Regulatory Effects of Glucose on the Catalytic Activity and Cellular Content of Glucokinase in the Pancreatic \(\beta\) Cell

**Study Using Cultured Rat Islets**

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Abstract

Glucose regulates the cellular content of glucokinase in the pancreatic \(\beta\) cell by altering the level of the enzyme. We investigated the existence of a second regulatory pathway, an alteration in the catalytic activity, by comparing \(V_{\text{max}}\) and protein levels of glucokinase in rat islets cultured under high glucose conditions (16.7 mM) for 6, 14, and 24 h. The \(V_{\text{max}}\) was increased by glucose at all time points. In contrast, glucokinase protein levels on Western blots were unchanged from the control value at 6 h but increased 40% at the later time points \((P < 0.0002)\). Further evidence for a dual regulatory system was obtained with a reversal protocol. After a 6-h incubation at high glucose, an additional 3-h incubation at 5.5 mM glucose restored glucokinase \(V_{\text{max}}\) to normal, but failed to change the \(V_{\text{max}}\) after a 24-h incubation at high glucose. Finally, 10 \(\mu\)M cycloheximide partially prevented the increase in glucokinase \(V_{\text{max}}\) induced by 24 h of high glucose, but had no effect at 6 h, suggesting the early increase in enzymatic activity did not require protein synthesis.

In summary, glucose regulates both the catalytic activity and cellular content of glucokinase in the \(\beta\) cell. Glucose-induced increases in glucokinase activity are an important element of the \(\beta\) cell adaptive response to hyperglycemia. (J. Clin. Invest. 1994. 94:1616–1620.) Key words: islets of Langerhans • insulin secretion • glucose phosphorylation • hexokinase • immunoblot

Introduction

In hepatocytes and pancreatic \(\beta\) cells, the high \(K_{\text{m}}\) glucose phosphorylation enzyme glucokinase is the rate-limiting step for glucose metabolism, and a key site of regulation for glucose-induced insulin secretion and hepatic glucose production (1, 2). The recent discovery that glucokinase gene mutations account for many of the cases of MODY (maturity-onset diabetes of youth) has pointed out the pivotal role played by this enzyme in glucose homeostasis (3, 4). Different isoforms with separate regulatory controls occur in the liver and \(\beta\) cell (5, 6). Interest has focused primarily on glucose as a regulatory factor of the \(\beta\) cell isofrom. Culturing rat islets in high glucose for 24 h causes the cellular content of this enzyme to increase without changing the mRNA level (7). Culture in low glucose has the opposite effect (7). This posttranscriptional regulation of the synthesis and/or degradation of glucokinase by glucose is to date the only known mode of regulation for the \(\beta\) cell isofrom.

We recently postulated that a second type of regulation occurs at the level of the catalytic activity of the enzyme. This hypothesis was based on our findings in two hyperinsulinemic rat models of increased activity of islet glucokinase without any change in the level of its protein measured by Western blot (8, 9). The present study was designed to test if glucose regulates the activity of this enzyme independent of changes in its cellular content by comparing glucokinase \(V_{\text{max}}\) and protein levels in rat islets cultured at high concentrations of glucose for 6, 14, or 24 h. A dual regulatory system was confirmed, with glucose increasing both the catalytic activity and cellular content of glucokinase over different time courses and with different susceptibilities to reversal.

Methods

**Isolation and culture of islets.** Male Sprague-Dawley rats (150–200 g) were anesthetized with pentobarbital sodium (100 mg/kg i.p.). Islets were isolated using an adaption of the method of Gotth et al. (10): pancreatic duct infiltration with collagenase, Histopaque gradient separation (Sigma Chemical Co., St. Louis, MO), and hand picking. Islets were cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 10% newborn calf serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin (all from Gibco Laboratories, Grand Island, NY) at 37°C in humidified air/5% CO\(_2\).

**Protocols.** (a) 18 h of 5.5 mM glucose followed by 16.7 mM glucose × 6 h; 16.7 mM glucose × 24 h; 5.5 mM glucose × 24 h. (b) 4 h of 5.5 mM glucose followed by 16.7 mM glucose × 14 h; 5.5 mM glucose × 18 h. (c) Groups of protocol a followed by an additional 3 h at 5.5 mM glucose. (d) 18 h of 5.5 mM glucose followed by 16.7 mM glucose × 6 h with or without 10 \(\mu\)M cycloheximide (Sigma Chemical Co.); 16.7 mM glucose × 24 h with or without 10 \(\mu\)M cycloheximide; 5.5 mM glucose × 24 h.

**Insulin secretion.** Islets were washed three times in KRB, 10 mM Hepes, 2.8 mM glucose, 0.5% BSA fraction V (Sigma Chemical Co.), and preincubated in the same buffer for 90 min at 37°C. Triplicate batches of 10 islets were transferred to glass vials containing 1 ml KRB + 2.8–27.7 mM glucose, and incubated 60 min in a 37°C shaking water bath. The medium was separated from the islets by gentle centrifugation (500–750 rpm 5 min at 10°C) and stored at −20°C pending measurement of insulin with an RIA that used charcoal separation (11) and rat insulin standards (Eli Lilly and Company, Indianapolis, IN). The three replicates at each glucose concentration were combined to give a single data point.

**Glucokinase enzymatic activity.** Islets were preincubated 90 min at 2.8 mM glucose, homogenized, and centrifuged 10 min at 12,000 \(g\) to remove mitochondrial-bound hexokinase (12). DNA content was
Glucokinase activity. The $V_{\text{max}}$ and $K_m$ values for glucokinase and hexokinase are listed in Table I. The phosphorylation data after 6- and 24-h incubations at high glucose are shown in Fig. 3. Hexokinase $V_{\text{max}}$ and $K_m$ were unaffected by the high glucose. In contrast, glucokinase $V_{\text{max}}$ was increased at all time points: 149±4% of control at 6 h ($P < 0.018$), 161±10% of control at 14 h ($P < 0.003$), and 168±16% of control at 24 h ($P < 0.027$). These values are not different from each other statistically. The $K_m$ for glucokinase was unchanged at any time point.

Glucokinase Western blot. To investigate the etiology of the increased glucokinase $V_{\text{max}}$, a time course of glucokinase protein level was constructed by Western blotting of islets cultured for 6, 14, 24, and 48 h at 16.7 mM glucose. After 6 h at high glucose, the level of glucokinase was unchanged from the control islets. In contrast, at 14, 24, and 48 h, glucokinase protein levels were increased ~40%. Finding divergent protein levels between 6 h and the later time points was surprising because the $V_{\text{max}}$ measurements were equal up through 24 h. We retested the observation with additional groups of islets (n = 5) studied after 6 and 24 h at 16.7 mM glucose, and we obtained the same result (Fig. 4). Combined densitometry readings from the five experiments are shown in Fig. 5. Glucokinase protein levels were 101±8% of control after 6 h of high glucose vs 144±3% of control with the 24-h incubation ($P < 0.0002$).

Reversal of the high glucose-induced increase in glucokinate activity. We next tested the reversibility of the increased activity of glucokinase after 6 and 24 h of high glucose by incubating for another 3 h at 5.5 mM glucose (Table I). Glucokinase $V_{\text{max}}$ decreased to basal control levels in the 6-h high glucose islets (112±8% of control, $P = NS$), as opposed to no change in the 24-h group (141±7% of control).
Table I. Kinetic Parameters for Glucokinase and Hexokinase in Cultured Islets

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Islet DNA</th>
<th>Hexokinase</th>
<th>Glucokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/islet</td>
<td>V_max</td>
<td>K_m</td>
</tr>
<tr>
<td></td>
<td>mol G-6-P/kg DNA/60 min</td>
<td>mM glucose</td>
<td>mol G-6-P/kg DNA/60 min</td>
</tr>
<tr>
<td>Protocol a: 24 h Total culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5 mM glucose (3)</td>
<td>19.3±1.9</td>
<td>1.32±0.13</td>
<td>0.09±0.13</td>
</tr>
<tr>
<td>6 h of 16.7 mM glucose (3)</td>
<td>18.8±3.2</td>
<td>1.49±0.31</td>
<td>0.08±0.03</td>
</tr>
<tr>
<td>24 h of 16.7 mM glucose (3)</td>
<td>20.1±3.7</td>
<td>1.33±0.09</td>
<td>0.10±0.04</td>
</tr>
<tr>
<td>Protocol b: 18 h Total culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5 mM glucose (4)</td>
<td>16.4±1.4</td>
<td>3.22±0.35</td>
<td>0.03±0.00</td>
</tr>
<tr>
<td>14 h at 16.7 mM glucose (4)</td>
<td>17.8±1.2</td>
<td>2.54±0.43</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>Protocol c: Reversal†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5 mM glucose (3)</td>
<td>13.6±1.8</td>
<td>4.47±0.31</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>6 h of 16.7 mM glucose (3)</td>
<td>13.8±1.6</td>
<td>4.70±0.57</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>24 h of 16.7 mM glucose (3)</td>
<td>15.3±2.9</td>
<td>3.90±0.28</td>
<td>0.02±0.00</td>
</tr>
</tbody>
</table>

Statistical significance was determined by comparing each group of islets cultured at 16.7 mM glucose against the time-matched group at 5.5 mM glucose. * P < 0.018, † P < 0.027, ‡ P < 0.003, †† P < 0.0046. V_max and K_m values for hexokinase and glucokinase were calculated as described in the text. † The reversal protocol entailed the culture conditions shown followed by an additional 3 h at 5.5 mM glucose.

16.7 mM glucose in the presence or absence of 10 μM cycloheximide. The previous findings concerning the glucokinase V_max and protein levels after 6 and 24 h of high glucose were replicated in the islets not exposed to cycloheximide (Table II). Cycloheximide did not prevent the increase in glucokinase V_max that occurred with the 6-h incubation at high glucose (Fig. 6); the V_max was similar with (144±14% of control) and without (136±4% of control) cycloheximide. In contrast, with 24 h of high glucose, cycloheximide halved the increase in glucokinase V_max (126±8% of control with cycloheximide and 158±7% of control without cycloheximide, P < 0.027). However, the V_max was still significantly increased (P < 0.035 vs 5.5 mM glucose islets), even though the rise in the glucokinase protein level had been totally blocked (102±10% of control) (Fig. 7). These data support the notion of dual regulatory effects of glucose to control the synthesis and catalytic activity of glucokinase. Protein synthesis is not required for the second effect.

Discussion

By mapping out a 24-h profile of glucokinase activity and protein levels plus the ED50 for glucose-induced insulin secretion in islets cultured at a high concentration of glucose, new insight was obtained into how β cells adapt to hyperglycemia. Two modes of regulation for glucokinase were noted, with relatively acute changes in the catalytic activity followed by a more chronic increase in the cellular content of the enzyme. Furthermore, the setpoint for insulin secretion was lowered in concert with the upregulated glucokinase activity, which fits with the well-known role of this enzyme in regulating the β cell sensitivity to glucose (2). Thus, our results revealed for the first time a multistep sequence of β cell adaptive responses that are initi-

**Figure 3.** Glucose phosphorylation to glucose-6-phosphate in rat islets during 90-min incubations at the glucose concentrations shown. Islets were cultured 6 h at 16.7 mM glucose preceded by 18 h at 5.5 mM glucose (□□□□□□ n = 3), 24 h at 16.7 mM glucose (■□□□□□ n = 3), or 24 h at 5.5 mM glucose (○○□□□□□ n = 3). (A) Incubations carried out at 0.03–0.5 mM glucose representing hexokinase activity. (B) Incubations carried out at 6–100 mM glucose representing glucokinase activity.

**Figure 4.** Western blot for glucokinase in cultured rat islets. Culture conditions: 6 h at 16.7 mM glucose preceded by 18 h at 5.5 mM glucose (A), 24 h at 5.5 mM glucose (B), 24 h at 16.7 mM (C). Protein aliquots (20 μg) were resolved by electrophoresis on a polyacrylamide gel and transferred onto nitrocellulose. Filters underwent sequential incubations with a polyclonal sheep antiserum raised against an E. coli-derived B1 isoform of rat glucokinase, then rabbit anti-sheep IgG. Bound antibody was detected with 125I-conjugated protein A.
ated by a high level of glucose. Almost immediately, insulin levels rise because of the direct stimulation of insulin secretion. If that degree of insulin release does not normalize the glucose concentration, a few hours later, the inductive effect on glucokinase catalytic activity causes a further increase in insulin output because of the increased β cell sensitivity to glucose. With persistent hyperglycemia, a third effect to augment the cellular content of glucokinase is added.

Glucose modulating the cellular content of glucokinase is a well-known regulatory mechanism for this enzyme (7, 8). In contrast, control of the catalytic activity of glucokinase separate from changes in the amount of enzyme is a new concept that is still controversial. Lynedjian et al. (18) proposed this possibility to explain the reduced glucokinase activity that occurs in islets from rats fasted for 72 h (19). We expanded the idea to insulin-resistant states by finding increased enzymatic activity of glucokinase without changes in the level of the protein measured by Western blotting in islets isolated from two hyperinsulinemic rat models (8, 9). The present study provided irrefutable evidence for the catalytic activity being a locus of regulation for this enzyme. The results with cycloheximide in the 6-

### Table II. Kinetic Parameters for Glucokinase and Hexokinase in Cycloheximide-treated Cultured Islets

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Hexokinase</th>
<th>Glucokinase</th>
<th>Protein on Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{max}$</td>
<td>$K_m$</td>
<td>$V_{max}$</td>
</tr>
<tr>
<td>Protocol d: cycloheximide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5 mM Glucose (3)</td>
<td>4.98±1.50</td>
<td>0.12±0.03</td>
<td>4.87±0.15</td>
</tr>
<tr>
<td>6 h of 16.7 mM (3)</td>
<td>4.91±1.27</td>
<td>0.12±0.03</td>
<td>6.66±0.18*</td>
</tr>
<tr>
<td>6 h of 16.7 mM</td>
<td>+ 10 μM Cycloheximide (3)</td>
<td>4.50±1.41</td>
<td>0.13±0.05</td>
</tr>
<tr>
<td>5.5 mM Glucose (4)</td>
<td>4.71±0.26</td>
<td>0.10±0.02</td>
<td>5.42±0.26</td>
</tr>
<tr>
<td>24 h of 16.7 mM (4)</td>
<td>4.81±0.46</td>
<td>0.11±0.05</td>
<td>8.54±0.40†</td>
</tr>
<tr>
<td>24 h of 16.7 mM</td>
<td>+ 10 μM Cycloheximide (4)</td>
<td>3.86±0.76</td>
<td>0.11±0.04</td>
</tr>
</tbody>
</table>

Statistical significance was determined by comparing each group of islets cultured at 16.7 mM glucose against the time-matched group at 5.5 mM glucose. * $P < 0.002$, † $P < 0.001$, ‡ $P < 0.035$, § $P < 0.02$.

h high glucose islets were the most telling. The concentration used (10 μM) is known to abolish islet protein synthesis (20). Still, the $V_{max}$ increased as with high glucose alone, which can only indicate an increase in the ratio of enzyme activity to protein level. The stimulus for the increased glucokinase activity in this study was a high level of glucose. Our previous studies made similar findings in normoglycemic rats: normoglycemic/hyperinsulinemic rats from a 48-h glucose infusion (8), and in spontaneously hypertensive rats (9), a model of insulin resistance (9), and this study with high glucose. Knowing the central role played by glucokinase in determining the β cell sensitivity for glucose and insulin output (2), these findings strongly suggest that the catalytic

![Figure 5](image-url)  
**Figure 5.** Combined data from five experiments for glucokinase protein level in cultured rat islets. Culture conditions: 24 h at 5.5 mM glucose, 6 h at 16.7 mM glucose preceded by 18 h at 5.5 mM glucose, and 24 h at 16.7 mM glucose. Glucokinase was quantified by Western blot. The glucokinase bands on each gel were measured by densitometry and expressed in relative terms against the islets cultured at 5.5 mM glucose (designated as 100%). * $P < 0.0002$ vs the 5.5 mM glucose islets.

![Figure 6](image-url)  
**Figure 6.** Effect of cycloheximide to prevent the high glucose–induced increase in glucokinase $V_{max}$. Islets were cultured for 6 h ($n = 3$) or 24 h ($n = 4$) at 16.7 mM glucose in the presence or absence of 10 μM cycloheximide. Glucokinase $V_{max}$ was measured as described in the text, and it was expressed as percentage of control islets (cultured at 5.5 mM glucose).
activity of glucokinase is a key locus of regulation in the $\beta$ cell for mediating adaptive changes in insulin output. Potential mechanisms include the inhibitory protein for glucokinase that has been identified in the liver (23, 24). However, our results virtually eliminated this possibility because that protein is a competitive inhibitor of glucokinase that alters the affinity (ED$_{50}$) for glucose, not the $V_{max}$ (24). An area of current speculation concerns a regulatory role for the $\beta$ cell glucose transporter GLUT-2 on glucokinase (25-27). Consistent with this, we observed that GLUT-2 levels were increased in tandem with an increased activity of glucokinase in islets isolated from spontaneously hypertensive rats (9). Nonetheless, the current results seem incompatible with the level of GLUT-2 being the regulatory factor because the increase in glucokinase activity with 6 h of high glucose was unaffected by the presence of the protein synthesis inhibitor cycloheximide. An alternate possibility not addressed in this study is a regulatory role for GLUT-2 based on the flux of glucose uptake through the transporter.

In summary, we have confirmed the presence of a dual regulatory system for glucokinase in the $\beta$ cell, with glucose-mediated control of the activity and cellular content of this enzyme. These results markedly strengthen the growing literature that suggests the catalytic activity of glucokinase is a key site of $\beta$ cell regulatory control, which, until recently, has gone unrecognized.

Acknowledgments

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References


