Glucose Regulates Glucokinase Activity in Cultured Islets from Rat Pancreas*

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In this study, we have used isolated pancreatic islets cultured for 7 days in 3 or 30 mM glucose to explore whether glucokinase is induced or activated by high glucose concentrations and has related enzyme activity to glucose-stimulated insulin release. Islets cultured in low glucose medium or low glucose medium plus 350 ng/ml insulin did not respond to high glucose stimulation. Islets cultured in medium containing high glucose concentrations showed a high rate of basal insulin secretion when perifused with 5 mM glucose, and the insulin release was greatly augmented in a biphasic secretion profile when the glucose concentration was raised to 16 mM. Islet glucokinase and hexokinase activities were determined by a sensitive and specific fluorometric method. Glucokinase activity was reduced to ~50% in islets cultured in low glucose medium with or without insulin present compared to results with fresh islets. However, islets cultured in 30 mM glucose showed that glucokinase activity was elevated to 286% compared to results with fresh islets. It is concluded that (a) glucose is the physiological regulator of glucokinase in the islet of Langerhans and that (b) the activity of glucokinase plays a crucial role in glucose-induced insulin secretion.

The precise mechanism by which glucose stimulates insulin secretion from pancreatic islets is not known, but it appears that increased glucose metabolism is causally involved (1, 2). Glucokinase (ATP:D-glucose 6-phosphotransferase, EC 2.7.1.2) determines the rate, concentration dependence, cooperativity, and anomic discrimination of glucose metabolism in the β-cells of pancreatic islets (2-6). Glucokinase is thus likely to function as the glucose sensor of the β-cell, relating extracellular glucose concentration to β-cell metabolism and insulin secretion (7). We reported (9) that the activity of this enzyme in islets might be regulated differently from that of liver glucokinase. Islet glucokinase activity appeared to be governed by serum glucose levels, whereas liver glucokinase activity seemed to be determined by serum insulin concentrations. Inducibility or activation by glucose rather than by insulin would be a plausible design feature for islet glucokinase as the proposed glucose sensor element of β-cells. In this study, we have used isolated pancreatic islets cultured for 7 days in 3 or 30 mM glucose to study directly glucose activation or inducibility of glucokinase and glucose-stimulated insulin release. The results support the hypothesis that glucose is the key regulator of glucokinase activity in the pancreatic islet based on mechanisms to be discovered.

MATERIALS AND METHODS

Islet Culture—Pancreatic islets were isolated from male rats (250-300 g) using collagenase digestion and harvesting islets on a Ficoll gradient (10). About 400-500 islets were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 2 mM glutamine, 10 mg/liter glutathione, and 50,000 units/liter penicillin/streptomycin. Various test substances were added to the culture medium as described under "Results." The culture medium was changed every 2nd day. After 7 days of culture, islets were used for assessing glucose-stimulated insulin release with a perifusion system or for determining glucokinase activity in tissue extracts. To evaluate the viability of the islet preparation, the glucose concentration in the medium was switched either from 3 to 30 mM or from 30 to 3 mM on the 7th day of culture, and culture was continued for another 4 days before studying insulin release with the perifusion system.

Islet Perfusion—A perifusion system described previously (11) was used. Islets were first perfused for 30 min with 5 mM glucose. This was followed by 30 min of perfusion with 16 mM glucose. Perifusate samples were collected at intervals and stored at -20 °C for later analysis of insulin by radioimmunoassay (12). After 60 min of perfusion, the filters containing the islets were quickly removed from the plastic chamber and frozen on a slab of dry ice within seconds. The frozen islets were stored at -80 °C for later determination of DNA and insulin content.

Glucokinase and Hexokinase Activity Determination—Approximately 300 fresh or cultured islets were homogenized after being washed free of glucose. Homogenization was performed with 300 μl of buffer (20 mM K2HPO4, containing 5 mM dithiothreitol, 1 mM EDTA, and 110 mM KCl) in a Kontes 18 glass homogenizer by 10 strokes of a machine-driven Teflon pestle (Bellco Glass homogenizer drive unit set on 3). This and all subsequent purification steps were performed at 2-4 °C. The homogenate was centrifuged at 12,000 × g for 30 min. The supernatant fraction was used for glucokinase and hexokinase determination by a fluorometric method (13). The reaction volume was 100 μl, and the assay was conducted for 90 min at 30 °C. The reaction mixture contained 30 mM Hepes/HCl, pH 7.0, 100 mM KCl, 7.4 mM MgCl2, 15 mM β-mercaptoethanol, 0.5 mM NAD+, 0.05% bovine serum albumin, 0.70 unit/ml glucose-6-P dehydrogenase from Leuconostoc mesenteroides, 5 mM ATP, and glucose at a range of 0.03-100 mM. Determination of the K0.5 values for hexokinase and glucokinase was routinely done with assay reagents containing five levels of glucose at a range of 0.03-0.3 mM and live

The abbreviation used is: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
levels of glucose at a range of 6–100 mM, respectively. According to this method (13), the reagent blanks consisting of the assay reagents with 0.5 or 100 mM glucose in the absence of tissue were included in all determinations. Each assay also had tissue blanks consisting of tissue plus assay reagent containing either 0.5 or 5 mM ATP in the absence of glucose. The reagent and tissue blanks were both subtracted from the total fluorescence of a corresponding complete reagent containing tissue. The sensitivity of this method is sufficient to accurately determine \( \approx 0.1 \mu M \) or, considering the assay volume, \( 10^{-10} \) mol of NADH. Average values of \( \approx 5 \times 10^{-11} \) mol of NADH were discounted. The kinetic constants of glucokinase and hexokinase were determined by Eadie-Scatchard plots (\( 1/[S] \) versus \( 1/[S] \)). The kinetic parameters of glucokinase and hexokinase were calculated according to the method of Spears et al. (14) with 10 correction cycles, which gives a good approximation for such parameters. To present the data in a manner comparable to other publications, the \( V_{max} \) values of glucokinase and hexokinase were extrapolated to a temperature of 37°C assuming a \( Q_0 \) of 2 (15).

Determination of Insulin and DNA Content in Islets—Frozen filters carrying the islets were sonicated in 1 ml of phosphate-buffered saline containing 50 kallikrein-inactivating units of Trasylol, pH 7.40. Immediately, 100 \( \mu l \) of the sonicate was transferred into 900 \( \mu l \) of 70% acid ethanol and kept at 4°C for 2 h. The acid ethanolic extract was then used for the insulin radioimmunoassay. The DNA content in the pancreatic islets was measured by a simple, rapid, and sensitive method (16). In the case of islets which were used for glucokinase determination, 10 \( \mu l \) of homogenate prepared as described above was diluted with 400 \( \mu l \) of phosphate-buffered saline for measuring DNA.

RESULTS

Insulin and DNA Content and Insulin Secretion of Freshly Isolated or Cultured Islets—Freshly isolated islets showed the typical biphasic profile of insulin release when stimulated with 16 mM glucose (Fig. 1). Islets cultured for 7 days in medium containing 3 mM glucose or 3 mM glucose plus 350 ng/ml insulin did not respond to high glucose concentrations. Islets cultured for 7 days in medium containing 30 mM glucose showed a high rate of basal insulin secretion when perfused with 5 mM glucose, and the insulin release was further augmented in a biphasic secretion profile when the glucose concentration was raised to 16 mM. When islets cultured for 7 days with 3 or 30 mM glucose and then incubated for an additional 4 days in culture medium in which the glucose level was switched from 3 to 30 mM or from 30 to 3 mM were functionally tested in a perfusion system, it was found that glucose responsivity had recovered after 4 days of culture in high glucose medium (98.2 ± 15.3% of fresh islets), but had been lost after 4 days in low glucose medium (data not shown).

The insulin content of the freshly isolated islets and of the islets cultured for 7 days in the various media showed no differences. The DNA content also appeared similar in all these islet groups (Table I).

Glucokinase and Hexokinase Activities in Freshly Isolated or Cultured Islets—The activities of glucokinase and hexokinase in islets harvested under different experimental conditions were determined by a sensitive and specific fluorometric method. The glucose phosphorylating activities in supernatants of islet homogenates were analyzed using Eadie-Scatchard plots. Two components were resolved. The high affinity component was identified as hexokinase, and the low affinity component as glucokinase. The \( V_{max} \) and \( K_m \) values of glucokinase were determined by the Eadie-Scatchard plot after correcting for glucose phosphorylation by hexokinase with an approximation which was calculated by the method of Spears et al. (14). An example illustrating the calculation of the \( V_{max} \) and \( K_m \) values of glucokinase and hexokinase is given in Fig. 2. Glucokinase activity was reduced to \( \approx 50\% \) in the islets cultured for 7 days in medium containing 3 mM glucose both with or without insulin present when compared to results with freshly isolated islets. However, the islets cultured in 30 mM glucose showed greatly elevated activity of glucokinase, i.e. 230% compared to fresh islets (Table I). The apparent \( K_m \) of glucokinase for glucose in freshly isolated islets was 17.3 mM, whereas in the islets cultured in high glucose medium, the \( K_m \) increased to 62.8 mM. The \( K_n \) values of glucokinase for glucose in the islets cultured in low glucose medium or low glucose medium plus insulin were 65.5 and 79.6 mM, respectively. Compared to islets isolated fresh, the activity of hexokinase was drastically decreased to \( \approx 13-19\% \) in the islets cultured in high or low glucose medium (Table I). The \( K_m \) of hexokinase for glucose in freshly isolated islets was 0.099 mM. Islets cultured for 7 days in high or low glucose medium seemed to increase the \( K_m \) of hexokinase for glucose to 0.203 ± 0.06 and 0.306 ± 0.08, respectively. However, statistical analysis showed no significant difference between the freshly isolated islets and the islets cultured either in high or low glucose medium.

DISCUSSION

Glucokinase in pancreatic islets appears to be the pace-maker of glycolysis and is conceptualized as the glucose sensor that governs insulin secretion at physiological glucose concentrations. Reduction of islet glucokinase activity has previously been observed in islets of fasted rats. After a 24-h fast, islet glucokinase activity was decreased by 20% and after a 72 h fast, by 81% (17). Glucokinase activity was greatly reduced by 71% in islet samples from rats which had severe prolonged hypoglycemia induced by an insulinoma, and the enzyme activity fully recovered within 24 h after tumor removal (9). It appeared reasonable to suggest that glucose is an important regulator of the activity of glucokinase. However, the in vivo models of extended fasting and severe hypoglycemia have obvious limitations. Therefore, a tissue culture model was used to study the direct effect of glucose on glucokinase activity and insulin secretion with minimal interference by other factors. A 7-day culture period was chosen and found suitable to test glucokinase inducibility or activation by glucose since the islets retained normal insulin and DNA content under the various culture conditions. To test the possible effect of glucose on the activity of glucokinase, extremes of
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Table I

Insulin and DNA content, insulin release function, and activities of glucokinase and hexokinase in freshly isolated and cultured islets from rat pancreas

|                   | Insulin content | DNA content | Insulin release | Glucokinase | Hexokinase |
|-------------------|----------------|-------------|----------------|-------------|------------|
| (n = 6)           | (n = 6)        | (n = 3)     |                | V_{max}     | V_{max}    |
| Freshly isolated islets |              |             |                |             |            |
| 3 mM glucose     | 1.83 ± 0.21   | 17.86 ± 0.79| 12.18 ± 0.10   | 5.22 ± 0.45 | 4.10 ± 0.51|
| 3 mM glucose + 350 ng/ml insulin | ND^a          | 18.31 ± 0.89 | 5.21 ± 0.80 | 2.55 ± 0.47 | 0.53 ± 0.16 |
| 30 mM glucose    | 1.48 ± 0.13   | 18.74 ± 1.50| 103.97 ± 9.80 | 12.32 ± 0.79 | 0.79 ± 0.22 |
| Islets cultured in: |              |             |                |             |            |
| 3 mM glucose     | 1.44 ± 0.17   | 19.24 ± 1.46| 100.00 ± 15.50| 5.22 ± 0.45 | 4.10 ± 0.51|
| 3 mM glucose + 350 ng/ml insulin | ND^a          | 18.76 ± 0.89 | 5.21 ± 0.80 | 2.55 ± 0.47 | 0.53 ± 0.16 |
| 30 mM glucose    | 1.48 ± 0.13   | 18.74 ± 1.50| 103.97 ± 9.80 | 12.32 ± 0.79 | 0.79 ± 0.22 |

The approximate V_{max} and K_m values of glucokinase and hexokinase were determined by the Eadie-Scatchard plot and corrected 10 times using the method of Spears et al. (14). All the V_{max} data presented here were extrapolated to a temperature of 37 °C assuming a Q_10 of 2.

^a The glucose-stimulated insulin release of freshly isolated islets was 293 ng/30 mm/min/100 islets.

^b n = 6.

^c n = 4.

^d ND, not determined.

^e p < 0.05 (compared with freshly isolated islets).

Previous biochemical studies of glucokinase indicated that glucokinase in pancreatic islets and in the liver might be differentially regulated. In the insulinoma-bearing rat, which showed severe hyperinsulinemia and hypoglycemia, liver glucokinase was increased nearly 4-fold, whereas islet glucokinase activity was reduced by 71% (9). Magnuson and Shelton (18) have reported that glucokinase DNA has tissue-specific promoters, one operative in β-cells with a unique translation start site compared with the promoter of liver glucokinase and leading to the production of a β-cell glucokinase with a different N-terminal sequence. Our data show a nearly 5-fold range of glucokinase activity as a function of the glucose concentration of the culture medium. It needed to be tested whether the high glucokinase activity in 30 mM glucose was due to stimulation of β-cells by high ambient insulin concentrations. Culture media of islets maintained in 30 mM glucose accumulated as much as 760 ng/ml insulin (data not shown). We chose to test the effect of 350 ng/ml insulin together with 3 mM glucose on glucokinase activity. This is about half the level found in the culture medium and at least 150 times the physiological level in the serum. The level of added insulin was maintained constant during organ culture (data not shown). Exogenous insulin did not influence glucokinase activity. The results demonstrate that it is glucose, and not insulin, which regulates glucokinase activity in the pancreatic islets. The insulin secretory response to glucose stimulation paralleled the change in glucokinase activity. Islets cultured in medium containing 30 mM glucose showed both high glucokinase activity and insulin secretion. On the other hand, glucokinase activity and insulin response were concomitantly reduced in the islets cultured in low glucose medium. However, it is noteworthy that the increases in glucokinase activity and insulin secretion lacked a one-to-one linear relationship. This indicates that besides glucokinase, other factors participating in the stimulation-secretion sequence of insulin release might be influenced by prolonged exposure to high glucose concentrations. A possible regulatory involvement of the glucose transporter of the β-cell membrane also needs to be investigated in this experimental system (19, 20).

Hexokinase activities in the supernatants of fresh islets and cultured islets were strikingly different. In the fresh islets, the activity of hexokinase amounted to 4.1 mol/kg of DNA/h, which is comparable to data published before (13), whereas in the cultured islets, hexokinase activity had dropped to 13–19% of the activity found in fresh islets. It seems that glucose concentrations were initially used in the culture medium. Islets cultured in 30 mM glucose responded promptly and biphasically to glucose stimulation, and islets that were cultured in low glucose medium and then cultured for 4 days in 30 mM glucose regained full glucose responsiveness. In additional experiments (not shown here), we perfused islets cultured for 7 days in 30 mM glucose with glucose-free Krebs buffer for 30 min and then switched to 16 mM glucose. Under this condition, basal insulin secretion from islets cultured in high glucose medium was the same as that from freshly isolated islets and showed the typical biphasic release of insulin when challenged with 16 mM glucose. This result suggests that the high basal insulin secretion from islets cultured in high glucose medium is due to an enhanced sensitivity to glucose. These functional results demonstrate that cultured islets are a suitable model to study the regulation of islet glucokinase.
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is not the determinant factor since hexokinase activity in islets cultured either in high or low glucose medium appeared similarly reduced. Hexokinase is usually associated with the particulate fractions of the tissue homogenates. We therefore measured hexokinase in the pellet of the islet homogenates and found a similar reduction as observed in the supernatant (data not shown). Thus, hexokinase appears greatly reduced in cultured islets. This may be due to loss of non-β-cells during culture. The data indicate that the glucokinase enzyme must play a key role in islet glycolysis and glucose-stimulated insulin secretion.

The apparent $K_m$ of glucokinase for glucose in the fresh islets was 17.3 mM. This value is close to the published values of 8–16 mM (13, 21). In islets cultured in 30 mM glucose, the apparent $K_m$ of glucokinase for glucose increased to 62.8 mM. The reason for this difference is not clear, and the metabolic significance needs to be investigated. Tippett and Neet (22) reported that long chain acyl-CoA specifically inhibits rat liver glucokinase. The inhibition was competitive with ATP or glucose and did not affect the $V_{max}$ or the Hill coefficient, indicating cooperativity with glucose. We have found that glucose stimulation for 30 min increases the long chain acyl-CoA level in perfused freshly isolated rat islets. High glucose concentrations in the culture medium might also raise the long chain acyl-CoA content in the cultured islets, leading to competitive inhibition of glucokinase and a $K_m$ shift. The Eadie-Scatchard plot indicated that glucokinase in the islets cultured in low glucose medium or low glucose medium plus insulin also showed a substantial increase in its apparent $K_m$ for glucose compared with glucokinase from freshly isolated islets. An explanation for this phenomenon is not obvious, and the metabolic significance of this drastic change remains to be explored.

Our data demonstrate that glucose is an important determinant of glucokinase activity in the islet of Langerhans. The mechanism of glucose regulation is not clear. Regulation of glucokinase activity by its substrate might occur at different levels of control. The promoter governing biosynthesis of β-cell glucokinase differs from the promoter controlling biosynthesis of liver glucokinase (18). Thus, glucose might influence transcription and regulate glucokinase at the mRNA level analogous to insulin’s effects on liver glucokinase mRNA. Lyndajian et al. (23) have recently reported that a regimen of 48 h of fasting followed by 6 h of refeeding did not alter the glucokinase mRNA level in the islets. This result indicates that (a) the glucose effect on the glucokinase mRNA might occur very slowly if it occurs at all or that (b) the glucose effect on glucokinase might be entirely translational or post-translational. The later explanation is supported by the drastically modified kinetic characteristics of glucokinase from cultured islets. Phosphorylation of glucokinase influences catalytic activity (8). This provides a potential mechanism to explain the activity changes of glucokinase as we observed.

On balance, we conclude that (a) glucose is the physiological regulator of glucokinase in the islets of Langerhans and that (b) the activity of glucokinase plays a crucial role in glucose-induced insulin secretion. This work greatly strengthens the concept that glucokinase serves as a β-cell glucose sensor and that the islet culture system might be very useful to further explore the adaptive nature of this crucial islet cell enzyme and the metabolism-dependent process of insulin synthesis and release.

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