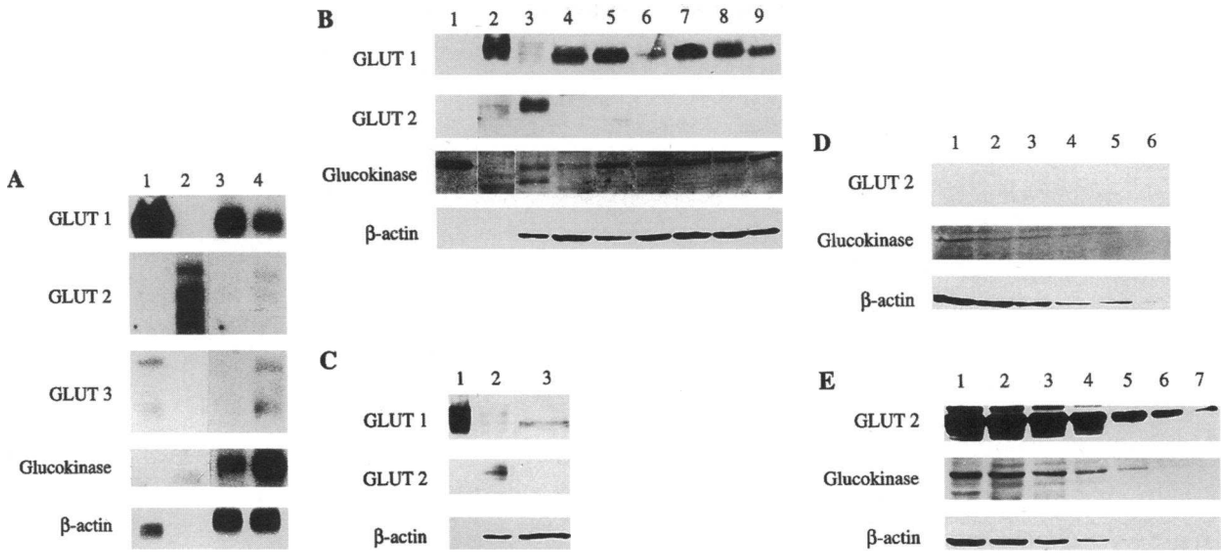


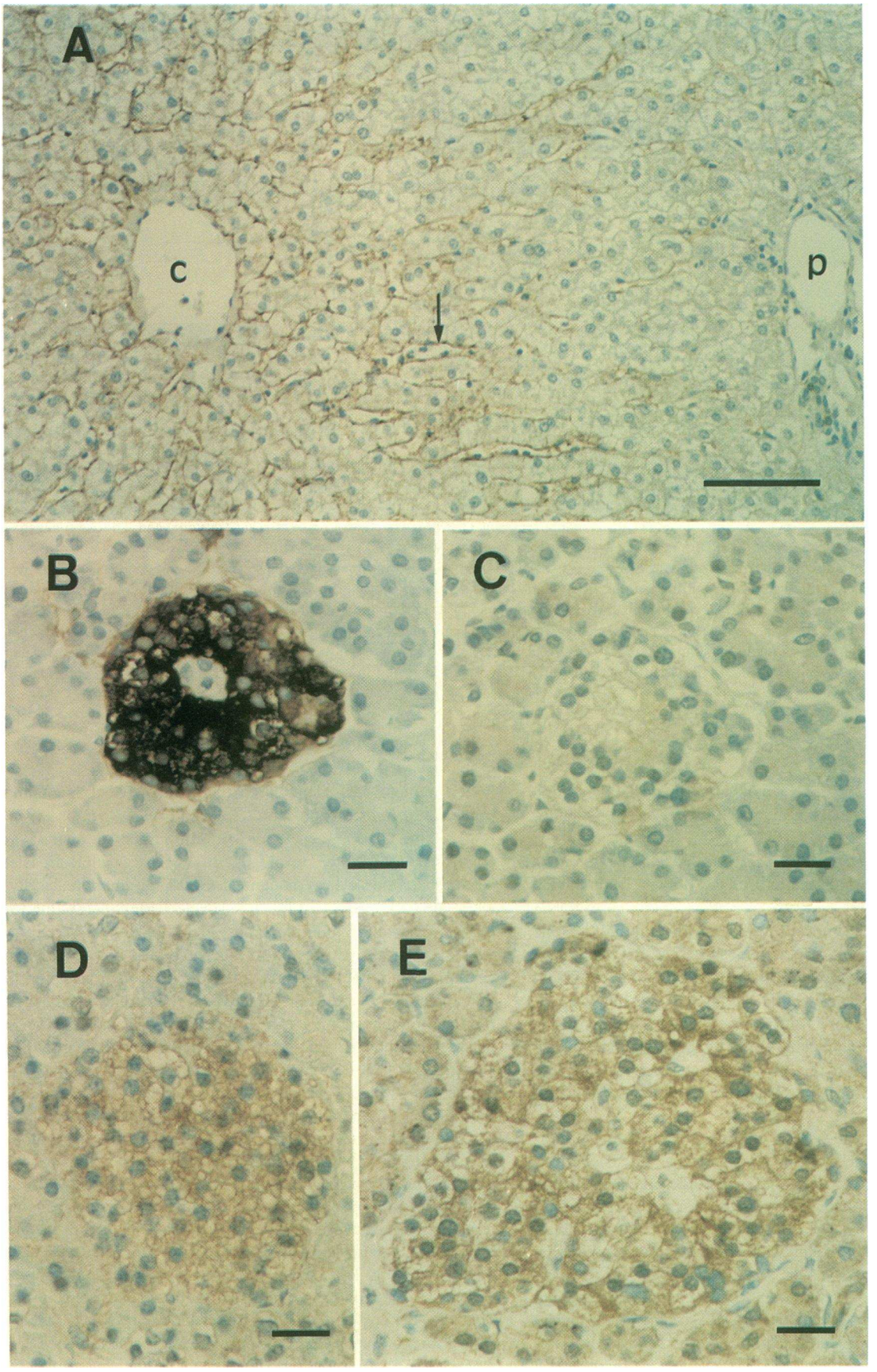
Figure 2. Glucose transporter and glucokinase expression in human islet cells. (*A*) Northern blots of total RNA from human placenta (lane *1*, 2.5 μg), liver (lane *2*, 2.5 μg), and two islet preparations (lanes *3* and *4*, 10 μg) hybridized with cDNA probes specific for human GLUT1, GLUT2, GLUT3, glucokinase, and β-actin. (*B*) Immunoblots of total protein extracted from cultured islet cells from six human pancreata (lanes *4–9*, 2.5 × 10^5 cell equivalents per lane). For GLUT1 immunoblotting, lane *2* represents the positive control (5 μg placenta protein) and lane *3* the negative control (20 μg of liver protein). The GLUT2 immunoblot was positive with liver protein (20 μg, lane *3*) but negative with placenta protein (20 μg, lane *2*). Glucokinase immunoreactive protein was detected using both 5 ng of purified rat liver glucokinase (lane *1*) and 25 μg of human liver protein (lane *3*) but undetectable using 25 μg of human muscle protein (lane *2*). Immunoblotting for β-actin was undetectable in 20 μg of human placenta protein (lane *2*) but positive with 20 μg of human liver protein (lane *3*). (*C*) Immunoblots of freshly isolated human islets (lane *3*, 2.5 × 10^5 cell equivalents) with 20 μg placenta protein (lane *1*) and 20 μg liver protein (lane *2*) as controls. Immunoblots of freshly isolated human (*D*) and rat (*E*) islet cells were examined undiluted (lane *1*, 5 × 10^5 islet cell equivalents) or 2-, 4-, 8-, 16-, 32-, and 64-fold diluted in sample buffer (lanes *2–7*) before electrophoresis. In a second experiment GLUT2 was still detected in a 128-fold diluted sample of rat islet cells (not shown).

K_m hexokinase activity observed in the islet cell preparation was mainly (85±5%; mean±SE; $n = 3$) localized in nonendocrine cells.

Glucose transporter and glucokinase gene expression by human islet cells. Northern blots of total RNA from human islets hybridized to GLUT1, GLUT3, and glucokinase cDNA probes; GLUT2 signal intensity was very low and only visible after prolonged autoradiographic exposure (Fig. 2 *A*). As positive control for the blots, human placenta RNA (2.5 μg) hybridized with the GLUT1 and GLUT3 probes, while human liver was strongly positive for GLUT2. The 2.5 μg liver RNA was close to, or below, the detection limit to visualize glucokinase and β-actin transcripts (Fig. 2 *A*); however, clear signals were observed after blotting 10 μg of liver total RNA (data not shown).

Quantification of the hybridization signals by phosphorimaging resulted in GLUT2 over β-actin signal intensity ratios which were at least two orders of magnitude higher in human liver tissue than in human islets. In contrast, glucokinase over β-actin signal ratios were comparable in human islets and liver. The mRNA hybridization data were confirmed by amplification of reverse-transcribed RNA (reverse transcriptase-PCR), performed with RNA from five human islet preparations: abundant GLUT1, GLUT3, and glucokinase cDNA fragments of appropriate length were reproducibly obtained after 40 cycles of amplification, while only weak GLUT2 signals were observed (data not shown). The sequence of the cloned 663-bp GLUT2 PCR fragment was identical to the published corresponding sequence of human pancreatic GLUT2 (27).

The low GLUT2 gene expression in human islet cells was confirmed at the protein level (Fig. 2 *B*). All six pancreatic islet cell preparations clearly contained GLUT1 immunoreactivity, while the GLUT2 protein was not detected. Immunoblotting for GLUT3 resulted in multiple bands, including a 45–48 kD protein that was also expressed in placenta (data not shown). In agreement with the RNA data, glucokinase protein was present in comparable quantities in human islet tissue and liver (Fig. 2 *B*). The immunostaining for GLUT1, GLUT2, and GLUT3 in pancreatic sections (Fig. 3) was in agreement with these in vitro data. For GLUT2 immunohistochemistry, human liver tissue served as positive control. Human liver GLUT2 immunostaining was observed on the sinusoidal face of the parenchymal cell membrane; staining was most intense in the perivenous area and declined towards the periportal zone (Fig. 3 *A*). This distribution may be related to metabolic zonation of the liver, with preferential postprandial glucose uptake in the perivenous region of the liver lobules (28). In contrast, sections from human donor pancreata (Fig. 3 *C*) were negative for GLUT2 immunostaining. This negative result was confirmed on a partial pancreatic resection from nondiabetic subject (data not shown). The islet sections were positive for GLUT1 and GLUT3, which were both preferentially localized in the plasma membrane (Fig. 3, *D* and *E*). These observations were further supported by immunoblots of freshly isolated human islets (Fig. 2 *C*) which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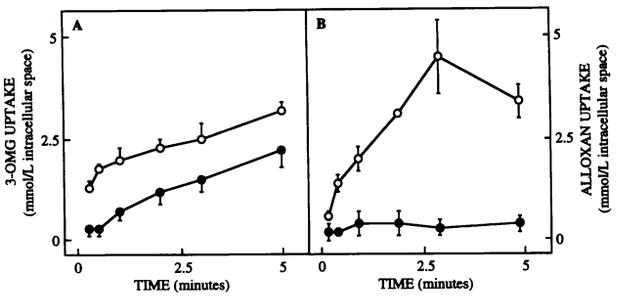

Figure 4. 3-OMG and alloxan uptake in human and rat islet cells. (*A*) 3-OMG uptake in rat (○, $n = 3$) and human (●, $n = 3$) islet cells. (*B*) Alloxan uptake in rat (○, $n = 4$) and human (●, $n = 3$) islet cells. Data represent mean values±SE.

were GLUT2 negative and GLUT1 positive. Two-step dilution series of human (Fig. 2 *D*) and rat (Fig. 2 *E*) islet cell protein allowed detection of rat GLUT2 in up to 64-fold diluted samples, while human islet GLUT2 was not detected, even in undiluted samples. In a second experiment, rat islet GLUT2 was detected after 128-fold dilution, while undiluted human islet GLUT2 was again not detected (data not shown). On the other hand, human and rat liver contained similar amounts of GLUT2 (data not shown) and human and rat islets exhibited comparable glucokinase abundancies (Fig. 2, *D* and *E*).

3-OMG and alloxan uptake in rat and human islet cells. Since human β-cells are less sensitive than rat islet β-cells to the toxic action of alloxan (29), a glucose-like molecule, we examined whether the low expression of GLUT2 results in low rates of alloxan uptake. We therefore compared the uptake kinetics of ^{14}C-labeled alloxan and ^{3}H-labeled 3-OMG in rat and human islet cells. Both compounds were transported 10 times more slowly in human than in rat islet cells (Fig. 4). In rat cells, alloxan uptake was competitively inhibited by unlabeled 3-OMG but not by L-glucose (data not shown), indicating that the agent indeed enters the cells via a glucose transporter. Since alloxan is oxidized rapidly in water to alloxanic acid (25), which can no longer enter the cells, intracellular concentration decreased in rat cells after a peak value at 3 min. As a consequence, high alloxan concentrations are rapidly reached in rat but not in human β-cells. The total area under the curve, assumed to represent a direct index of cytoplasmic exposure to the toxin, was therefore at least 10-fold lower in human β-cells than in rat β-cells (Fig. 4 *B*).

Discussion

The present study describes some metabolic properties of human pancreatic islet cells. Cellular glucose utilization and oxidation were proportional to extracellular glucose concentrations within the physiological range between 3 and 10 mmol/liter. This metabolic feature was previously observed in purified rat β-cells (20, 30) and seems therefore essential for a tissue equipped with a metabolic glucose sensor. Glucose phosphorylation via glucokinase parallelled the overall glycolytic flux in intact human islet cells. This glucokinase activity was similar to that found in rat β-cells (20). In line with a previous observation in microdissected human islets (31), human islet cell extracts were found to contain substantial amounts of low-K_m hexokinase activity. This low-K_m hexokinase activity is probably of minor importance for the glucose sensor of normal β-cells in vivo since (*a*) chromatographically purified hexokinase was 90% saturated at 1 mmol/liter substrate; (*b*) low-K_m phosphorylation was 80% suppressed by 2.5 mmol/liter glucose-6-phosphate, which might be representative for intact cells containing glucokinase and exposed to glucose levels above 5 mmol/liter; (*c*) the hexokinase activity of the islet cell preparation is mainly localized in nonendocrine cells as evaluated after fluorescence-activated sorting of endocrine and nonendocrine cells.

In contrast to the similarities at the level of glycolysis and glucokinase activity, the 3-OMG uptake rates by human islet cells were ~ 10 times lower than those observed in rat islet cells (3, 20). Because 3-OMG uptake in human islet cells still exceeded metabolic flux through glycolysis by a factor of five, it can be proposed that transport is not rate limiting for overall glucose metabolism. The 10-fold lower rates of 3-OMG transport in human islet cells as compared to rat islet cells were correlated to low levels of the high capacity glucose transporter GLUT2 mRNA in human islet cells. These results were confirmed both at the protein level and at the level of functional activity. The observed K_m for zero-*trans* 3-*O*-methyl glucose uptake (3 mmol/liter) is indeed much lower than the described K_m for rat GLUT2 (32) and seems more compatible with GLUT1 and/or GLUT3 (33, 34).

Since culture of isolated rat islets is known to induce GLUT1 immunoreactivity and reduce GLUT2 expression (35), we considered the possibility that culture of human islets had down-regulated GLUT2 to levels below the detection limits of our assays and up-regulated GLUT1 in parallel. Immunohistochemical analysis of tissue sections did not reveal GLUT2 positivity in human islets, suggesting that the in vitro data reflect a biological phenomenon rather than an artifact of the isolation procedure. Also arguing against the role of cell manipulation is the fact that protein expression studies in freshly isolated human islets gave similar results when compared to cultured islets. The same observations were made for cold-preserved and freshly fixed tissue and for donors with different antemortem conditions, indicating that these variables had no major influence on islet and liver GLUT2 immunostaining. It seems therefore reasonable to conclude that human islet cells express little GLUT2 in comparison to GLUT1 or GLUT3 and that the human β-cell GLUT2 expression level is markedly lower than in rat

Figure 3. Immunohistochemical localization of glucose transporters in human pancreas and liver sections. (*A*) Control immunostaining of human liver with anti-GLUT2 (1:50). Note staining of the sinusoidal face of the cell membrane of parenchymal cells (*arrow*) which is more intense around the central vein (*c*) than around the portal triad (*p*). (*B* and *C*) Consecutive sections of human pancreas, showing the same islet, stained for insulin (1:1,000, *B*) and GLUT2 (1:10, *C*). (*D* and *E*) Two islets from the same pancreas were immunostained for GLUT1 (1:200, *D*) and GLUT3 (1:100, *E*). Scale bars: 200 μm in (*A*) and 50 μm in (*B–E*).

β-cells and in human liver cells. The physiological consequence of this observation is not yet clear, particularly since the role of GLUT2 in the rat β-cell glucose sensor (20) and the reason for its down-regulation in animal models of NIDDM (36, 37) are topics of debate. The presence of GLUT3 protein in human β-cells is in agreement with a reverse transcriptase-PCR study, which demonstrated amplified GLUT3 cDNA in a human islet preparation (38). The present immunolocalization of GLUT3 in normal human islets is compatible with the idea that this transporter is widely distributed in man, in contrast to the mouse where GLUT3 is restricted to the central nervous system (39). The GLUT3 expression and islet cell membrane localization may also explain, at least in part, the observed low K_m of 3-OMG uptake of human β-cells. The relative contributions of GLUT1 and GLUT3 in the overall uptake kinetics remain, however, unclear. Future experiments using antisense oligonucleotides or other gene manipulations of human islet cells may sort out this complicated issue.

The interspecies differences in GLUT2 expression do well explain the major difference in sensitivity of human and rat β-cells for the cytotoxic action of streptozotocin (29). Interestingly, this nitroso urea-derivative of glucose has been reported to enter GLUT2-expressing rodent β-cells more rapidly than GLUT2-negative cells (40), suggesting that the low GLUT2 expression in human β-cells may be the cause for the low in vivo toxicity of streptozotocin in man. Since human β-cells are also resistant to the cytotoxic action of alloxan (29), another rodent diabetogenic agent, uptake kinetics of radiolabeled alloxan and 3-OMG were compared in rat and human islet cells. Uptake was indeed 10 times slower than in rat β-cells and could be stereospecifically competed for with 3-OMG. The different alloxan uptake kinetics in human islet cells resulted in a lower intracellular exposure level to the toxin and can therefore explain their differential sensitivity to the drug as compared to rat islet cells. It is of course conceivable that the differential alloxan toxicity in rat and man is the result of additional mechanisms such as increased expression of DNA repair or cellular defense proteins in human β-cells.

In conclusion, the frequently cited hypothesis that defects in pancreatic beta-cell GLUT2 expression contribute to the pathogenesis of human NIDDM (3) may need to be reconsidered. On the other hand, our observations provide direct support to the view that glucokinase is implicated in the glucose sensor of human β-cells (4). They may form a basis for the concept that reduced glucokinase expression or activity can reduce the glucose responsiveness in human β-cells. That such conditions occur in human disease has been suggested by genetic studies of maturity onset diabetes of the young, an autosomal dominant form of diabetes (13, 41).

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References

1. Cerasi, E. 1975. Mechanisms of glucose stimulated insulin secretion in health and in diabetes: some re-evaluations and proposals. *Diabetologia.* 11:1–13.

2. MacDonald, M. J. 1990. Elusive proximal signals of β-cells for insulin secretion. *Diabetes.* 39:1461–1466.

3. Unger, R. H. 1991. Diabetic hyperglycemia: link to impaired glucose transport in pancreatic β cells. *Science (Wash. DC).* 251:1200–1205.

4. Matschinsky, F. M. 1990. Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. *Diabetes.* 39:647–652.

5. Hughes, S. D., J. H. Johnson, C. Quaade, and C. B. Newgard. 1992. Engineering of glucose-stimulated insulin secretion and biosynthesis in non-islet cells. *Proc. Natl. Acad. Sci. USA.* 89:688–692.

6. Hughes, S. D., C. Quaade, J. H. Johnson, S. Ferber, and C. B. Newgard. 1993. Transfection of AtT-20ins cells with GLUT-2 but not GLUT-1 confers glucose-stimulated insulin secretion. *J. Biol. Chem.* 268:15205–15212.

7. Ferber, S., H. BeltrandelRio, J. Johnson, R. J. Noel, L. E. Cassidy, S. Clark, T. C. Becker, S. Hughes, and C. B. Newgard. 1994. GLUT-2 gene transfer into insulinoma cells confers both low and high affinity glucose-stimulated insulin release. Relationship to glucokinase activity. *J. Biol. Chem.* 269:11523–11529.

8. Valera, A., G. Solanes, J. Fernandez-Alvarez, A. Pujol, J. Ferrer, G. Asins, R. Gomis, and F. Bosch. 1994. Expression of GLUT-2 antisense RNA in β cells of transgenic mice leads to diabetes. *J. Biol. Chem.* 269:28543–28546.

9. Efrat, S., M. Leiser, Y. J. Wu, D. Fusco-DeMane, O. A. Emran, M. Surana, T. L. Jetton, M. A. Magnuson, G. Weir, and N. Fleischer. 1994. Ribozyme-mediated attenuation of pancreatic β-cell glucokinase expression in transgenic mice results in impaired glucose-induced insulin secretion. *Proc. Natl. Acad. Sci. USA.* 91:2051–2055.

10. Johnson, J. H., A. Ogawa, L. Chen, L. Orci, C. B. Newgard, T. Alam, and R. H. Unger. 1990. Underexpression of β cell high Km glucose transporters in noninsulin-dependent diabetes. *Science (Wash. DC).* 250:546–549.

11. Ogawa, A., J. H. Johnson, M. Ohneda, C. T. McAllister, L. Inman, T. Alam, and R. H. Unger. 1992. Roles of insulin resistance and β-cell dysfunction in dexamethasone-induced diabetes. *J. Clin. Invest.* 90:497–504.

12. Orci, L., R. H. Unger, M. Ravazzola, A. Ogawa, I. Komiya, D. Baetens, H. F. Lodish, and B. Thorens. 1990. Reduced β-cell glucose transporter in new onset diabetic BB rats. *J. Clin. Invest.* 86:1615–1622.

13. Matschinsky, F., Y. Liang, P. Kesavan, L. Wang, P. Froguel, G. Velho, D. Cohen, M. A. Permutt, Y. Tanizawa, T. L. Jetton, et al. 1993. Glucokinase as pancreatic β cell glucose sensor and diabetes gene. *J. Clin. Invest.* 92:2092–2098.

14. Chiu, K. C., M. A. Province, and M. A. Permutt. 1992. Glucokinase gene is genetic marker for NIDDM in American blacks. *Diabetes.* 41:843–849.

15. Elbein, S. C., M. D. Hoffman, A. Matsutani, and M. A. Permutt. 1992. Linkage analysis of GLUT1 (HepG2) and GLUT2 (Liver/Islet) genes in familial NIDDM. *Diabetes.* 41:1660–1667.

16. Mueckler, M., M. Kruse, M. Strube, A. C. Riggs, K. C. Chiu, and M. A. Permutt. 1994. A mutation in the Glut2 glucose transporter gene of a diabetic patient abolishes transport activity. *J. Biol. Chem.* 269:17765–17767.

17. Pipeleers, D. 1994. Treatment of diabetes by islet cell transplantation. *In* Biomedical and Health Research. Vol. 3, Advances in Medical Biology. C. Baya, editor. IOS Press, Amsterdam. 185–197.

18. Eizirik, D. L., G. S. Korbutt, and C. Hellerström. 1992. Prolonged exposure of human pancreatic islets to high glucose concentrations in vitro impairs the β-cell function. *J. Clin. Invest.* 90:1263–1268.

19. Heimberg, H., A. De Vos, D. Pipeleers, B. Thorens, and F. Schuit. 1995. Differences in glucose transporter gene expression between rat pancreatic α- and β-cells are correlated to differences in glucose transport but not in glucose utilization. *J. Biol. Chem.* 270:8971–8975.

20. Heimberg, H., A. De Vos, A. Vandercammen, E. Van Schaftingen, D. Pipeleers, and F. Schuit. 1993. Heterogeneity in glucose sensitivity among pancreatic β-cells is correlated to differences in glucose phosphorylation rather than glucose transport. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:2873–2879.

21. Trus, M. D., W. S. Zawalich, P. T. Burch, D. K. Berner, V. A. Weill, and F. M. Matschinsky. 1981. Regulation of glucose metabolism in pancreatic islets. *Diabetes.* 30:911–922.

22. Thorens, B., H. F. Lodish, and D. Brown. 1990. Differential localization of two glucose transporter isoforms in rat kidney. *Am. J. Physiol.* 259:C286–C294.

23. Slieker, L. J., K. L. Sundell, W. F. Heath, H. E. Osborne, J. Bue, J. Manetta, and J. R. Sportsman. 1992. Glucose transporter levels in tissues of spontaneously diabetic Zucker fa/fa rat (ZDF/drt) and viable yellow mouse (A^{vy}/a). *Diabetes.* 41:187–193.

24. Iynedjian, P. B., G. Möbius, H. J. Seitz, C. B. Wollheim, and A. E. O. Renold. 1986. Tissue-specific expression of glucokinase: identification of the gene product in liver and pancreatic islets. *Proc. Natl. Acad. Sci. USA.* 83:1998–2001.

25. Weaver, D. C., M. L. McDaniel, and P. E. Lacy. 1978. Alloxan uptake by isolated rat islets of Langerhans. *Endocrinology.* 102:1847–1855.

26. Ling, Z., R. De Proft, and D. Pipeleers. 1993. Chronic exposure of human pancreatic beta-cells to high glucose increases their functional activity but decreases their sensitivity to acute regulation by glucose. *Diabetologia.* 36:74*a* (*Abstr.*)

27. Permutt, M. A., L. Koranyi, K. Keller, P. E. Lacy, D. W. Scharp, and M. Mueckler. 1989. Cloning and functional expression of a human pancreatic islet glucose-transporter cDNA. *Proc. Natl. Acad. Sci. USA.* 86:8688–8692.

28. Jungermann, K., and N. Katz. 1989. Functional specialization of different hepatocyte populations. *Physiol. Rev.* 69:708–764.

29. Eizirik, D. L., D. G. Pipeleers, Z. Ling, N. Welsch, C. Hellerström, and A. Andersson. 1994. Major species differences between humans and rodents in the susceptibility to pancreatic β-cell injury. *Proc. Natl. Acad. Sci. USA.* 91:9253–9256.

30. Gorus, F., W. J. Malaisse, and D. Pipeleers. 1984. Differences in glucose handling by pancreatic A- and B-cells. *J. Biol. Chem.* 259:1196–1200.

31. Bedoya, F. J., J. M. Wilson, A. K. Ghosh, D. Finegold, and F. M. Matschinsky. 1986. The glucokinase glucose sensor in human pancreatic islet tissue. *Diabetes.* 35:61–67.

32. Johnson, J. H., C. B. Newgard, J. L. Milburn, H. F. Lodish, and B. Thorens. 1990. The high K_m glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has identical primary sequence. *J. Biol. Chem.* 265:6548–6551.

33. Wheeler, T. J., and P. C. Hinkle. 1981. Kinetic properties of the reconstituted glucose transporter of human erythrocytes. *J. Biol. Chem.* 256:8907–8914.

34. Gould, G. W., H. M. Thomas, T. J. Jess, and G. I. Bell. 1991. Expression of human glucose transporters in Xenopus oocytes: kinetic characterization and substrate specificities of the erythrocyte, liver, and brain isoforms. *Biochemistry.* 30:5139–5145.

35. Tal, M., Y. Liang, H. Najafi, H. F. Lodish, and F. M. Matschinsky. 1992. Expression and function of GLUT-1 and GLUT-2 glucose transporter isoforms in cells of cultured rat pancreatic islets. *J. Biol. Chem.* 267:17241–17247.

36. Thorens, B., Y. J. Wu, J. L. Leahy, and G. C. Weir. 1992. The loss of GLUT2 expression by glucose-unresponsive β cells of db/db mice is reversible and is induced by the diabetic environment. *J. Clin. Invest.* 90:77–85.

37. Ogawa, Y., Y. Noma, A. M. Davalli, Y. J. Wu, B. Thorens, S. Bonner-Weir, and G. C. Weir. 1995. Loss of glucose-induced insulin secretion and GLUT2 expression in transplanted β-cells. *Diabetes.* 44:75–79.

38. Seino, Y., T. Yamamoto, K. Inoue, M. Imamura, S. Kadowaki, H. Kojima, J. Fujikawa, and H. Imura. 1993. Abnormal facilitative glucose transporter gene expression in human islet cell tumors. *J. Clin. Endocrinol. Metab.* 76:75–78.

39. Gould, G. W., A. M. Brant, B. B. Kahn, P. R. Shepherd, S. C. McCoid, and E. M. Gibbs. 1992. Expression of the brain-type glucose transporter is restricted to brain neuronal cells in mice. *Diabetologia.* 35:304–309.

40. Schnedl, W. J., S. Ferber, J. H. Johnson, and C. B. Newgard. 1994. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. *Diabetes.* 43:1326–1333.

41. Velho, G., P. Froguel, K. Clement, M. E. Pueyo, B. Rakotoambinina, H. Zouali, P. Passa, D. Cohen, and J. J. Robert. 1992. Primary pancreatic beta-cell secretory defect caused by mutations in glucokinase gene in kindreds of maturity onset diabetes of the young. *Lancet.* 340:444–448.