β-Cell adaptation in 60% pancreatectomy rats that preserves normoinsulinemia and normoglycemia

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β-Cell adaptation in 60% pancreatectomy rats that preserves normoinsulinemia and normoglycemia. Am J Physiol Endocrinol Metab 278: E68–E73, 2000.—Islet β-cells are the regulatory element of the glucose homeostasis system. When functioning normally, they precisely counterbalance changes in insulin sensitivity or β-cell mass to preserve normoglycemia. This understanding seems counter to the dogma that β-cells are regulated by glycemia. We studied 60% pancreatectomy rats (Px) 4 wk post surgery to elucidate the β-cell adaptive mechanisms. Nonfasting glycemia and insulinemia were identical in Px and sham-operated controls. There was partial regeneration of the excised β-cells in the Px rats, but it was limited in scope, with the pancreas β-cell mass reaching 55% of the shams (40% increase from the time of surgery). More consequential was a heightened glucose responsiveness of Px islets so that glucose utilization and insulin secretion per milligram of islet protein were both 80% augmented at normal levels of glycemia. Investigation of the biochemical basis showed a doubled glucokinase maximal velocity in Px islets, with no change in the glucokinase protein concentration after adjustment for the different β-cell mass in Px and sham islets. Hexokinase activity measured in islet extracts was also minimally increased, but the glucose 6-phosphate concentration and basal glucose usage of Px islets were not different from those in islets from sham-operated rats. The dominant β-cell adaptive response in the 60% Px rats was an increased catalytic activity of glucokinase. The remaining β-cells thus sense, and respond to, perceived hyperglycemia despite glycemia actually being normal. β-Cell mass and insulin secretion are both augmented so that whole pancreas insulin output, and consequently glycemia, are maintained at normal levels.

--glucokinase; glucose metabolism; glycolysis; glucose 6-phosphate; islets of Langerhans; insulin secretion

THE IsLET β-CELL through its secretion of insulin regulates the storage and metabolism of cellular fuels. Not surprisingly, the regulatory system is complex, with multiple factors affecting insulin secretion, proinsulin synthesis, and the mass of pancreatic β-cells. The best studied factor is glucose; all of these functional aspects are glucose responsive, and it is well accepted that glycemia is the major regulator of β-cell function. However, the primary effect of β-cell activity is to maintain a normal metabolic milieu. When β-cells function normally, insulin secretion precisely meets tissue insulin needs so that normoglycemia is maintained (16). Moreover, whole body insulin sensitivity varies throughout life (puberty, pregnancy, and aging are insulin-resistant states), yet most humans do not develop diabetes. As such, an unanswered question in regard to the glucose homeostasis system is how β-cell adaptation occurs in the absence of ongoing changes in glycemia.

Insight has come from studies of rodents with genetic-based insulin resistance or pregnancy; their β-cells are supersensitive to glucose so that insulin secretion is augmented at normoglycemia (4, 6–8, 11, 30). Kahn et al. (15) reported the same finding in healthy humans made insulin resistant by a 14-day nicotinic acid infusion. Thus a disassociation of the normal coupling between glucose concentration and insulin secretion characterizes the β-cell adaptation to insulin resistance. The mechanism of this effect is unknown. We studied nondiabetic insulin-resistant spontaneously hypertensive rats (SHR) and found that catalytic activity of the β-cell glucose sensor enzyme, glucokinase, was enhanced (6). However, alternate mechanisms have been reported in other models (3, 34, 40). In particular, a recent suggestion is that elevated β-cell fatty acid (FA) metabolism mediates the heightened glucose sensing for insulin secretion, because hypertriglyceridemia commonly is found with insulin resistance (32), and culturing islets with FA shifts to the left the glucose concentration–insulin secretion curve (12, 28, 42). It remains unclear how to reconcile these different findings.

Little is known about how glucose homeostasis is preserved when the β-cell mass is lowered. Biobreeding rats before onset of autoimmune diabetes have a left-shifted glucose concentration–insulin secretion curve (36). We made the same observation after a 60% pancreatectomy in rats (20). Thus a variable β-cell sensitivity to glucose also operates when the β-cell mass is lowered. Whether the biochemical details are the same as for insulin-resistant states is not known.

The current study investigated rats after a 60% pancreatectomy (Px); these rats maintain normal

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METHODS

60% Px model and islet isolation. One hundred-gram Sprague-Dawley rats underwent 60% Px by use of our previously described method (19). Briefly, the portion of the pancreas bordered by the spleen and stomach extending to the small flap of pancreas attached to the pylorus was removed by use of gentle abrasion with cotton applicators. The removed portion was 57 ± 3% of the pancreas weight (19). Control (sham) rats underwent laparotomy and mobilization of the pancreas with gentle rubbing between the fingers. Postoperatively, all rats received standard chow and tap water ad libitum until being studied 4 wk after the surgery. Islets were isolated using an adaptation of the Gotoh method (10): pancreas duct infiltration with collagenase (Serva, Heidelberg, Germany), Histopaque gradient separation (Sigma, St. Louis, MO), and hand picking. Islet DNA content was measured by the Labarca method (17), protein by a commercial kit (Bio-Rad, Hercules, CA) with bovine albumin as standard, and insulin content after acid ethanol extraction with an insulin RIA (1). Freshly isolated islets were used in all experiments.

Oral glucose tolerance test and meal challenge. Both tests were preceded by an overnight fast. Px and sham rats were administered 1 g/kg of glucose (0.5 g/ml) by gavage tube. Blood for plasma glucose determination was obtained by tail snipping at 0, 30, 60, and 120 min. The meal challenge was performed 3 days later. Px and sham rats were given free access to chow at 9:00 AM (time0), and plasma glucose values were measured at 0, 30, 60, and 120 min.

Islet insulin secretion and glucose utilization. Islets under 30 min of preincubation in warmed and oxygenated Krebs-Ringer buffer (KRB) with 2.8 mM glucose and 0.5% BSA. Insulin secretion was assessed using triplicate batches of 10 islets in 0.2 ml glass vials containing 1 ml of KRB with 0.5% BSA and 2.8, 5.5, 8.3, or 16.7 mM glucose for 60 min in a 37°C shaking water bath. Medium was separated by gentle centrifugation and stored at −20°C pending insulin measurement by RIA (1). Islet glucose usage was measured as previously described (6) under the same experimental conditions with a method based on quantifying conversion of d-[5-3H]glucose (NEN, Boston, MA) to [3H]H2O (2).

Islet glucokinase/ hexokinase kinetics. Glucose phosphorylation was measured in islet extracts as previously described (6) with a method based on quantifying conversion of NAD+ to NADH by exogenous glucose-6-phosphate dehydrogenase (22). Islet homogenates were centrifuged at 12,000 g for 10 min, and the supernatants were incubated at 10 glucose concentrations (0.03–100 mM) to measure glucose phosphorylation. Maximal velocity (Vmax) and Michaelis-Menten constant (Km) values for glucokinase and hexokinase were calculated by linear regression from an Eadie-Scatchard plot (volume/substrate concn) and 10 cycles of the method of Spears et al. (35) to identify each enzyme’s activity.

Glucokinase immunoblots. Glucokinase immunoblots were performed as described (6) using sheep antiserum raised against an Escherichia coli-derived B1 isoform of rat glucoki-

RESULTS

General characteristics. Body weight and nonfasting glycemia, insulinemia, triglycerideremia, and plasma free fatty acids were equal in the 60% Px and sham-operated rats (Table 1). An oral glucose tolerance test and a meal challenge were performed to confirm the normoglycemia of the 60% Px rats; glycemic responses were identical in the two groups (Fig. 1).

Table 1. General characteristics of rats 4 wk after sham operation or 60% Px

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<thead>
<tr>
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<th>Sham</th>
<th>60% Px</th>
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<tr>
<td>Weight, g</td>
<td>320 ± 7 (12)</td>
<td>311 ± 8 (12)</td>
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<tr>
<td>Serum glucose, mmol/l</td>
<td>8.0 ± 0.2 (6)</td>
<td>7.8 ± 0.3 (5)</td>
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<tr>
<td>Plasma insulin, pmol/l</td>
<td>287 ± 16 (6)</td>
<td>308 ± 21 (5)</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/l</td>
<td>1.44 ± 0.11 (6)</td>
<td>1.65 ± 0.16 (5)</td>
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<tr>
<td>Plasma free fatty acids, meq/l</td>
<td>0.39 ± 0.06 (6)</td>
<td>0.38 ± 0.05 (5)</td>
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Values are means ± SE of normally fed rats at 9:00 AM; nos. of rats are shown in parentheses. Px, pancreatectomy. No measure was statistically significantly different between Px and sham rats.
One possibility for these results was regeneration of the excised β-cells. We had previously addressed that issue 7 wk after 60% Px: β-cell mass was 55% of the sham rats, which is a 40% increase from the postsurgery period, and islet non-β-cell mass was unchanged at 45% of control, which suggests that the islet regeneration was β-cell specific (19). We now report identical findings in isolated islets from 60% Px rats 4 wk after the surgery. Islet DNA, protein, and insulin content were 40% increased compared with the sham-operated animals (Table 2). Consequently, the 60% Px induced some β-cell regeneration, but it was incomplete.

**Islet glucose sensing/response.** We assessed insulin secretion and glucose utilization in isolated islets from the 60% Px and sham-operated rats (Fig. 2); results are expressed per milligram of protein to compensate for the different islet cell mass. Both parameters showed an upregulated response in the Px islets, with no change in the dose giving a half-maximal response (ED₅₀), and a 70–80% increase at 8.4 mM glucose, which approximates the usual nonfasting plasma glucose level of the Px and sham rats (Table 1).

The pattern of the increase suggested augmented glucokinase activity in the Px islets, which was confirmed by measuring glucose phosphorylation in isolated islet extracts over a range of 0.03–100 mM glucose (Fig. 3; derived enzyme kinetics shown in Table 3). Glucose phosphorylation over the range of glucose concentrations that approximated normoglycemia was 60% increased in the Px islets, in close agreement with the results that modulate a protein's function. Striking differences were seen compared with the denaturing gel; the Px band intensity was now less than that of the sham, confirming the presence of a glucokinase post-translational change in Px islets. Thus the doubled glucokinase Vₘₐₓ per kilogram DNA in the Px islets stemmed from an enhanced catalytic activity of this enzyme, as opposed to a changed β-cell expression.

**DISCUSSION**

This study has identified the β-cell adaptive responses in 60% Px rats that kept insulinemia, and thus glyceremia and triglyceridemia, indistinguishable from sham-operated rats. One element was a limited regeneration of the excised β-cells, which brought the pancreas β-cell mass from 40% of normal at the time of surgery to slightly more than one-half of normal several weeks later (19). The more consequential effect was a changed relationship between the glucose concentration and islet glucose utilization so that normal...
levels of glycemia elicited a raised flux of islet glucose metabolism, and consequently greater than normal insulin secretion. The latter reflects β-cell glycolytic flux being a key regulator of glucose-induced insulin secretion through well characterized effects on β-cell ion channels and membrane potential (27, 31). The heightened glycolytic flux stemmed from an enhanced activity of glucokinase, which is the main regulator of β-cell glucose usage and is termed the β-cell glucose sensor (26). The mechanism was an increased catalytic activity of this enzyme, as opposed to a change of its β-cell concentration, which was confirmed by the novel observation of divergent denaturing and nondenaturing glucokinase immunoblots in the Px islets. Crucial to the workings of this regulatory system was the observation that the degree of change in glucokinase activity was small; glucose phosphorylation and, consequently, glucose metabolism were 60–80% increased in Px islets at the physiologically relevant 8.4 mM glucose, compared with the multifold changes in gene expression that make up many cellular regulatory systems, such as the fivefold increase in proinsulin gene expression reported after short-term exposure of β-cells to a high glucose concentration (21). The reason is that glucokinase is the rate-limiting β-cell glycolytic enzyme and is without allosteric or end-product influences (26, 27). Its activity thus directly modulates β-cell glucose utilization and insulin secretion (38). This effect was evident in the current study by the close agreement between the degree of changes in glucokinase activity, glucose utilization, and insulin secretion in the Px islets. As such, insulin release at 8.4 mM glucose also increased 80%, which is very significant physiologically. By this reasoning, it is of interest that the rise in β-cell mass to 55% of normal (19), together with the 92% increased glucokinase activity per cell mass shown in Table 3, results in whole pancreas glucokinase activity in the Px rats being identical to that in the sham rats. Our results are consistent with the recent study of Martín et al. (25), which concluded that an upregulation of islet glucose metabolism was the basis for the β-cell glucose hypersensitivity in normoglycemic 60% Px mice, although the study provided no insight as to the mechanism (25). Note, glucose utilization was assessed in this study only as conversion of [5-3H]glucose to [3H]H2O, and a measure of affected pathways and metabolites is needed to fully understand how the change in glucokinase activity affects islet-cell glucose metabolism, analogous to the studies of others (38, 39).

Our finding of increased glucokinase catalytic activity in the Px islets agrees with our studies of normoglycemic insulin-resistant SHR rats (6) and rats that were hyperinsulinemic and normoglycemic secondary to glucose infusions (5). Making the same observation in three diverse rat models of successful β-cell adaptation suggests that variable glucokinase activity is a core β-cell adaptive response of the glucose homeostasis system that is initiated by changing metabolic demands for insulin secretion of multiple types. The basis for this effect is not known, although evidence for a
Glucokinase and hexokinase maximal velocity \( V_{\text{max}} \) (mol glucose 6-phosphate (G-6-P) kg DNA \(^{-1}\) 60 min \(^{-1}\)) and dose giving half-maximal response (ED\(_{50}\), mM glucose) were measured in islet extracts \((n=5 \text{ experiments})\) with the method described in the text. Values are means ± SE. Statistical significance was determined by unpaired Student's t-test. NS, nonsignificant.

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<thead>
<tr>
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<th>Hexokinase</th>
<th>Glucokinase</th>
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<tr>
<td></td>
<td>( V_{\text{max}} )</td>
<td>ED(_{50})</td>
</tr>
<tr>
<td>Sham</td>
<td>3.5 ± 0.2</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>60% Px</td>
<td>5.2 ± 0.5</td>
<td>0.08 ± 0.01</td>
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<tr>
<td>P</td>
<td>0.003</td>
<td>NS</td>
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Glucokinase and hexokinase maximal velocity \( V_{\text{max}} \) and ED\(_{50}\) (mM glucose) for 60 min, followed by measurement of G-6-P content, as described in the text.
adaptive responses to occur when glycermia is unaltered.

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REFERENCES


