

The glucose sensor protein glucokinase is expressed in glucagon-producing α -cells

(islets/metabolism/hormones/diabetes)

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ABSTRACT Expression of glucokinase in hepatocytes and pancreatic β -cells is of major physiologic importance to mammalian glucose homeostasis. Liver glucokinase catalyzes the first committed step in the disposal of glucose, and β -cell glucokinase catalyzes a rate-limiting step required for glucose-regulated insulin release. The present study reports the expression of glucokinase in rat glucagon-producing α -cells, which are negatively regulated by glucose. Purified rat α -cells express glucokinase mRNA and protein with the same transcript length, nucleotide sequence, and immunoreactivity as the β -cell isoform. Glucokinase activity accounts for more than 50% of glucose phosphorylation in extracts of α -cells and for more than 90% of glucose utilization in intact cells. The glucagon-producing tumor MSL-G-AN also contained glucokinase mRNA, protein, and enzymatic activity. These data indicate that glucokinase may serve as a metabolic glucose sensor in pancreatic α -cells and, hence, mediate a mechanism for direct regulation of glucagon release by extracellular glucose. Since these cells do not express *Glut2*, we suggest that glucose sensing does not necessarily require the coexpression of *Glut2* and glucokinase.

Control of blood glucose levels in mammals is dependent on hormonal and metabolic communication between pancreatic islets of Langerhans and the liver. Acute changes in blood glucose concentration are detected by the endocrine pancreas, which responds by secreting a hormonal mixture that is rich in either glucagon (fasting state) or insulin (during or just after meals). Glucagon stimulates the liver to mobilize glucose from intracellular glycogen stores, while insulin increases postprandial glucose extraction from the portal vein (1). Since glucose itself is the main physiological activator of insulin release (2) and a direct inhibitor of glucagon release (3–5), the system is controlled via short feedback loops that are typical for homeostasis in general.

Glucokinase (hexokinase IV) is expressed in liver and islets of Langerhans where it has been proposed to regulate hepatic glucose disposal and pancreatic glucose sensing (6, 7). Unlike the other mammalian hexokinases, which all have high affinity for glucose, the K_m of glucokinase is in the millimolar range. Consequently, glycolytic flux becomes proportional to the extracellular glucose concentration in glucokinase-expressing cells as long as glucose uptake is not rate-limiting for its further metabolism (6, 7). Furthermore, the enzyme is not sensitive to feedback inhibition by glucose 6-phosphate (G6P), allowing the liver to sustain high metabolic flux despite elevated intracellular concentration of G6P (7). Glucokinase gene expression level in β -cells is correlated to cellular glucose sensitivity both *in vivo* (8, 9) and *in vitro* (10), suggesting that

the enzyme is a constituent of the β -cell glucose sensor. Analogous to the situation in β -cells, it is conceivable that glucokinase expression in pancreatic α -cells is required for glucose to suppress glucagon release. This effect is of major physiological importance because glucagon secretion is required to prevent or correct hypoglycemia and needs to be suppressed during hyperglycemia. However, little is known about the mechanisms whereby glucose regulates glucagon-producing α -cells. The presence of glucokinase has been described in the α -cell-enriched islets of streptozotocin-treated rats (11), but the periphery of normal rat pancreatic islets appeared negative for glucokinase immunostaining (12). Our laboratory has developed methods to isolate rat α -cells by autofluorescence-activated cell sorting (13). We have shown (14) that fluorescence-activated cell sorter-purified rat α - and β -cells differ in their glucose transporter gene expression but not in glucose-dependent rate of glycolysis, suggesting that glucose uptake is not rate-limiting for glucose metabolism in both cell types. In the present study, we explored the possibility that the similarity in overall glucose utilization by α - and β -cells is caused by a common level of glucokinase expression in both cell types. The demonstration of functional glucokinase expression in α -cells indicates that the concept of glucokinase as a glucose sensing device can be extended to glucagon-producing cells.

MATERIALS AND METHODS

Purification and Culture of Cells from the Endocrine Pancreas. Purified islet β -cell and non- β -cell preparations were isolated from male Wistar rats after dissociation to single cells with trypsin/DNase and autofluorescence-activated cell sorting for FAD scatter at 2.8 mM glucose (13). The purified β -cell fraction contained $93 \pm 3\%$ β -cells and $2 \pm 1\%$ α -cells. Pancreatic polypeptide (PP) cells and δ -cells were each represented by less than 3% of the cells. The endocrine non- β -cell fraction was composed of $69 \pm 6\%$ α -cells, $12 \pm 4\%$ β -cells, and 5–10% PP-cells or δ -cells. The non- β -cell fraction was further purified to α -cells by sorting for NAD(P)H autofluorescence after incubation at 20 mM glucose (13). The resulting α -cell preparation included $87 \pm 6\%$ α -cells, $4 \pm 3\%$ β -cells, and less than 5% PP-cells or δ -cells. Estimation of the purity of the preparations ($n \geq 6$) was done by light microscopic immunocytochemistry and electron microscopy. Cells were more than 90% viable as assessed by neutral red uptake. Cell culture was in Ham's F-10 containing 1% bovine serum albumin, 2 mM glutamine, 50 μ M 3-isobutyl-1-methylxanthine, and 6 mM glucose (15). MSL-G2-IN and MSL-G-AN

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Abbreviation: G6P, glucose 6-phosphate.

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were transplantable islet-cell tumors procured from NEDH rats (16). Liver and brain were obtained from adult male Wistar rats.

mRNA Analysis. Total RNA (5 μ g) was isolated, submitted to gel electrophoresis, and blotted as described (10). Autoradiographic exposure times required to obtain the indicated signals were 3 h for insulin, 1 day for β -actin, 2 days for Glut2, and 10 days for glucokinase.

Analysis of Amplified cDNA. Total RNA (0.5 μ g) extracted from α -cells, β -cells, MSL-G-AN, MSL-G2-IN, and control tissues (brain and liver) was reverse-transcribed and amplified according to the protocol of the GeneAmp RNA-PCR kit in a GeneAmp 9600 thermocycler (Perkin-Elmer/Cetus) with blanks in each assay. Specific primer sequences were glucokinase B1-5' (codons 9-15; 5'-AGGCCACCAAGAAG-GAAAAG), glucokinase B1/L1-3' (codons 97-104; 5'-TTGTCTTCAGCTCCACTGC), and glucokinase L1-5' (codons 9-15; 5'-GAGCCCAGTTGTTGACTCTG), yielding an amplified fragment of 285 bp. Specific primers for control amplifications were Glut2-5' (codons 79-84; 5'-GCCTGG-GAAGAAGAGACT), Glut2-3' (codons 252-258; 5'-AGCTTTTCTTGGCCCTGA; 538-bp fragment); insulin II-5' (codons 7-14; 5'-TAATGCCCTGCTGGCCCTGCT), insulin II-3' (codons 105-111; 5'-CCTAGTTGCAGTAGT-TCTCCA; 315-bp fragment), β -actin-5' (codons 249-255; 5'-ACTATCGGCAATGAGCGGTTTC), and β -actin-3' (codons 338-344; 5'-AGAGCCACCAATCCACACAGA; 288-bp fragment). The cycling profile was 2 min at 95°C followed by 1 min at 94°C, 1.5 min at 65°C, and 1.5 min at 72°C for 10 cycles and 0.5 min at 94°C, 1 min at 60°C, and 1.5 min at 72°C for 10-25 cycles. Mixed oligonucleotide primed amplification of cDNA was performed using two degenerate primer sets, 5'-primers [5'-GACAC(ACG)GT(AGT)G(CG)(CGT)AC(ACT)ATGAT; codons 205-211] and 3'-primers [5'-AT(CGT)CC(AG)C(CT)(ACG)A(CT)(C-G)AT(CT)TTCTC; codons 290-296], yielding an amplified fragment of 275 bp. The cycle profile was as for the specific amplification except for a lowering of the annealing temperature, 10 cycles of 1.5 min at 50°C and 30 cycles of 1 min at 55°C. PCR-derived fragments were eluted from agarose gel and TA-cloned in pCRII (Invitrogen). Clones were sequenced via the dideoxynucleotide method using modified T7 DNA polymerase (Pharmacia).

Protein Analysis. Protein electrophoresis and blotting were performed as described (14). The blotted filters were incubated with anti-glucokinase (1:5000 dilution), anti-Glut2 (1:4000 dilution), anti-insulin (1:100 dilution), or anti-glucagon (1:1000 dilution) antiserum for 60 min at room temperature. Sheep anti-rat glucokinase antiserum was directed against the purified enzyme (17), polyclonal rabbit anti-Glut2 antibodies were directed against the carboxyl-terminal 25 residues of rat Glut2 (18). Guinea pig anti-porcine insulin was a polyclonal antiserum and anti-porcine glucagon was a mouse monoclonal antibody (Novo-Nordisk, Copenhagen).

Immunocytochemistry. For glucokinase immunocytochemistry, freshly isolated purified islet non- β -cells were fixed for 4 h in 4% phosphate-buffered formaldehyde, dehydrated in ethanol, and embedded in paraffin. Sections (3-4 μ m) from the cell pellets were treated with methanol/hydrogen peroxide to block endogenous peroxidase activity and with 10% horse serum to block nonspecific binding of the antibodies. Affinity-purified anti-glucokinase serum (19) was purchased from H. Seitz (Institut für Physiologische Chemie, Univeristäts-Krankenhaus Eppendorf, Hamburg, Germany) and used for all immunocytochemical experiments. The affinity-purified serum recognized one 55-kDa protein on Western blots of total liver and islet protein but did not react with protein extracts from muscle or brain (data not shown). For immunocytochemistry, incubation with the primary antibody (overnight, 4°C, at a dilution of 1:5 or 1:10) was followed by a 2-h

exposure to a biotinylated donkey anti-sheep IgG diluted 1:300 (Amersham). The reaction was visualized after binding of streptavidin-peroxidase complex (Dako), with diaminobenzidine as chromogen. Two types of negative control reaction were used: (i) 200 μ l of affinity-purified glucokinase antiserum was preabsorbed to 25 μ g of purified rat liver glucokinase and incubated for 2 h at room temperature; after centrifugation for 10 min at 4°C, the supernatant fraction was further used for immunocytochemistry; and (ii) the primary antiserum was omitted. Both types of controls resulted in very weak background staining over the whole section (data not shown). For double immunostaining in a second sequence, rabbit anti-glucagon (1:500 dilution) was applied to the same section overnight. Positive reaction was visualized by immunofluorescence using a donkey anti-rabbit IgG, labeled with rhodamine, at a dilution of 1:10 (Jackson ImmunoResearch).

Glucose Utilization and Glucose Phosphorylation. Glucose utilization was measured in batches of 1×10^5 α -cells per 100 μ l of Earle's/Hepes buffer during 2-h incubations at 37°C as conversion of D-[5-³H]glucose into tritiated water (10). The high- K_m glucose phosphorylation that was not suppressible by its reaction product was determined radiometrically on cell homogenates in the presence of 20 mM glucose and 2.5 mM G6P, as described (10). The dose-response curve of glucose phosphorylation was determined fluorimetrically in homogenates of isolated β -cells (2×10^6 cells per ml) and non- β -cells (4×10^6 cells per ml) as described by Trus *et al.* (20). Glucokinase activity was calculated by subtracting the glucose phosphorylating activity at 0.5 mM glucose, which approximates the low- K_m hexokinase activity, from the total activity measured at substrate concentrations higher than 5 mM. Glucokinase and low- K_m hexokinases from MSL-G-AN were partially purified by DEAE column chromatography as described (21). Individual 1-ml column fractions were analyzed for glucose phosphorylation by using the fluorimetric technique.

RESULTS

Presence of Glucokinase mRNA in Purified Rat α -Cells.

The expression of glucokinase mRNA was examined by Northern blots of total RNA from purified α -cells, probed with rat insulinoma glucokinase cDNA (22). The blots also contained total RNA from rat liver and purified β -cells that express glucokinase (23). Brain RNA was used as negative control. Fig. 1 shows a transcript of the same length in both islet α - and β -cells, while the mRNA that hybridized with the glucokinase cDNA probe in liver had a lower molecular weight. The relative abundance of glucokinase mRNA over β -actin mRNA was 0.2, 0.9, and 0.3 in three preparations of freshly isolated α -cells and increased to 7.5, 3.3, and 0.6, respectively, after overnight culture in Ham's F-10 medium. On the contrary, glucokinase over β -actin mRNA signal ratios from two β -cell preparations (2.0 and 1.5, respectively) decreased to 1.8 and 0.4 after overnight culture. Despite this differential response of glucokinase mRNA abundance in cell culture, it could be argued that the observed glucokinase mRNA signal in α -cells was due to β -cells contaminating the purified α -cell preparations. We therefore rehybridized the blots with rat Glut2 and insulin II cDNA probes, which both recognize abundant β -cell-specific mRNAs. As is shown in Fig. 1, hybridization with these probes revealed weak or no autoradiographic signals with α -cell RNA. The ratio of glucokinase over Glut2 signal was 100-fold higher in α -cells than in β -cells. Glucokinase mRNA was also detected in the glucagon-secreting tumor MSL-G-AN (14), which is devoid of insulin-producing cells as demonstrated by the fact that neither Glut2 nor insulin mRNA were detected (Fig. 1). The positive control in this experiment was the insulin-producing tumor MSL-G2-IN (16), which expressed glucokinase, Glut2, and insulin. Further characterization of the glucokinase transcript in isolated α -cells was

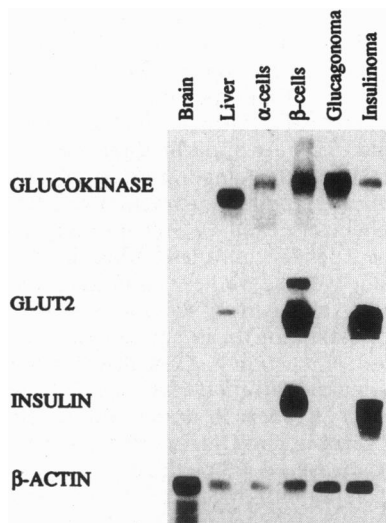


FIG. 1. Glucokinase gene expression in brain, liver, α - and β -cells, glucagonoma MSL-G-AN, and insulinoma MSL-G2-IN. Total RNA (5 μ g) was submitted to gel electrophoresis, blotted, and hybridized with cDNA probes specific for glucokinase, Glut2, insulin, and β -actin. The RNA blot shown here is representative for three experiments.

performed through reverse transcription-coupled PCR and DNA sequencing. Liver glucokinase (L1) and β -cell-specific glucokinase (B1) differ in their 15 amino-terminal residues (22), allowing selective amplification of their mRNAs by using isoform-specific primers. This method was validated by amplifying cDNAs from α -cells and MSL-G-AN and from control tissues, using primer sets specific for hexokinase I, Glut2, insulin, and β -actin. Target sequences for hexokinase I-specific primers were located in the same region as the glucokinase primers. After 35 cycles, a signal corresponding to hexokinase I became apparent in brain and in the tumor cell lines (data not shown), while a signal corresponding to glucokinase L1 was detected in liver (Fig. 2). Amplification of cDNA from purified α -cells and MSL-G-AN tumor cells generated a 285-bp PCR fragment with the β -cell-specific glucokinase primers but not with the liver-specific primer set (Fig. 2). The nucleotide sequences derived from the amplified α -cell and MSL-G-AN cDNAs were identical to the sequence of the β -cell-specific fragment (data not shown), which was the same as the pub-

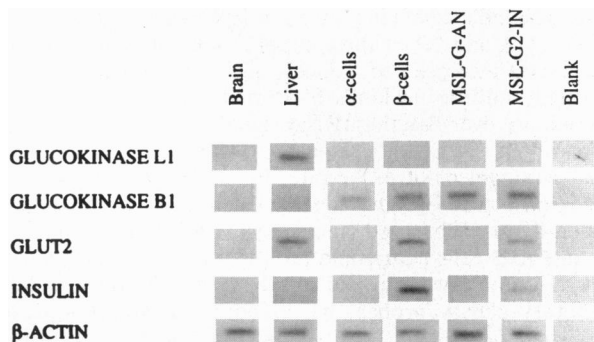


FIG. 2. Reverse transcription-coupled of glucokinase transcripts in purified α - and β -cells. Total RNA was reverse-transcribed and amplified using liver-specific (L1) or islet-specific (B1) glucokinase primers. As cell-specific controls, Glut2, insulin, or β -actin transcripts were amplified. Amplification of cDNA from purified α -cells and MSL-G-AN tumor cells generated a 285-bp PCR fragment with the β -cell-specific glucokinase primers but not with the liver-specific primer set. Liver, β -cells, and MSL-G2-IN were Glut2-positive after 30 cycles. β -cells and MSL-G2-IN were insulin-positive after 20 cycles. The β -actin signal was similar in all cells and tissues under examination.

lished glucokinase cDNA derived from insulinoma (22). Degenerated primers were designed to anneal to highly conserved regions in the 6th, 8th, 14th, and 16th exons of the known hexokinase isoforms. Analysis of nucleotide sequences of the amplified fragments demonstrated tissue-specific regulation of the hexokinase I-IV gene expression: the mRNA pool extracted from brain contained hexokinase I; hexokinase I and glucokinase L1 were found among liver mRNAs; and hexokinase I and glucokinase B1 were amplified from α - and β -cell cDNA. No new members of the hexokinase family were isolated with this technique (data not shown).

Presence of Glucokinase Protein in Purified Rat α -Cells. To assess whether glucokinase mRNA is translated in α -cells, protein blots containing α -cell extracts were incubated with a polyclonal antibody directed against rat liver glucokinase (17). Presence of Glut2 and insulin was a marker for β -cell contamination, while glucagon immunoreactivity served as a positive control for α -cell protein. Since the endocrine non- β -cells of rat islets are several times smaller than β -cells, a higher number of α -cells were blotted to obtain overall protein load that was comparable to β -cells, as was assessed by Ponceau staining (data not shown) and the β -actin signals. Fig. 3 illustrates the detection of a single 55-kDa immunoreactive glucokinase in extracts from liver, α -cells, and β -cells, while rat brain was negative. The antibody also recognized the 55-kDa protein in the glucagonoma MSL-G-AN and insulinoma MSL-G2-IN. Densitometric analysis of the relative abundance of glucokinase over Glut2 protein was 9.8 ± 3.8 for α -cells ($n = 3$) and 0.20 ± 0.04 for β -cells ($n = 8$, $P < 0.001$, using unpaired two-tailed Student's t test). Purified β -cells contained a large store of immunoreactive insulin while MSL-G2-IN have a low insulin storage capacity (O.M., unpublished data). Immunoreactive glucagon was detected both in MSL-G-AN and α -cells.

The cellular localization of glucokinase immunoreactivity was analyzed by double immunostaining for glucokinase and glucagon on paraffin sections from flow-sorted non- β -cells. In this cell preparation, glucokinase immunoreactivity was located in 85–90% of the cells, whereas approximately 80% of the cells reacted positively for glucagon. Although there was variation in staining intensity of individual cells, double staining for glucokinase and glucagon revealed that most, if not all, α -cells contained glucokinase (Fig. 4). Preabsorption of glucokinase antiserum to the peptide resulted in weak back-

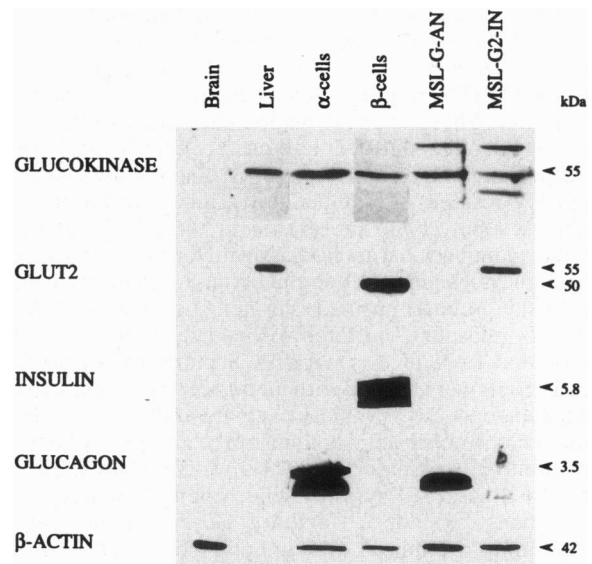


FIG. 3. Glucokinase protein expression in α -cells. Protein blots were incubated with anti-glucokinase, anti-Glut2, anti-insulin, or anti-glucagon sera. The blots are representative of at least three experiments.

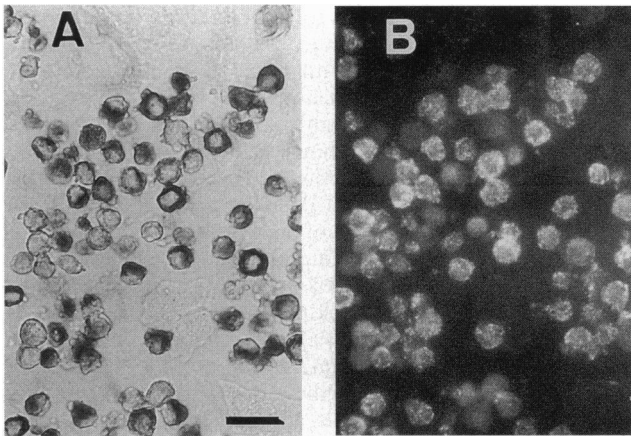


FIG. 4. Double immunocytochemical staining on a paraffin section from flow-sorted islet non- β -cells (approximately 70% α -cells), isolated from rat pancreatic islets. Coexpression of glucokinase (A) and glucagon (B) is demonstrated by ABC-immunoperoxidase cytochemistry (A) and rhodamine immunofluorescence (B). (Bar = 25 μ m.)

ground staining, similar to what was obtained when only the secondary antibody was applied (data not shown). After double staining for somatostatin or pancreatic polypeptide and glucokinase, double-positive cells could not be convincingly demonstrated (data not shown).

Glucokinase Enzymatic Activity in Purified Rat α -Cells and MSL-G-AN Cells. In contrast to the other members of the hexokinase family, catalysis via glucokinase is characterized by its high K_m for glucose (>10 mM) and resistance to feedback inhibition by G6P (7). To distinguish between low- K_m and high- K_m hexokinases, total glucose phosphorylation in α -cells and β -cells was measured fluorimetrically over a broad range of glucose concentrations at 30°C (Fig. 5 A and B). Eadie-Hofstee analysis of the data (Fig. 5 C and D) clearly showed that glucose phosphorylation in both cell preparations presents two kinetic components. The calculated $K_{m1} = 0.10 \pm 0.03$ and $K_{m2} = 14 \pm 4$ mM for high- and low-affinity hexokinases in α -cells (mean \pm SD of four experiments) were comparable to the corresponding affinities measured in β -cells ($K_{m1} = 0.17$

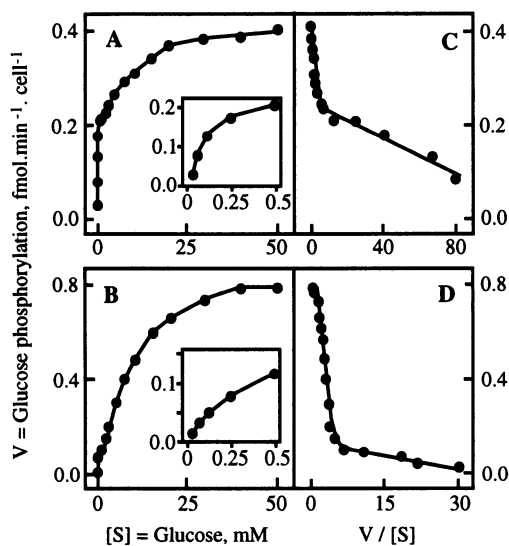


FIG. 5. Low- and high- K_m glucose phosphorylation in purified α - and β -cells. Concentration dependency of glucose phosphorylation was measured fluorimetrically in α -cells (A) and β -cells (B). (Inset) Enzymatic velocities at low substrate levels. (C and D) Eadie-Hofstee analysis of the data in A and B, respectively. The experiment shown is representative of four experiments.

± 0.04 and $K_{m2} = 17 \pm 2$ mM, respectively). Radiometric assays were run in parallel at 1 or 20 mM glucose in the presence or absence of 2.5 mM G6P, allowing to discriminate between G6P-sensitive hexokinases and G6P-resistant glucokinase (24). The phosphorylation activity in α -cells measured at 20 mM glucose in the presence of G6P (0.20 ± 0.07 pmol per min per 10^3 cells; mean \pm SD; $n = 4$) was very similar to the enzymatic activity measured via the fluorimetric method (0.15 ± 0.03 pmol per min per 10^3 cells; mean \pm SD; $n = 4$) and represented 70% of total glucose phosphorylation at this substrate level.

To measure glucokinase activity in MSL-G-AN homogenates (Fig. 6), prior column fractionation of the samples was required because of the dominant low- K_m hexokinase activity (90% of total glucose phosphorylation). The peak fraction of glucokinase enzyme activity displayed sigmoidal substrate kinetics (Fig. 6B) and revealed maximal abundance of glucokinase immunoreactivity on protein blots (data not shown). In a second set of experiments, glucokinase activity was measured in α -cells at 37°C, to allow direct comparison with total glucose utilization by intact cells without correction for temperature differences. Both total glucose utilization and glucokinase activity increased in parallel to the substrate concentration. Moreover, the absolute rates of glucose utilization and flux through glucokinase were very similar: total glucose utilization in purified α -cells was 0.08 ± 0.01 , 0.13 ± 0.02 , and 0.16 ± 0.02 pmol per min per 10^3 cells at 5, 10, and 20 mM glucose, while the corresponding rates of glucokinase activity were respectively 0.07 ± 0.03 , 0.11 ± 0.03 , and 0.16 ± 0.02 pmol per min per 10^3 cells (mean \pm SD of three or four experiments). The observed glucokinase activity in purified α -cells was also very similar to that in rat β -cells when data were expressed per liter of intracellular space. Glucokinase activities in rat β -cells were 0.25 ± 0.06 and 0.51 ± 0.09 mmol per min per liter (mean \pm SD, $n = 10$) at 5 and 10 mM glucose, while the corresponding activities in rat α -cells were 0.26 ± 0.11 and 0.41 ± 0.11 mmol per min per liter (mean \pm SD, $n = 3$).

DISCUSSION

Glucose exerts a direct and inverse regulation upon hormone secretion by pancreatic α - and β -cells (3–5, 25). Analogous to the metabolic control of insulin release (2, 26), it can be postulated that the mechanism of glucose suppression of glucagon release is related to a substrate-induced metabolic flux, generating inhibitory signal(s) for exocytosis. Compatible with this concept is the recently observed similarity in overall glycolytic flux measured in purified rat α - and β -cells (14). Moreover, glucose transport is not rate-limiting for glucose metabolism in both cell types (14). The rate of glucose

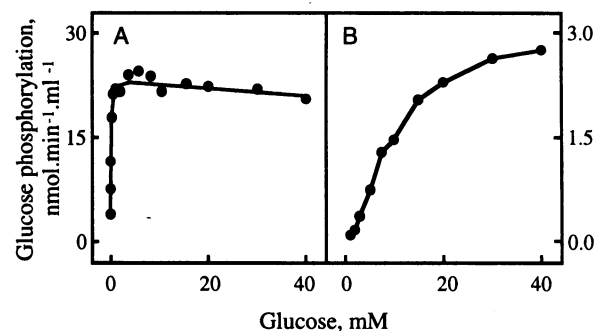


FIG. 6. Glucose phosphorylation activities of partially purified low- K_m hexokinases and high- K_m glucokinase from MSL-G-AN tumor cells. Approximately 3 mg of protein homogenate was loaded on the column and eluted as 1-ml fractions via a stepwise KCl gradient. Fractions 9 (A) and 11 (B) contained peak hexokinase and glucokinase activities, respectively, which were determined fluorimetrically.

utilization in α - and β -cells is linear between 5 and 10 mM substrate, the physiological plasma concentration range, and therefore, well adapted for metabolic signal transduction. The present study extends this similarity by showing that both rat α - and β -cells express functional glucokinase in quantities that render this enzyme rate-limiting for glycolysis. Therefore, the substrate dependency of glucose utilization in α - and β -cells may be directly determined by the kinetics of glucose phosphorylation in both cell types. The kinetic properties of glucokinase in both cell types are not distinguishable, both in terms of high- K_m and resistance to G6P inhibition.

An obvious point of concern in the interpretation of these data is the possibility that the detection of glucokinase mRNA, protein, or activity in fluorescence-activated cell sorter-purified α -cell preparations is due to contaminating β -cells. However, this possibility is unlikely since β -cell contamination should be most easily detected with specific markers that are highly expressed in β -cells. The relative intensity of the glucokinase over the Glut2 immunoreactive signal was at least 50-fold higher in α -cells than in β -cells. If contaminating β -cells would account for all glucokinase expression in α -cell preparations, such β -cells should be expressing very low amounts of insulin and Glut2. Second, overnight culture of pure α -cells caused at least a 3-fold increase in glucokinase mRNA levels, while the mRNA abundance in purified β -cells rather decreased. The down regulation of β -cell glucokinase mRNA after a 1-day culture is in agreement with previous data from islets cultured for 7 days (27). Preliminary experiments indicate that the rapid increase of glucokinase mRNA in α -cells is not paralleled by a similar change in glucokinase protein (data not shown). Third, glucokinase mRNA, protein, and functional activity were detected in the glucagon-producing tumor MSL-G-AN, which contains no differentiated β -cells (16) and which was negative for β -cell markers such as Glut2 and insulin. Finally, immunocytochemical colocalization of glucagon and glucokinase in flow-sorted rat α -cells directly shows that the two proteins are expressed in the same cells. Thus, these arguments strengthen the conclusion that functional glucokinase expression has been demonstrated in rat pancreatic α -cells.

Glucokinase gene expression was observed in rare neuroendocrine cells from the gastrointestinal mucosa and ventromedial hypothalamus, loci that contain cells that are glucose-sensitive in terms of electrical activity and hormone release (28). In the same study, a transgenic mouse expressing the growth hormone gene under control of the rat β -cell glucokinase promoter displayed growth hormone immunoreactivity in up to 75% of the α -cells. These data and our observations indicate that expression of glucokinase is associated with cells that sense glucose to regulate their physiological function. On the basis of enzyme kinetics (7, 27), *in vivo* (8, 9) and *in vitro* (10, 27) gene expression studies, and observed mutations in patients with maturity-onset diabetes of the young (29), it can be proposed that glucokinase is one of the basic units that constitute the glucose sensor. A metabolic or flux-generating type of glucose sensor produces metabolic signal(s) at a rate that is proportional to the extracellular glucose level. Rapid transport of glucose from the cellular interstitium to the cytosol is required for accurate sensing to avoid a delay in equilibration between extracellular and intracellular glucose levels and to avoid a sink in free cytosolic glucose when the sugar is phosphorylated. Rapid glucose transport is ensured in rat β -cells by the low-affinity/high-capacity glucose transporter Glut2 (18). In rat α -cells, where transport is one order of magnitude slower than in β -cells and Glut2 is not expressed (14), glucose uptake via Glut1 is still 5-fold more rapid than glucose utilization. The similarity in glucose utilization by α - and β -cells (14) suggests that Glut1 activity in α -cells is sufficient to maintain equilibrium of glucose levels over the cell membrane. Recent studies in AtT-20 cells have raised the

point that the glucose sensing role of Glut2 can be dissociated from its transport function. Transfection of glucose-unresponsive AtT-20 cells with Glut1 or Glut2 resulted in engineered cell lines with similar increase in glucose transport, while only the Glut2 transfectants acquired functional glucose responsiveness (30). The authors proposed that a second—isoform-specific and still hypothetical—function of Glut2 contributes to the glucose sensor (31). A possible mechanism may be the structural organization of proteins involved in proximal glycolysis close to the plasma membrane where later events in exocytosis occur. This hypothesis is in agreement with the well known coexpression of glucokinase and Glut2 in pancreatic β -cells and may be compatible with data recently obtained from human islets, where Glut2 is expressed at much lower level than in rat islets (32). On the other hand, our present data from purified rat islet α -cells suggest that cells expressing glucokinase can be glucose responsive without demonstrable participation of Glut2. It remains to be investigated whether the molecular structure of a glucose sensor in cells detecting decreased glucose levels is identical to that of cells that react to elevated glucose concentrations.

It is a matter of debate whether or not low- K_m hexokinases, which represent 50% or more of total glucose phosphorylating activity in extracts of nontumoral islet cells (33), play any role in glucose sensing. On the basis of kinetic properties, the low- K_m hexokinases are poorly equipped to “measure” the range of physiological glucose concentrations since they are all saturated at 1 mM glucose. Furthermore, allosteric inhibition may ensure the inactivation of low- K_m hexokinase in cells with sufficient glucokinase activity to generate the required G6P. Such situation is most likely present in rat α -cells, where the glycolytic flux at 0.5 mM glucose (0.02 ± 0.01 pmol per 10^3 cells per min) is about 10 times lower than the V_{max} of hexokinase activity (0.18 ± 0.15 pmol per 10^3 cells per min; mean \pm SD of four experiments). The low- K_m hexokinase activity was more variable in individual cell preparations than glucokinase activity. Assay variance can be excluded as a reason for this difference, leaving open the possibility that the source of low- K_m hexokinase is a non- α -cell type contaminating to variable extent the enriched α -cell preparations. In cells with hexokinase levels exceeding glucokinase by one order of magnitude or more, overall glycolysis will be much more dependent on hexokinase, resulting in a glucose response that is saturated at subphysiological glucose concentrations. This situation is typically observed in islet cell lines (30, 34) and may also be present in the MSL-G-AN and MSL-G2-IN tumor cells.

This study indicates that the functional cooperation of hepatocytes, islet β -cells, and glucagon-producing α -cells in the control of blood glucose levels is associated with the common property of these cells to express glucokinase. The present *in vitro* data suggest that abnormalities in glucokinase gene expression induce not only disturbed glucose stimulation of insulin release but may also cause defects in glucose suppression of glucagon release. Such defects have been observed in maturity-onset diabetes of the young-type diabetic families with congenital glucokinase gene mutations (35).

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