The Glucokinase Glucose Sensor in Human Pancreatic Islet Tissue

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SUMMARY

The enzyme glucokinase controls glucose metabolism in islets and is proposed to be the glucose sensor in pancreatic β-cells. This concept was developed from studies with rodents and it remained to be explored whether it also applies to man. Studies in man were hampered, however, by the difficulty in obtaining well-preserved pancreatic islet tissue and also because the high activity of hexokinase made it difficult to measure glucokinase. To overcome these obstacles, quantitative histochemical sampling techniques were developed allowing precise dissection of pure human islet tissue and a newly designed radiometric microassay was used, avoiding hexokinase interference, and providing the sensitivity necessary to measure the relatively low glucokinase activity in small samples of tissue obtained from brain-dead tissue donors. The present data indicate that glucokinase is present in human pancreatic islet tissue and is not found in the exocrine pancreas. The enzyme's V_{max} with D-glucose as substrate was similar to the V_{max} for glucose utilization reported previously for intact, isolated human islets and the enzyme's K_m for D-glucose was about 5 mM. Since glucokinase was also present in islet tissue of hamster, mouse, and rat, it is suggested that the glucokinase-glucose sensor concept has general applicability and that it could explain many aspects of the physiology and pathology of glucose homeostasis. This well-defined pancreatic islet glucokinase-glucose sensor should, therefore, be incorporated in any comprehensive model of glucose homeostasis. DIABETES 1986; 35:61–67.

We have recently proposed that the enzyme glucokinase (ATP-D-glucose-6-phosphotransferase, E.C. 2.7.1.1) plays a dual role in a two-stage feedback loop that maintains glucose homeostasis:[1] the enzyme might function as glucose sensor in the pancreatic β-cell and it might control the hepatic glucose uptake and storage in the form of glycogen. Much evidence has been accumulated since then supporting the glucokinase-glucose sensor role in pancreatic β-cells,[2–5] whereas the proposed regulatory role of liver glucokinase remains under debate.[6,7]

Our concept about the glucose sensor role of glucokinase in the β-cell was developed from studies with rodents and tissues from rodents. It remained to be explored whether this novel viewpoint could be applied to glucose homeostasis in man. Biochemical studies of glucokinase in human islet tissue were stifled and delayed because it was difficult to procure well-preserved pancreas tissue and because human islet tissue has relatively high activity of hexokinase, which interferes with the analysis of glucokinase.[8] To overcome these obstacles, we have used quick-frozen pancreata obtained from brain-dead tissue donors and have applied appropriate modifications of quantitative histochemical techniques to study this important question of physiologic chemistry.

In the present publication, we report our attempts to measure glucose phosphorylation in human pancreatic islet tissue and compare the results obtained with human material with results from three other species (hamster, mouse, and rat). The data to be presented show that glucokinase is present in the islet tissue of all four species investigated here, which suggests that the glucokinase-glucose sensor concept has general significance for physiology and pathology of glucose homeostasis.

MATERIALS AND METHODS

TISSUE COLLECTION AND SAMPLING

The majority of human pancreas specimens were collected by the Organ Procurement Center at the University of Pitts-

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TABLE 1
Case reports for pancreas donors

Donor	Sex	Age (yr)	Duration of pancreas anoxia	Plasma glucose at time of pancreas sampling (mg/dl)	Cause of death
1*	Male	12 $\frac{8}{12}$	60 s	478	Head injury; left parietal and temporal hematoma
2*	Male	14	15 s	626	Self-inflicted gunshot wound of head
3*	Male	18	15 s	540	Subarachnoid hemorrhage and subdural hematoma secondary to motor vehicle accident
4‡	Male	19	12 h	ND	Head injuries secondary to motor vehicle accident
5*	Female	20	25 s	485	Head injuries secondary to motor vehicle accident
6*	Female	21 $\frac{6}{12}$	60 s	ND	Cerebrovascular accident
7*,†	Male	36	60 s	105	Died 1 day after removal of sellar tumor and cyst; 10-yr history of acromegaly
8§	Female	73	4 h	ND	NA

ND, not determined; NA, not applicable.
*Donors received Lasix, mannitol, and glucocorticoid, pre- and intraoperatively.
†Intermittent glycosuria 18 mo before death; required insulin treatment 1 mo before death (75 U NPH/40 U regular before breakfast and 40 U NPH/40 U regular before dinner); received insulin infusion (10 U/h) while organs were removed.
‡Received Decadron, mannitol, and Lasix while at hospital.
§Surgical biopsy obtained from exploratory surgery after recurrent, reactive hypoglycemia.

burgh (from donors 1–3 and 5–7, see Table 1). Families of the donors had given permission for obtaining portions of pancreas for research purposes. Pancreas samples were obtained from five male and three female donors ranging in age from 12 yr and 8 mo to 73 yr (Table 1). Most donors had been involved in accidents that resulted in brain death. Donor 7 had died after removal of a sellar tumor. Specimen 8 was obtained by surgical biopsy. Donor 1 had been septic and required antibiotics before organ donation. Donor 7 had a 10-yr history of acromegaly with development of glycosuria 18 mo before organ donation. One month before organ donation, he developed symptomatic diabetes requiring insulin treatment; he was treated with an intravenous (i.v.) infusion of insulin (10 U/h) during the surgical removal of organs for transplantation. Donor 8 had a 15-yr history of reactive hypoglycemia; she underwent partial pancreatectomy 5 yr before episodes of dizziness, grand mal, and recurrent hypoglycemia (20–60 mg/dl) started. She was a heavy smoker (two packs of cigarettes a day) and she was being treated with Dilantin. Before organ removal, donors 1–3 and 5–7 received 1500–2000 mg of Solu-Medrol or a similar glucocorticoid and fluids containing glucose, Lasix, and mannitol. A small segment of either the body or the tail of each pancreas was excised while exposing the kidneys, handed to one of the investigators, immediately frozen in Freon 12 that had been cooled with liquid N_2 to its freezing point. The samples were stored at −70°C until used. These specimens were ischemic for times ranging from 15 s to 2 min. Specimen 4 was provided by Dr. A. Naji, University of Pennsylvania, and was stored on ice for 12 h until it was frozen in Freon 12. Specimen 8 was a surgical biopsy obtained from Dr. G. Boden, Temple University, and was stored on ice for 4 h until it was frozen.

To prepare tissue for analysis of glucokinase in microscopic samples, 20-μm-thick sections of the pancreas specimens were cut at −20°C and the sections were freeze dried for 48 h. Alternate sections from specimens 1–3 and 5–7 were saved and stained with thionin eosin. Detailed histologic maps of stained sections were drawn with the aid of a camera lucida attached to a dissecting microscope using procedures developed for studying brain tissue with quantitative histochemical methods.[9] The maps were superimposed on the adjacent, unstained, freeze-dried sections, allowing identification and dissection of islet tissue. On the average, 15–20 samples from 6 to 10 randomly chosen slices were dissected from each pancreas using a 20–30× magnification setting of a stereomicroscope. With two specimens (nos. 4 and 8), islets were recognizable without the use of maps of adjacent structures. It is not clear why islets were visible in some cases and not in others. The dry weights were determined on a quartz fiber, fish-pole balance.[10] Sample weights varied between 0.06 and 0.18 μg. A similar number of pieces were dissected from the exocrine portion of each pancreas and processed to be used as a control for the analysis of hormone content and enzyme activities. Exposure time of the tissue to air while identifying and dissecting the tissue ranged from 95 min to 8 h and affected neither the enzymatic activities nor the hormonal content of the samples.

For the studies with rodents, pancreata from male Wistar rats weighing 300–350 g; from adult, normal Chinese hamster (AA or M line), diabetic hamster (XA or AC line);[11,12] and from adult mouse (CBA/J or C58/J line)[13] were removed and processed for quantitative histochemical analysis as described above.

ASSAYS FOR GLUCOSE PHOSPHORYLATION

Radiometric method. The radiometric assay for glucokinase assay has been described elsewhere.[8] It is based on the liberation of ^3H_2O from D-[2^{3}H(N)]glucose-6-phosphate, the product of the glucokinase reaction. Exogenous phospho-

glucose isomerase is added to catalyze the generation of ^3H_2O from glucose-6-P. In the presence of 10 mM glucose-6-phosphate, the hexokinase activity of human islets that might interfere was largely inhibited (97.2 ± 0.3%, N = 6) without affecting glucokinase. The sensitivity of the assay was greatly increased by using small volumes with an oil-well procedure.

The actual glucokinase assay was carried out in 0.3 μl of 50 mM Hepes,* pH 7.6, containing 500 mM KCl, 10 mM DTT, 10 mM G-6-P, 8 mM $MgCl_2$, 0.1% BSA, 0.5 or 20 mM D-glucose, and 0.6–0.7 μCi of D-[2^3H(N)]glucose, specific activity 24 Ci/mmol. After preincubating the samples for 30 min, the reaction was started by adding 0.03 μl of 62 mM ATP and incubation was continued for an additional 40 min at room temperature. At this point, the reaction was stopped by adding 1.95 μl of 50 mM Hepes, pH 7.6, containing 1.5 M D-glucose and 110 mM EDTA. The isomerization of glucose-6-phosphate to fructose-6-phosphate was accomplished by adding of 0.5 μl of phosphoglucose isomerase (350 U/ml). After 2-h incubation at room temperature, the reaction was stopped by 5 μl of 0.33 N HCl. The efficiency of the isomerization step was checked routinely using 0.5 μCi of D-[2^3H(N)]glucose-6-phosphate. Under these conditions of assay, >96% of D-[2^3H(N)]glucose-6-phosphate was isomerized leading to the production of ^3H_2O. ^3H_2O was separated from the radioactive substrate D-[2^3H(N)]glucose by a diffusion step. The efficiency of the diffusion step was checked routinely using ^3H_2O standards and was always >90%. Tissue blanks (reagent without ATP) and reagent blanks (complete reagent without tissue in the presence and absence of ATP) were also included routinely for both 0.5 and 20 mM glucose. The performance of the microassay was checked routinely with a biologic standard using aliquots of a rat liver extract containing glucokinase. The glucokinase activity of this extract had been determined fluorometrically and radiometrically and aliquots of the extract were stored at −80°C. Each frozen aliquot was employed only once.

The total capacity for glucose phosphorylation by glucokinase in microdissected pieces of human islets (i.e., V_{max}) was calculated using the Michaelis-Menten equation. The K_m of human islet glucokinase was obtained with tissue dissected from one single human pancreas. The Michaelis constant for D-glucose applied in these computations was determined by least-squares linear regression of Hanes-Woolf plots for velocities measured at concentrations of glucose over the range of 1.7–44 mM. The activities were corrected for temperature (37°C) assuming a Q_{10} of 2.1.[4]

Fluorometric cycling method. The fluorometric cycling assay for hexokinase and glucokinase has been described previously.[14] In brief, pooled, dissected, freeze-dried islet samples weighing 1 μg were placed in an oil well and extracted with 5.5 μl of 50 mM Hepes, pH 7.7, containing 120 mM KCl, 0.5 mM EDTA, and 0.2% BSA for 30–40 min at room temperature. Aliquots of the islet extract (0.3 μl) were placed in wells and covered with oil and 0.3 μl of 50 mM Hepes, pH 7.7, 120 mM KCl, 16 mM $MgCl_2$, 14.8 mM 2-mercaptoethanol, 10 mM ATP, 1 mM NAD^+, 200 mM or 1 mM D-glucose, and 20 μg/ml (10 U/ml) of glucose-6-phosphate dehydrogenase (*Leuconostoc mesenteroides)* were added. The reaction was carried out at room temperature for 90 min. Tissue blanks (islet suspension plus assay medium without ATP), reagent blanks (complete extraction medium and assay medium with and without ATP), and glucose-6-phosphate standards prepared in extraction medium were also included. At the end of the incubation, the reaction was stopped by adding 1 μl of 0.2 N NaOH to each well. The rack was then heated at 60°C for 20–25 min.

The incubation mixture was then transferred quantitatively to fluorometer tubes with 50 μl of cycling reagent containing 100 mM Tris, pH 8.1, 300 mM ethanol, 2 mM mercaptoethanol, 0.02% BSA, 2 mM oxalacetate, 75 μg/ml alcohol dehydrogenase, and malate dehydrogenase, 12 μg/ml. The tubes were incubated at 25°C for 60 min and, for terminating the reaction, were heated in a boiling water bath for 2 min.

Indicator reagent (1 ml) containing 50 mM AMP-2 buffer, pH 9.9, 100–200 μM NAD^+, 10 mM glutamate, malate dehydrogenase (10 μg/ml), and glutamate-oxalacetate transaminase (2 μg/ml) was added to each tube. Another set of tubes was included to run malate standards. This reaction was carried out for 35–40 min. NADH formed by the indicator reaction was measured fluorometrically.

Glucose-6-phosphate standards were used for calculating the rates of phosphorylation of glucose, and malate standards were used to calculate the number of cycles during the cycling reaction. Apparent glucose phosphorylating activity was calculated for reagent and tissue blanks.

HORMONE CONTENT OF ISLET TISSUE

Pieces of freeze-dried tissue weighing 0.05–0.15 μg were transferred to the bottom of 12 × 75-mm glass tubes. Loading was carried out using a low magnification setting of a stereomicroscope. The hormone extraction was performed by adding to each tube 0.5 ml of cold 0.2 M glycine, pH 8.8, and 0.25% HSA. After letting the tubes stand in ice for 30 min, 0.5 ml of the same buffer was added to each tube. The tube was then capped, vortexed, and stored at −20°C until the day of radioimmunoassay.

Glucagon and insulin in human pancreas samples were measured by radioimmunoassay[15,16] using porcine insulin and a mixture of bovine and porcine glucagon as standards. Rat insulin was used as standard when measuring insulin content in pieces of rat pancreas.

CHEMICALS

Yeast phosphoglucose isomerase, glucose-6-phosphate dehydrogenase (*L. mesenteroides*), and alcohol dehydrogenase were from Boehringer-Mannheim (Indianapolis, Indiana). The suspensions were spun down at 12,000 rpm for 15 min at 4°C, the supernatants removed, and the pellets dissolved in a medium containing 20 mM Tris HCl, pH 8.2, with 0.2% BSA resulting in stock solutions with 350 U/ml of phosphoglucose isomerase, 3250 U/ml of glucose-6-phosphate dehydrogenase, and 25 mg/ml of alcohol dehydrogenase, respectively. Malate dehydrogenase and glutamate-oxalacetate transaminase were from Boehringer-Mannheim and were treated in the same way as glucose-6-phosphate de-

*Abbreviations: AMP-2, 2-amino-2-methyl-1-propanol; ATP, adenosine-5-triphosphate; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetracetic acid; G-6-P, glucose-6-phosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; HSA, human serum albumin; NAD, β-nicotinamide-adenine dinucleotide; NADH, β-nicotinamide-adenine dinucleotide, reduced; and Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

TABLE 2
Insulin and glucagon contents in microdissected human and rat islet tissue*

	Insulin (ng/μg dry wt)	Glucagon (ng/μg dry wt)
Human specimen†		
1	58 ± 9 (4)	4.7 ± 1.1 (4)
2	25 ± 4 (5)	13.7 ± 3.5 (5)
3	38 ± 8 (4)	7.9 ± 3.2 (4)
5	63 ± 2 (4)	7.6 ± 1.2 (4)
6	76 ± 9 (3)	6.3 ± 0.8 (3)
7	19 ± 2 (4)	1.9 ± 0.5 (4)
Mean ± SEM	46.5 ± 9.2 (6)	7.0 ± 1.6 (6)
Rat specimen		
1	86 ± 7 (5)	2.9 ± 1.4 (5)
2	102 ± 3 (4)	2.5 ± 0.8 (4)
3	96 ± 6 (6)	1.9 ± 0.4 (6)
Mean ± SEM	94.7 ± 4.7 (3)	2.4 ± 0.3 (3)

*All of the values represent the means ± SEM, with the number of determinations in parentheses.
†The hormone contents of tissues dissected with the aid of histologic maps are shown here.

hydrogenase to achieve concentrations of 10 and 5 mg/ml, respectively. D-[2-^{3}H(N)]glucose and ^3H_2O were from New England Nuclear (Boston, Massachusetts). Glucose-6-phosphate, ATP, DTT, 2-mercaptoethanol, malate, bovine serum albumin, human serum albumin, glutamate, oxalacetate, Hepes, Tris, NAD^+, EDTA, insulin, and glucagon were from Sigma (St. Louis, Missouri); 2-amino-2-methyl-1-propanol was from Eastman Kodak (Rochester, New York); and D-glucose was from Pfanstiel Laboratories (Waukeegan, Illinois).

Other chemicals were from Mallinkrodt (St. Louis, Missouri), Fisher (Fairlawn, New Jersey), and Sigma.

ANIMALS

Wistar albino rats were obtained from Hilltop Laboratories (Scottdale, Pennsylvania). Chinese hamsters (AA, M, XA and AC lines) were from Dr. G. Gerritsen, Upjohn Laboratories (Kalamazoo, Mississippi). Mice (CBA/J or C58/J lines) were provided by the Jackson Laboratory (Bar Harbor, Maine).

RESULTS

HORMONE CONTENT OF MICRODISSECTED HUMAN ISLETS AS INDICATOR FOR RELIABLE SAMPLING

The reliability of the microscopic sampling of islet tissue needed to be evaluated. Measurements of the hormone contents of random samples seemed most useful for this purpose. Five to six islet pieces were dissected from random slices of six of eight pancreata and extracted in assay buffer for measuring insulin and glucagon by radioimmunoassay. Five to six samples of exocrine tissue from each of the six pancreata were also dissected and extracted for the same purpose. Twenty-four of 31 islet pieces had insulin and glucagon in them, whereas all exocrine tissue samples were negative (Table 2). The hormone contents of specimens 4 and 8 were not measured because islets were identified without difficulty. Insulin and glucagon contents of rat islet samples were measured for comparison. In analyzing three rat pancreata, 15 of 15 islet pieces contained insulin and glucagon, whereas exocrine tissue was always negative. The insulin content of pieces of human islets was one-half of the insulin content of rat islet tissue (46.5 ± 9.2 versus 94.7 ± 4.7 ng/μg dry wt). By contrast, the glucagon content of human islets was three times higher than the glucagon content of rat islets (7.0 ± 1.6 versus 2.4 ± 0.8 ng/μg dry wt). The insulin/glucagon ratio was six times higher in rat islets than in human islets (39.1 versus 6.6). The results of these hormone measurements attest to the reliability of the dissection procedure.

GLUCOSE PHOSPHORYLATION OF ISLET TISSUE

Studies with the radiometric assay. Five islet samples from each pancreas were analyzed for glucokinase (Table 3). Twenty-five of 30 contained glucokinase activity. This success rate of 80% is similar to the outcome of the hormone analysis of human islets (see above). Even though 20% of the individual samples lacked glucokinase, the enzyme was present in all six pancreas specimens tested. The average computed maximal activity was 63.5 ± 11.6 mmol/kg dry wt × h, about one-half of the values found in rat islets (134.2 ± 4.3 mmol/kg dry wt × h). It needs to be appreciated that this calculation was made from the results obtained with 0.5 and 20 mM glucose and using 5.1 and 14.5 mM as K_m values for human and rat islet tissue, respectively. Table 4 illustrates the complexity of the radiometric glucokinase analysis. It is noteworthy that reagent blanks are substantial. The glucokinase measurements are based on an increment of 50% or more above the overall blank and are, therefore, reliable.

TABLE 3
Radiometric analysis of glucokinase in pancreatic islet tissue of man and rat

	Glucokinase activity* (mmol/kg dry wt × h at 37°C)
Human specimen	
1	47.3 ± 12.0 (4)
2	47.1 ± 18.7 (3)
3	41.7 ± 7.0 (4)
5	63.2 ± 18.6 (4)
6	118.5 ± 13.0 (5)
7	63.2 ± 19.2 (4)
Mean ± SEM	63.5 ± 11.6 (6)
Rat specimen	
1	125.1 ± 3.2 (5)
2	140.8 ± 5.7 (5)
3	142.6 ± 9.1 (5)
4	122.4 ± 5.4 (5)
5	140.2 ± 7.1 (5)
Mean ± SEM	134.2 ± 4.3 (5)

Results of the analysis of six human pancreata and five rat pancreata are presented. The means ± SEM are recorded and the number of samples analyzed is indicated in parentheses. It needs to be appreciated that for each pancreas altogether, four groups of 3–5 samples each were analyzed to obtain tissue blanks at 0.5 and 20 mM glucose (studies with the human tissue) or at 0.5 and 30 mM glucose (studies with the rat tissue) (i.e., in the absence of ATP), and to measure the rates of glucose phosphorylation at 0.5 and 20 or 0.5 and 30 mM glucose (i.e., in the presence of ATP).
*The activities are expressed as V_{max} and were calculated according to the Michaelis-Menten equation using a K_m for D-glucose of 5.1 mM for human glucokinase (present report) and 14.5 mM for rat islet glucokinase (see ref. 8).

TABLE 4
Example of radiometric measurement of glucokinase in human pancreatic tissue*

Condition	H_2O produced (fmol/40 min at 20.5°C)
(1) 0.6 mM D-glucose	
(a) Reagent blank (−ATP)†	69 ± 6 (3)
(b) Islet tissue blank (−ATP)‡	130 ± 14 (3)§
(c) Islet tissue glucose phosphorylation‖	203 ± 20 (4)§
(2) 20 mM D-glucose	
(a) Reagent blank (−ATP)†	2062 ± 77 (3)
(b) Islet tissue blank (−ATP)‡	687 ± 87 (3)§
(c) Islet tissue glucose phosphorylation‖	960 ± 127 (4)§
(2c − 1c) Islet high-K_m glucose phosphorylation¶	757 ± 107 (4)

*Tissue samples were dissected from specimen 3 and analyzed according to the assay described in MATERIALS AND METHODS. The glucokinase activity was 41.7 ± 7.0 mmol/kg dry wt × h (37°C). It is emphasized that this specimen had the lowest glucokinase activity.
†The reagent blanks are the same in the presence and absence of ATP in the medium.
‡Corrected for reagent blank.
§The weight of the samples ranged from 0.10 to 0.17 μg. The tissue activities were corrected for weight and expressed per 0.1 μg dry wt to allow approximate comparisons.
‖Corrected for reagent blank and tissue blank.
¶Islet tissue glucose phosphorylation at 20 mM glucose (2c) corrected for islet tissue glucose phosphorylation at 0.6 mM (1c).

To investigate the glucose dependence of glucokinase in human islets, 33 pieces of islet tissue from donor 6 were dissected and assayed for glucokinase over a range of D-glucose concentrations from 1 to 44 mM (Figure 1). Least-squares linear regression analysis of the linear portion of the data indicate a K_m for D-glucose of 5.1 mM and a V_{max} of 72.0 mmol/kg dry wt × h (37°C).

Studies with the fluorometric assay. In initial attempts to study glucose phosphorylation in pancreatic islet tissue with quantitative histochemical methods, a fluorometric procedure was applied. The assay involved the use of oil wells to handle small volumes and enzymatic cycling to achieve the needed sensitivity.[10,14] This approach was useful when measuring hexokinase activity but generated reliable measurements of glucokinase activity only with islet tissue from Chinese hamsters and mice (Table 5). Normoglycemic and diabetic Chinese hamsters showed the highest glucokinase activities. Diabetes had little impact on the glucokinase activity. Since the β-cell population in these diabetic animals drops to about 50% of the normal group, the possibility that glucokinase is also present in non-β-cells should be considered; further studies with islet preparations enriched with non-β-cells (i.e., islet cell purification by fluorescence-activated cell sorting or α-cell-rich islets from rodents with experimental diabetes) are necessary to support this hypothesis.

The hexokinase activities of two mouse strains were very similar regardless of the nutritional state of the animals. Glucokinase activities in the fed state were also the same in the two strains but fell by one-third when animals were fasted. The decrease of glucokinase due to fasting is of a similar magnitude as that observed in two strains of rats.[17] The two mouse strains were chosen because Coleman[18] had reported that liver glucokinase in the two strains differed by a factor of two and it seemed attractive to explore whether a similar difference manifested itself in the pancreatic islets. Since this was not so, it seems that the level of glucokinase in islet tissue and liver was regulated independently. The results from fasted mice help to illustrate that the fluorometric microassay can provide reproducible results when the relative glucokinase activities are in the range of about 30% of the total phosphorylation activity.

The hexokinase activity of rat and human islets was about twice that found in the mouse. It is also noteworthy that the hexokinase activity of the exocrine tissue of rats was almost three times the activity of rat islet tissue. Therefore, even minor contaminations of endocrine with exocrine tissue could interfere with the quantitation of islet hexokinase and more significantly with measurements of islet glucokinase. The apparent glucokinase activity in rat and human islets was only a minor fraction of the total glucose phosphorylating activity, i.e., 10–16%. In the absence of detailed substrate dependency studies of glucose phosphorylation, such a small increment of glucose phosphorylation when the substrate is raised from 0.5 to 100 mM is not sufficient evidence for the presence of glucokinase in a given tissue. However, the radiometric microassay, which is not compromised by the hexokinase background, allowed us to apply quantitative his-

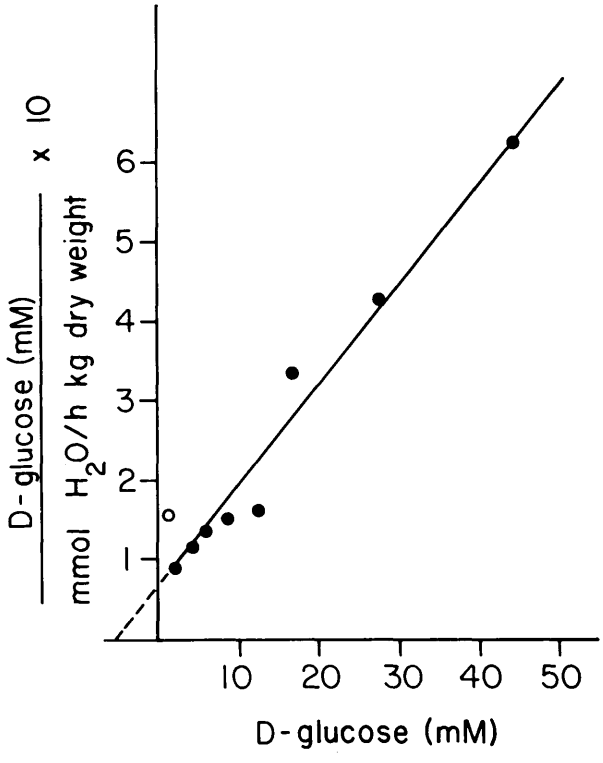

FIGURE 1. Hanes-Woolf plot of the glucose dependency of glucokinase in microdissected pieces from human pancreatic islets. Freeze-dried pieces of human islets weighing 0.05–0.150 μg and dissected from specimen 6 were used. The assays at each glucose concentration were performed in quintuplicate as described in MATERIALS AND METHODS and the results were corrected for temperature (37°C) assuming a Q_{10} of 2 .1. Glucokinase activity was corrected for the hexokinase component as described in the text. The velocity at 0.9 mM glucose (○) was not considered when computing kinetic parameters for glucokinase because it was considered indicative of deviation from Michaelis-Menten behavior.

TABLE 5
Fluorometric analysis of glucose phosphorylation by islet tissue of different species

Species	Hexokinase (HK) (mmol/kg islet dry tissue × h at 37°C)	Glucokinase (GK) (mmol/kg islet dry tissue × h at 37°C)	(GK/HK + GK) × 100
Chinese hamster, fed			
Normal AA-line	275 ± 50 (10)*	175 ± 23 (10)	39
M-line	400 ± 33 (15)	204 ± 36 (14)	34
Diabetic† XA-line	212 ± 40 (7)	186 ± 14 (7)	47
AC-line	651 ± 45 (11)	197 ± 25 (10)	24
Mouse			
CBA/J, fed	179 ± 19 (8)	103 ± 8 (8)	37
72-h-fasted	175 ± 27 (7)	67 ± 8 (7)	28
C58/J, fed	149 ± 11 (8)	94 ± 9 (8)	39
72-h-fasted	139 ± 25 (3)	64 ± 6 (3)	32
Rat, fed‡	345 ± 69 (6)	65 ± 14 (6)	16
Man‡			
Specimen 4	349	40	10
Specimen 8	358	68	16

The data were obtained with the fluorometric oil-well assay using enzymatic cycling of pyridine nucleotides to achieve the necessary sensitivity. Hexokinase was measured with 0.5 mM and glucokinase with 100 mM glucose in the medium. The glucokinase values were obtained by difference.
*Numbers in parentheses refer to number of animals analyzed.
†Fed blood sugar values were: 523 ± 26 mg/dl (AC-line), 562 ± 32 mg/dl (XA-line), and <200 mg/dl in the AA- and M-lines.
‡Hexokinase of exocrine samples from rat pancreas was 1210 ± 177 mmol/dry tissue but glucokinase was not detectable; i.e., with 100 mM glucose in the medium, glucose phosphorylation was 2.5 ± 4.5% less than at 0.5 mM with glucose. In exocrine samples of the human pancreas, hexokinase activities were 351 and 485 mmol/kg dry tissue and the apparent relative contributions of glucokinase to total glucose phosphorylation as measured at 100 mM glucose were 9.5% and 5.3%, respectively.

tochemical techniques for measuring glucokinase in pancreatic islets of rat and man (Table 3).

In summary, during the last 4 yr using two analytic methods, we have collected quantitative histochemical data showing the presence of glucokinase in the pancreatic islets of four mammalian species: hamster, mouse, rat, and man. The glucokinase activities differed by a factor of about three, varying from 60 to 200 mmol/kg dry tissue × h, with the lowest activity found in man and the highest observed in the Chinese hamster.

DISCUSSION

Methodologic considerations. We are aware of only five direct biochemical studies of the glucostat function of human pancreatic islet tissue.[19–23] The reason for this limited information is that human pancreatic tissue is scarce and that bulk preparations of human pancreatic islet tissue risks contamination by exocrine tissue. The quantitative histochemical sampling procedure applied here avoids many problems inherent in common methods of islet isolation. However, this approach suffers from the fact that the sample collected for analysis represents only a minute fraction of the total islet organ. In quantitative terms, only about five parts per million were randomly selected to measure hormone content or glucokinase activity in islet tissue. This biopsy approach seems justified, however, on general principles and judging from the similarity of results that were obtained by analyzing insulin and glucokinase in rat islets sampled by microdissection from freeze-dried sections on one hand or obtained by mass isolation using collagenase on the other (compare data in Table 3 with previously published results).[8] The success rate of collecting islet tissue by microdissection using maps of adjacent stained sections as guide is high, 80 times higher than can be expected from random dissection of pancreatic tissue. The approach could probably achieve the theoretical 100% yield if staining were performed with an islet-specific, immunohistochemical method for insulin.

The use of two analytic procedures for measuring glucokinase histochemically as practiced in this study is explained historically. The fluorometric assay was developed first[14] and generated reliable data when applied to liver tissue, in which 60–90% of the glucose phosphorylating activity is contributed by glucokinase. This assay also proved reliable and useful in studies of hamster and mouse islet tissue, in which glucokinase contributes 25–50% to glucose phosphorylation. However, for measurements in rat and human islets, with a hexokinase component of 80–90% of the total phosphorylation capacity, a new assay had to be developed that largely eliminated the hexokinase interference. It is conceivable that the fluorometric oil-well assay could be improved by inclusion of antibodies inhibiting hexokinase. Such a modification could provide the nearly limitless sensitivity that characterizes pyridine nucleotide-dependent cycling assays.

Glucokinase is the pacemaker of glycolysis in human pancreatic islets. An extensive quantitative histochemical study on the enzyme pattern of human islets of Langerhans was reported several years ago by Gepts et al.,[19] but the publication lacked information on important rate-limiting enzymes of glycolysis. A significant report relevant for human islet cell glucose sensing is that of Ashcroft et al.,[20] who studied glucose metabolism in intact islets isolated with collagenase from one single pancreas removed surgically from a 4-yr-old girl with the diagnosis of idiopathic hypoglycemia. The rate of glucose utilization (measured as the rate of ^3H_2O production from [5-^{3}H]glucose) was 56.6 ± 10.4 pmol/islet/h at 20 mM glucose, which is 50% lower than the rate of glucose usage in intact, isolated rat islets reported by Trus et al.:[2] glucose utilization was increased sixfold by raising

extracellular glucose from 3.3 to 20 mM in the presence of 5 mM caffeine. Andersson et al.[21] studied the oxygen consumption of human islets maintained in culture for 7 days: 16.7 mM glucose increased the oxygen uptake by 31%. This stimulatory action of glucose is one-half of that reported for corresponding manipulation of rodent islets.[22]

The results of measuring glucose use and glucose-stimulated oxygen consumption in isolated human islets have to be viewed in light of current concepts about the nature of regulation of glycolysis in islet tissue. Glucokinase is assumed to determine the metabolic flux through the glycolytic pathway. Studies in rat islet tissue indicate that the enzyme phosphorylates glucose with a V_{max} of 130–160 pmol/islet/h (37°C) and a K_m of 8–14.[2,3] In addition, it has been shown that glucose dependency of islet glucokinase is sigmoidal.[3] Glucose use by intact rat islets has virtually identical characteristics.[2] Glucokinase in human islets of Langerhans had an apparent V_{max} of 63.5 ± 11.6 mmol/kg dry wt × h (37°C). The Hanes-Woolf plots of results from the glucose concentration dependency study of glucokinase in islet samples from the pancreas donor 6 indicates a V_{max} of 72.0 mmol/kg dry wt × h (37°C) and a K_m for glucose of 5.1 mM (Figure 1). This K_m value is somewhat lower than the value reported for rat islet glucokinase. It needs to be appreciated that these are the results from one single pancreas. Nevertheless, the apparent K_m is in the range of physiologic blood glucose concentrations in man and is in agreement with the studies recently reported by Harrison et al.,[23] who showed that both glucose oxidation and insulin secretory response to glucose of human isolated islets were sigmoidal with a threshold at 2–4 mM and half-maximal response at 5 mM. Although human islet glucokinase seems to deviate from Michaelis-Menten behavior, the data are insufficient to decide whether human islet glucokinase is sigmoidal or not. More extensive studies seemed unwarranted because they exceded the capacity of our microdissection procedure; three 7-h sessions of dissection were needed to collect the pool of samples necessary to perform one single K_m determination.

The fact that the apparent V_{max} for glucokinase found in human islets extracts is similar to the V_{max} for glucose utilization reported previously for intact, isolated human islets and the finding of a K_m for glucose within the range of physiologic blood glucose concentrations both in human islet extracts and intact islets strongly suggest that this enzyme could determine glucose use in the β-cell of the human islet, as previously proposed for rat islets.

It has been speculated that a specific failure of the β-cell glucose sensor could be the basis of certain forms of type II diabetes.[24] It is appealing to hypothesize that abnormalities in the regulation of the biosynthesis or degradation of islet glucokinase or of the allosteric and hysteretic regulation of this enzyme[25] could be involved in the pathogenesis of selected cases of type II diabetes. The present report discusses the conceptual basis for thinking along such lines and describes the application of new micromethods for studying the human islet glucose sensor.

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