Glucose Regulates in Vivo Glucose-6-phosphatase Gene Expression in the Liver of Diabetic Rats*

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Overproduction of glucose by the liver is the major cause of fasting hyperglycemia in both insulin-dependent and non-insulin-dependent diabetes mellitus. The distal enzymatic step in the process of glucose output is catalyzed by the glucose-6-phosphatase complex. We show here that 90% partially pancreatectomized diabetic rats have a 5-fold increase in the messenger RNA and a 3-4-fold increase in the protein level of the catalytic subunit of glucose-6-phosphatase in the liver. Normalization of the plasma glucose concentration in diabetic rats with either insulin or the glycosuric agent phlorizin normalized the hepatic glucose-6-phosphatase messenger RNA and protein within ~8 h. Conversely, phlorizin failed to decrease hepatic glucose-6-phosphatase gene expression in diabetic rats when the fall in the plasma glucose concentration was prevented by glucose infusion. These data indicate that in vivo gene expression of glucose-6-phosphatase in the diabetic liver is regulated by glucose independently from insulin, and thus prolonged hyperglycemia may result in overproduction of glucose via increased expression of this protein.

Excessive production of glucose is the major cause of fasting hyperglycemia in human (1, 2) and experimental diabetes mellitus (3, 4). The hydrolysis of hepatic glucose-6-phosphate is the "final common pathway" for the release of glucose into the circulation. Recent experimental evidence supports the notion that this final step is rate-determining for the increased rate of hepatic glucose output in diabetic states. In fact, marked changes in the rate of formation of hepatic glucose-6-phosphate through gluconeogenesis fail to alter hepatic glucose production (5–9), and in experimental diabetes hepatic glucose production is markedly elevated in the presence of a significant decrease in the hepatic glucose-6-phosphate pool (4, 10).

The catalytic portion of the glucose-6-phosphatase (Glc-6-Pase) complex has recently been cloned (11–13). Studies in Fao rat hepatoma cells (13) indicate that insulin down-regulates Glc-6-Pase mRNA levels, while glucocorticoid induces it. Haber et al. (14) have recently reported a marked increase in hepatic Glc-6-Pase mRNA and protein in diabetic BB rats. In the same study, in vivo treatment with 0.5 unit of insulin normalized the plasma glucose concentration and the hepatic Glc-6-Pase mRNA levels in diabetic rats within 4 h. Similarly, Liu et al. (15) have shown increased hepatic Glc-6-Pase mRNA and activity in streptozotocin-induced diabetic rats. Since experimental diabetes and insulin treatment are associated with simultaneous and opposite changes in the plasma glucose and plasma insulin concentrations, we have attempted to discern the relative role of glucose and insulin in the short-term regulation of the hepatic Glc-6-Pase gene expression in (90% partially pancreatectomized) diabetic rats.

Our results confirm that prolonged insulin deficiency and hyperglycemia (experimental diabetes) cause a marked increase in the hepatic Glc-6-Pase mRNA and protein and indicate that short-term (~8 h) correction of hyperglycemia in diabetic rats leads to normalization of the hepatic gene expression of this enzyme, regardless of the circulating insulin concentrations.

MATERIALS AND METHODS

Animals—Four groups of male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were studied: group I, controls (n = 9); group II, 90% partially pancreatectomized rats (n = 10); group III, 90% partially pancreatectomized rats acutely treated with low dose insulin (n = 10); group IV, 90% partially pancreatectomized rats acutely treated with phlorizin (n = 16). At 3–4 weeks of age, all rats (80–100 g) were anesthetized with pentobarbital (50 mg/kg of body weight, intraperitoneally), and in groups II–IV, 90% of their pancreas was removed according to the technique of Foglia (16), as modified by Bonner-Weir et al. (17). Immediately after surgery rats were housed in individual cages and subjected to a standard light (6 a.m. to 6 p.m.)–dark (6 p.m. to 6 a.m.) cycle. All rats received ad libitum water and standard rat laboratory chow (Ralston-Purina, St. Louis, MO). After surgery rats were weighed twice weekly, and tail vein blood was collected for the determination of nonfasting plasma glucose and insulin concentrations at the same time (8 a.m.). Five weeks following pancreatectomy rats were anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg of body weight), and indwelling catheters were inserted into the right internal jugular vein and in the left carotid artery, as described previously (18, 19). Food intake was carefully monitored following catheter placement, and only rats gaining weight and eating >12 g of chow during the night prior to the study were included in the study.

Insulin/Phlorizin Administration—Studies were performed in awake, unstressed, chronically catheterized rats. At ~7 a.m. food was removed, and rats received either a vehicle subcutaneous injection (groups I, II, and III) and a variable intra-arterial insulin infusion (group III) or a subcutaneous injection of phlorizin (group IV, 0.3 mg/kg of body weight) and an intra-arterial infusion of saline (groups I, II, and IV). The insulin infusion (group III) was designed to gradually lower the plasma glucose concentration in a similar fashion to that observed with a single injection of phlorizin (group IV). The average rate of insulin infusion was 1.8 ± 0.3 milliunits/kg/min during the first 3 h and 0.9 ± 0.3 milliunits/kg/min thereafter. To discern the effect of the normalization of the plasma glucose concentration from the potential direct effect of phlorizin on Glc-6-Pase gene expression, a subgroup (n = 6) of phlorizin-treated diabetic rats also received glucose infusions designed to maintain hyperglycemia throughout the in vivo study. Plasma sam-

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1 The abbreviations used are: Glc-6-Pase, glucose-6-phosphatase; FFA, free fatty acid(s); MOPS, 4-morpholinepropanesulfonic acid.
In Vivo Glucose-6-phosphatase Gene Expression

TABLE I
General basal characteristics of control and diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight</th>
<th>Glucose</th>
<th>Insulin</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>9</td>
<td>326 ± 13</td>
<td>8.2 ± 0.2</td>
<td>43 ± 5</td>
<td>149 ± 10</td>
</tr>
<tr>
<td>2. Pancreatectomized</td>
<td>9</td>
<td>301 ± 12</td>
<td>22.3 ± 1.7*</td>
<td>27 ± 5*</td>
<td>175 ± 13</td>
</tr>
<tr>
<td>3. Pancreatectomized/insulin</td>
<td>10</td>
<td>352 ± 9</td>
<td>23.1 ± 1.4*</td>
<td>25 ± 6*</td>
<td>163 ± 12</td>
</tr>
<tr>
<td>4. Pancreatectomized/phlorizin</td>
<td>10</td>
<td>328 ± 10</td>
<td>22.8 ± 1.1*</td>
<td>21 ± 5*</td>
<td>171 ± 13</td>
</tr>
</tbody>
</table>

* p < 0.01 versus control.

RESULTS AND DISCUSSION

Studies of the physiologic regulation of hepatic Glc-6-Pase have been consistently demonstrated in experimental diabetes mellitus (4, 10, 21–23). This increase correlates with the plasma glucose concentrations in these animals (10, 15) and can be decreased or normalized by insulin treatment (10, 22, 23). The recent cloning of the human, rat, and murine Glc-6-Pase cDNAs (11–14) allows one to examine the physiologic regulation of its gene expression. Although insulin per se decreases Glc-6-Pase mRNA in Fao rat hepatoma cells (13) and in BB diabetic rats (14), the physiologic in vivo regulation of Glc-6-Pase gene expression remains to be delineated. In fact, though in two recent reports insulinopenic diabetes was associated with a marked increase in Glc-6-Pase mRNA in the liver (14, 15), the potential regulatory role of prolonged hyperglycemia per se on Glc-6-Pase gene expression has not been studied. Thus, we quantitated the hepatic mRNA and protein levels of Glc-6-Pase in diabetic rats following normalization of the plasma glucose concentration, with or without insulin administration.

Basal Metabolic Parameters—There were no significant differences in the mean body weights between the control and the diabetic groups (Table I). The diabetic animals were carefully matched for their metabolic parameters prior to assignment to the three experimental protocols. In fact, the non-fasting plasma glucose concentrations during the 2-week period prior to the in vivo studies were significantly and similarly elevated in the diabetic groups, compared with the control group (p < 0.01). The non-fasting plasma insulin concentrations were significantly decreased in all diabetic rats (p < 0.01), while the plasma glucagon concentrations were unchanged.
steady state plasma glucose, FFA, insulin, and glucagon concentrations during the in vivo studies.

Plasma samples for the determination of plasma FFA, insulin, and glucagon concentrations were sampled at 30-min intervals during the last 2 h of the in vivo studies. Plasma samples for the determination of plasma glucose concentrations were sampled at 10-min intervals during the last 2 h of the in vivo studies. Values displayed in this table represent the average levels during this time period. The plasma glucose concentration in group 4 refers to that measured in the 10 rats not infused with glucose (see "Materials and Methods" and Fig. 3B).

**TABLE II**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose</th>
<th>FFA</th>
<th>Insulin</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>8.2 ± 0.2</td>
<td>0.79 ± 0.09</td>
<td>41 ± 5</td>
<td>158 ± 11</td>
</tr>
<tr>
<td>2. Pancreatectomized</td>
<td>21.0 ± 1.3</td>
<td>1.18 ± 0.12</td>
<td>25 ± 6</td>
<td>175 ± 18</td>
</tr>
<tr>
<td>3. Pancreatectomized/insulin</td>
<td>9.1 ± 0.5</td>
<td>0.75 ± 0.06</td>
<td>58 ± 9</td>
<td>166 ± 16</td>
</tr>
<tr>
<td>4. Pancreatectomized/phlorizin</td>
<td>9.1 ± 0.7</td>
<td>0.26 ± 0.19</td>
<td>21 ± 7</td>
<td>219 ± 19</td>
</tr>
</tbody>
</table>

* a p < 0.01 versus control.

**Fig. 2.** Effect of diabetes and of the normalization of plasma glucose concentrations with either insulin or phlorizin on hepatic Glc-6-Pase mRNA. A, Northern analysis of Glc-6-Pase mRNA in liver freeze-clamped in situ at the completion of the in vivo studies. Northern blots were probed with 32P-labeled Glc-6-Pase cDNA. 20 μg of each liver RNA sample were used. B, Ethidium-stained ribosomal RNA bands. The figure depicts Glc-6-Pase mRNA in groups (n = 3) of samples obtained from control rats (CON), untreated diabetic rats (PANX), insulin-treated diabetic rats (INS), and phlorizin-treated diabetic rats (PHLO). Rats were sacrificed and liver freeze-clamped in situ following 2 h at the desired plasma glucose levels (as displayed in Table II). All tissue determinations were performed on samples obtained at the completion of these in vivo studies. Analysis was performed several times for all rats included in Tables I and II with similar results.

**Fig. 3.** Effect of diabetes and of the normalization of plasma glucose concentrations with either insulin or phlorizin on hepatic Glc-6-Pase protein. A, Immunoblot of liver microsomal fraction of control rats (CON), untreated diabetic rats (PANX), insulin-treated diabetic rats (INS), and phlorizin-treated diabetic rats (PHLO). B, Immunoblot of liver microsomal fraction of phlorizin-treated diabetic rats in which the plasma glucose concentration is maintained at diabetic levels of ~21 mM (Glc +) or decreased and maintained at normoglycemic levels (Glc ─). Rats were sacrificed and liver freeze-clamped in situ following 2 h at the desired plasma glucose levels (as displayed in Table II). All tissue determinations were performed on samples obtained at the completion of these in vivo studies. Analysis was performed several times for all rats included in Tables I and II with similar results.

The administration of insulin, but not of phlorizin, normalized the plasma insulin levels. Plasma glucagon was not affected by the insulin infusion; however, the acute normalization of the plasma glucose concentration with phlorizin moderately increased the plasma glucagon concentration (Table II). The circulating FFA levels were increased in the diabetic rats compared with control rats. Normalization of glycemia with insulin, but not with phlorizin treatment, restored the plasma FFA concentrations to the normal values (Table II). Thus, this in vivo experimental manipulation allows us to examine diabetic rats in the presence of high glucose and low insulin (group II), low glucose and high insulin (group III), and low glucose and low insulin (group IV). To investigate the potential direct effect of phlorizin administration on Glc-6-Pase mRNA and protein, a subgroup of diabetic rats received phlorizin injection and variable glucose infusions designed to maintain the plasma glucose concentration at the diabetic level. In this group an average rate of 30.9 ± 2.5 mg/kg/min was required to maintain the plasma glucose concentration at 20.4 ± 2.2 mM. The latter value was similar to the plasma glucose concentrations at baseline (Table I) and during the in vivo studies (Table II) in the untreated diabetic rats (group I).

**Effect of Short-term Correction of Hyperglycemia on Hepatic Glc-6-Pase mRNA Levels**—The relative abundance of Glc-6-Pase mRNA was determined by Northern blot analysis of total RNA obtained from liver samples at the end of the in vivo studies. We used a 1.25-kilobase pair cDNA probe that recognized the region encoding the catalytic portion of the Glc-6-Pase. Glc-6-Pase mRNA concentrations in liver of non-diabetic rats were used as control (set at 100%). Multiple densitometric scanning of different Northern blots (example is shown in Fig. 2) shows that the diabetic rats manifested a ~5-fold increase in Glc-6-Pase mRNA concentrations as compared with non-diabetic rats. As expected, low dose insulin administration almost completely reversed this marked increase in the Glc-6-Pase messenger RNA. However, since the infusion of insulin concomitantly decreases the plasma glucose and FFA concentrations and increases the plasma insulin concentration, we wished to examine the effect of the normalization of blood glucose concentration per se on hepatic Glc-6-Pase gene expression. Thus, we induced a similar gradual decline in the plasma glucose concentration with the glycosuric agent, phlorizin, which normalized the plasma glucose but not the plasma insulin and FFA levels in diabetic rats. Phlorizin treatment causes the same marked reduction in Glc-6-Pase mRNA as seen with insulin. When glucose was infused to prevent the decline in the plasma glucose concentration, phlorizin administration did not alter the Glc-6-Pase mRNA in diabetic liver (not shown). These results suggest that correction of hyperglycemia per se is able...
to suppress the marked diabetes-induced increase in Glc-6-Pase mRNA.

Effect of Short-term Correction of Hyperglycemia on Hepatic Glc-6-Pase Protein Levels—We next examined whether this marked changes in mRNA levels were reflected in a comparable decrease in the protein concentration (Fig. 3, A and B). The amount of Glc-6-Pase enzyme in liver microsomes was assessed using polyclonal antibodies against the catalytic portion of Glc-6-Pase. Diabetic rats show larger than a 3-fold increase in Glc-6-Pase protein levels, as compared with the control rats. Normalization of the plasma glucose concentration with either insulin or phlorizin similarly decreases the hepatic Glc-6-Pase protein levels. However, maintenance of hyperglycemia following phlorizin administration completely prevented the marked decrease in the hepatic Glc-6-Pase protein level.

Thus, although the changes in hepatic Glc-6-Pase mRNA observed with diabetes and following normalization of the plasma glucose levels tend to be more dramatic than those observed in the protein levels, there is an excellent correspondence of mRNA and protein levels in all experimental conditions. The finding of such a marked effect of extracellular glucose on the mRNA and protein levels of this key hepatic enzyme should not be interpreted as contradictory to the previously demonstrated regulation by insulin (13, 14). In fact, it is likely that both hyperinsulinemia and the restoration of normoglycemia may independently contribute to the physiologic regulation of Glc-6-Pase gene expression in the diabetic liver. Regarding the mechanism by which changes in the plasma glucose concentration may regulate gene expression, it is noteworthy that several key enzymes in carbohydrate and lipid metabolic pathways are transcriptionally regulated by extracellular glucose (24–27). However, it is likely that such regulation requires changes in the pool of phosphorylated glucose and may better correlate with the concentrations of glucose 6-phosphate or of other intracellular metabolites rather than with the extracellular glucose levels per se (25–27). Finally, it should be emphasized that the Glc-6-Pase complex is regulated at multiple levels (10, 28–31), which include, but are not limited to, the gene expression of its catalytic subunit.

In conclusion, we provide evidence for a stimulatory effect of hyperglycemia per se on hepatic Glc-6-Pase gene expression in diabetic rats. This may represent a molecular mechanism by which prolonged hyperglycemia favors the persistence of excessive hepatic glucose output in diabetes mellitus.

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REFERENCES