Role of Glucokinase and Glucose-6-phosphatase in the Acute and Chronic Regulation of Hepatic Glucose Fluxes by Insulin

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Increased hepatic glucose production (HGP) is the major cause of fasting hyperglycemia in all forms of diabetes. Glucokinase (GK) and glucose-6-phosphatase (Glc-6-Pase) are the proximal and the distal enzymatic steps, respectively, in the regulation of HGP. We examined the impact of changes in GK and Glc-6-Pase activities on in vivo hepatic glucose fluxes in diabetic (D) and control (C) rats. In particular, the acute regulation by insulin was investigated using the euglycemic hyperinsulinemic clamp technique in conscious rats.

In experimental diabetes (6 weeks): (a) GK mRNA was decreased by \(-40\%\) (b) the \(V_{max}\) of GK was markedly decreased (\(-46\text{versus } 9 \mu\text{mol/g wet weight/min}\)) and that of Glc-6-Pase was 2-fold increased (\(-30\text{versus } 15 \mu\text{mol/g wet weight/min, D versus C}\)), while (c) the \(K_m\) of GK (\(-10 \text{mM}\)) and Glc-6-Pase (\(-1.5 \text{mM}\)) were unchanged. HGP was increased by 65% in diabetes and correlated highly with the ratio of Glc-6-Pase/GK (\(r = 0.81, p < 0.01\)).

Following acute hyperinsulinemia (2 h): (a) GK mRNA increased by \(-2\text{-fold in both C and D; (b) GK V}_{max}\) did not change in C, but doubled to near-normal in D; (c) Glc-6-Pase \(V_{max}\) decreased by 23% in C and by 34% in D; (d) the \(K_m\) of GK decreased by \(-40\% (p < 0.01)\) in C. Acute hyperinsulinemia almost completely inhibited HGP in both C and D, and no correlation was demonstrated between HGP and the ratio of Glc-6-Pase/GK in these groups. Our data suggest that GK and Glc-6-Pase are important determinants of fasting HGP in diabetes. However, acute changes in Glc-6-Pase and GK activities can account for only a small portion of the in vivo inhibition of hepatic glucose flux by insulin, suggesting additional mechanisms for the short-term regulation of HGP.

Fasting hyperglycemia, the hallmark of diabetes mellitus, is largely the result of glucose overproduction by the liver (1). Hepatic glucose production (HGP) \(*\) is the balance between the fluxes through glucokinase (GK) and glucose-6-phosphatase (Glc-6-Pase). GK is markedly decreased (2, 3), and Glc-6-Pase activity is markedly increased (4) in insulin-deficient diabetic rats. These observations suggest that these enzymes are important regulators of HGP in diabetes.

Insulin may supress HGP, through "direct" or "indirect" effects (5–9). "Directly," insulin changes hepatic enzyme activities through alterations in phosphorylation state (e.g. glycogen synthase, pyruvate dehydrogenase), and/or gene expression (e.g. pyruvate kinase, phosphoenolpyruvate carboxykinase). Insulin may also act "indirectly" by changing substrate concentrations and their "mass" effect on hepatic glucose fluxes. Insulin treatment of diabetic rats increases GK mRNA (10–12), corrects GK activity (2, 3, 10), and tends to normalize Glc-6-Pase activity (4). Yet, the effect of acute and chronic changes in the circulating insulin concentrations on both in vitro enzyme activities and in vivo hepatic glucose fluxes have not been examined simultaneously. In previous studies, insulin was injected in diabetic rats, and both plasma insulin and glucose concentrations changed dramatically (2–4). Thus, the independent effects of insulin and glucose could not be delineated under steady state conditions. Furthermore, the hormonal and neuronal counter-regulation to hypoglycemia, triggered by the injection of insulin, may have affected GK and Glc-6-Pase activities (2–4). Most importantly, the potential impact of these changes on the in vivo hepatic glucose metabolism has not been examined.

In this study, we compared the in vivo hepatic glucose fluxes with the in vitro GK and Glc-6-Pase activities in control and mildly insulin-deficient diabetic rats to examine their acute and chronic regulation by insulin. For this purpose, we measured hepatic glucose fluxes by combining isotope dilution techniques with the euglycemic hyperinsulinemic clamp and with the assessment of the specific activities of key hepatic substrates. Insulin's direct effects on hepatic GK and Glc-6-Pase activities and its indirect effects through changes in substrate levels were correlated to the hormone's action on HGP, total glucose output (TGO), and glucose cycling (GC).

EXPERIMENTAL PROCEDURES

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§ The abbreviations used are: HGP, hepatic glucose production; GK, glucokinase; Glc-6-Pase, glucose-6-phosphatase; Glc-6-P, glucose 6-phosphate; TGO, total glucose output; GC, hepatic glucose cycling; MOPS, 4-morpholinoisopropanesulfonic acid.

Animals—Four groups of male Sprague-Dawley rats (Charles River Breeding Laboratories) were studied: 1) C, controls (n = 10); 2) C + I, controls infused with insulin (n = 11); 3) D, diabetic (90% pancreatectomized rats; n = 9); 4) D + I, diabetic, infused with insulin (n = 7). At 3–4 weeks of age, all rats (50–100 g) were anesthetized with pentobarbital (50 mg/kg body weight intraperitoneally), and, in the diabetic groups, 90% of their pancreas was removed according to the technique of Foglia (13), as modified by Bonner-Weir et al. (14). Immediately after surgery, rats were housed in individual cages and subjected to a standard light (6 a.m. to 8 p.m.)–dark (8 p.m. to 6 a.m.) cycle. After surgery, rats were weighed twice weekly, and tail vein blood was collected for the determination of fasting and nonfasting plasma glucose and insulin concentrations. Five weeks following pancreatectomy, rats were anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg body weight), and indwelling catheters were inserted into the right internal jugular vein and in the left carotid artery, as previously described (15, 16).

Insulin and Traer Infusion—Studies were performed in awake, unstressed, chronically catheterized rats using the euglycemic hyperinsu-
linemic clamp technique in combination with [3-3H]glucose infusion as previously described (15-17). All rats were fasted for 6 h before the in vivo studies. In groups C + I and D + I, a primed-continuous infusion of regular insulin (18 milliunits/kg/min) was administered, and a variable infusion of a 25% glucose solution was started at time zero and periodically adjusted to clamp the plasma glucose concentration at ~ 8 mM (euglycemic clamp; Fig 1). [3-3H]Glucose (DuPont NEN) was administered as a primed-continuous infusion of [3-3H]glucose (15 µCi of bolus, 0.4 µCi/min) throughout the study. Plasma samples for determination of [3H]glucose specific activity were obtained at 10-min intervals throughout the basal period and the insulin infusions. Plasma samples for determination of plasma insulin concentrations were obtained at times 0, 5, 10, 20, 30, 60, 90, and 120 min during the study. The total volume of blood withdrawn was ~ 3.0 ml/study, to prevent volume depletion and anemia, a solution (1:1, v/v) of ~ 4.0 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 units/ml) was infused. All determinations were also performed on portal vein blood obtained at the end of the experiment. At the end of the insulin infusion, rats were anesthetized (pentobarbital 60 mg/kg body weight, intravenously), the abdomen was quickly opened, portal vein blood was obtained, and liver was freeze-clamped in situ with aluminum tongs precoated in liquid nitrogen. The time from the injection of the anesthetic until freeze-clamping of the liver was less than 45 s. All tissues were immediately placed in liquid nitrogen for subsequent analyses.

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committees of the Albert Einstein College of Medicine.

Isolation of RNA—RNA was extracted from five rats of each of the four groups. Samples of freshly excised liver tissue weighing 150 mg were homogenized with RNA STAT-60 Tel-Test "B" (18). The RNA was extracted with chloroform, precipitated in isopropanol, and washed with 70% ethanol. It was dissolved in diethyl pyrocarbonate water and stored in 2 volumes of ethanol and 10% 2 x KAc at ~ 80 °C.

Preparation of GK cDNA Probe—To generate a GK cDNA probe based on previous rat, GK cloning (26), a 498-base pair fragment was amplified from a reverse transcriptase reaction on the isolated rat RNA. Polymerase chain reaction was performed with rat liver GK oligonucleotides of both sense and antisense). Vent (New England Biolabs) heat-stable polymerase was used. Small fragments were sampled from different parts of the liver to a total weight of approximately 100 mg/tube. Liver homogenates were prepared in 50 mM Hepes, 100 mM KCl, 7.5 mM MgCl2, and 2.5 mM dithioerythritol. Homogenates were centrifuged at 100,000 g for 30 min at 4 °C. The precipitated and resolved RNA was transferred to a nylon membrane. The membrane was probed with PhosphorAnalyzer (Molecular Dynamics), normalized for tubulin and separate bands that may be of a close mRNA size. The rRNA (18S and 28S) were homogenized with RNA STAT-60 Tel-Test "B" (18). The RNA was extracted with chloroform, precipitated in isopropanol, and washed with 70% ethanol. It was dissolved in diethyl pyrocarbonate water and stored in 2 volumes of ethanol and 10% 2 x KAc at ~ 80 °C.

Analytical Procedures—Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments) and plasma insulin by immunomunassay using rat and porcine insulin standards. Plasma [3H]glucose radioactivity was measured in duplicate on the supernatants of Ba(OH)2 and ZnSO4 precipitates of plasma samples after evaporation to dryness to eliminate tritiated water. Liver Glc-6-P concentrations were measured spectrophotometrically as described by Micheal (22). Hepatic uridine diphosphoglucose (UDP-glucose) concentration and specific activity were measured following two sequential chromatographic separations, as previously reported (15, 17, 23).

Terminology—In the present manuscript, the term Total Glucose Output (TGO) is intended as total in vivo flux through glucose-6-phosphate, and the term Hepatic Glucose Production (HGP) is intended as an estimate of contribution of plasma glucose to hepatic glucose production.

Calculations—The HGP was calculated as the difference between the tracer-derived rate of appearance and the infusion rate of glucose. The percent of the hepatic Glc-6-P pool directly derived from plasma glucose was calculated as the ratio of [3H]UDP-glucose and plasma [3H]glucose specific activities (15, 17). This ratio also measures the percent contribution of plasma glucose to in vivo Glc-6-P flux (i.e. glucose cycling). Since the flux through Glc-6-P (total glucose output) is the sum of the HGP plus GC (and GC = [3H]glucose specific activity/ plasma [3H]glucose × total glucose output), the equation can be solved to calculate both glucose cycling (GC) and total glucose output (TGO): TGO = HGP/1 - [3H]UDP-glucose specific activity/plasma [3H]glucose specific activity (i.e. glucose cycling). Assumptions and limitations of this methodology have been discussed in previous publications (15, 17). The intracellular concentration of hepatic Glc-6-P (pmol) was estimated from the tissue concentration (µmol/kg wet weight) assuming that 70% of liver weight is water and 30% of liver water is extracellular.

RESULTS

Basal Metabolic Parameters—The studies were performed in control and diabetic rats. There were no differences in the mean body weight (322 ± 7 and 305 ± 12 g) and liver weight (13.2 ± 0.8 and 13.6 ± 1.0 g) between control and diabetic rats, respectively. The nonfasting plasma glucose concentrations during the 2 weeks the mice in vivo studies were significantly elevated in the diabetic group (18.5 ± 1.3 and 7.7 ± 0.4 mm, D versus C). Following 24 h of fasting, the plasma insulin concentration was similar in the control and diabetic groups (156 ± 18 and 120 ± 36 pm, respectively).

Hyperinsulinemic Euglycemic Clamp Studies (Fig. 1)—In
vivo hyperinsulinemia was induced in the C + I (2360 ± 252 pm) and D + I groups (2324 ± 330 pm) in order to concomitantly examine the effect of insulin per se on in vivo hepatic glucose fluxes and in vitro enzymes (Fig. 1A). In D + I, a low dose insulin infusion was begun 90 min before the start of the study in order to slowly bring the plasma glucose concentration to near-normal levels prior to the start of the insulin clamp study. During hyperinsulinemia, the plasma glucose concentration was maintained at normal fasting glucose levels in C + I (7.4 ± 0.4 mm) and D + I (7.9 ± 0.6 mm) (Fig. 1B). Steady state conditions were achieved within ~50–60 min in each study, and in vivo hepatic glucose fluxes were calculated from plasma frequency sampled during the last 30 min of each study.

GK mRNA (Table I, Fig. 2)—GK mRNA was decreased by ~40% in this mildly hypoinsulimic diabetic rat model. Euglycemic hyperinsulinemia (2 h) caused an ~2-fold increase in GK mRNA in both C + I and D + I. These observations confirm that insulin exerts a potent long- and short-term regulatory role in GK gene expression. However, to investigate the biochemical consequences of this insulin-induced alteration in gene transcription, we also examined the kinetics of GK activity in the four groups.

**Table I**

<table>
<thead>
<tr>
<th>Kinetic parameters for glucokinase (GK) and glucose-6-phosphatase (Glc-6-Pase) and results of the Northern blot analysis of GK mRNA</th>
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<td></td>
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<tr>
<td>GK</td>
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<tr>
<td>K&lt;sub&gt;M&lt;/sub&gt; (nm)</td>
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<tr>
<td>mRNA</td>
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<tr>
<td>Glc-6-Pase</td>
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<tr>
<td>K&lt;sub&gt;M&lt;/sub&gt; (nm)</td>
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<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; (μm/ol/g of liver/min)</td>
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<tr>
<td>Glc-6-Pase/GK ratio</td>
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<sup>a</sup> p < 0.01 versus C.  
<sup>b</sup> p < 0.01 versus D.

**Fig. 1.** Plasma insulin (A) and plasma glucose (B) concentrations in the four groups before and during the euglycemic hyperinsulinemic clamp. C (n = 10) = control, C + I (n = 11) = control infused with insulin for 2 h, D (n = 9) = diabetic, D + I (n = 7) = diabetics infused with low dose insulin (1 milliunit/kg/min) for 90 min to bring glucose concentrations to near-normal and then infused with insulin for 2 h as C + I.

**Glucoinase and Glc-6-Pase Activities (Table II)—**In the attempt to approximate the in vivo fluxes through GK and Glc-6-Pase, we also calculated the GK and Glc-6-Pase activities at the in vivo hepatic GK and Glc-6-P concentrations. Portal vein and hepatic glucose concentrations were >2-fold increased, and hepatic Glc-6-P was 38% decreased in D. These differences in substrate concentrations partially buffered the marked alterations in GK and Glc-6-P V<sub>max</sub> values in D versus C. In fact, although still significantly decreased, due to the mass effect of Glc, the GK activity was only 22% lower in D versus C. Similarly, taking into account the hepatic Glc-6-P concentrations, the Glc-6-Pase activity increased by less than 60% in D. Accordingly, when Glc-6-P/GK ratios were calculated at the in vivo substrate concentrations, a 2-fold increase was observed in D.

Acute hyperinsulinemia led to a 25–28% increase in GK activity in both C and D (Table II). In C, the insulin-induced decrease in the hepatic Glc-6-P concentrations added to the decrease in Glc-6-Pase V<sub>max</sub> and resulted in a 47% suppression of the basal Glc-6-Pase activity. The ratios of Glc-6-Pase/GK activities at the in vivo substrate concentrations were 53% and...
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Figs. 2 and 3. Northern blot analysis for GK. Equal amounts of total individual RNAs were pooled for the four groups and processed as described under "Experimental Procedures." GK probe was used together with tubulin for quantity control. The experiment was repeated five times with reproducible results. A photograph was obtained from PhosphorAnalyzer. C = 6-h fasted controls; C + I = controls after 2 h of euglycemic hyperinsulinemia; D = 6-h fasted diabetics; D + I = diabetics after 2 h of euglycemic hyperinsulinemia.

55% decreased compared to basal levels in C + I and D + I, respectively.

Hepatic Glucose Fluxes (Table III)—In D, under basal conditions, HGP was increased by 65%, TGO by 97%, and GC by almost 3-fold. GC accounted for a small portion of TGO (23%) and of GK activity (13%) in C. However, in D, GC accounted for 35% of TGO and 48% of GK activity measured at the in vivo substrate concentration.

HGP, TGO, and GC were almost completely suppressed after 2 h of euglycemic hyperinsulinemia in both C and D.

In Figs. 4 and 5, three bars are displayed for each experimental group corresponding to the percent change from C in V_max activity at the in vivo substrate concentrations, and in vivo fluxes.

Glc-6-Pase and TGO (Fig. 4)—TGO is the in vivo flux through Glc-6-Pase and is a function of the enzyme activity and of the substrate (Glc-6-P) concentration. In D, Glc-6-Pase V_max, Glk-6-Pase activity, and TGO were similarly increased by approximately 2-fold.

Insulin decreased Glc-6-Pase V_max by 23% and the activity at the in vivo Glc-6-P levels by 47% in C + I, while the TGO decreased by 77%. Similarly in D + I, Glc-6-Pase V_max decreased by 32% and the activity by 44% in D + I, but the TGO was suppressed by 87%. Thus, following chronic hyperinsulinemia, which is expected to correlate with the in vivo HGP.

Fig. 6B. Taken together, this suggests a potential role of the coordinated alterations in these hepatic enzyme activities in the chronic regulation of hepatic glucose fluxes by insulin.

Acute insulin administration decreased the ratio of Glc-6-Pase/GK V_max values by 23% in C + I and by 68% in D + I (compared with C and D, respectively). The Glc-6-Pase/GK activity at the in vivo substrate concentration was approximately 50% decreased in both C + I and in D + I (compared with C and D, respectively). HGP was decreased by 86% in C + I, and 86% in D + I (compared with C and D, respectively). However, HGP and the ratios of Glc-6-Pase/GK were not correlated during the insulin infusion studies.

DISCUSSION

Insulin may regulate hepatic glucose flux through effects on hepatic enzyme activities and through alterations in the delivery of energy and substrates to the liver (5–9). Glc-6-Pase and GK are believed to be the determining steps for the net dephosphorylation of glucose 6-phosphate to glucose, i.e., HGP. Since increased hepatic glucose production is the major cause of fasting hyperglycemia in all forms of diabetes (1), it is important to delineate the role, if any, of the direct effects of insulin on hepatic GK and Glc-6-Pase. This study examined the in vivo hepatic glucose fluxes and the in vitro kinetics of GK and Glc-6-Pase in relation to their chronic and acute regulation by insulin.

Rat and human GK genes were cloned and sequenced (24, 25). The gene for liver GK is about 20 kilobases long, appears as a single copy, and contains 12 exons (26, 27). Surprisingly, analysis of glucokinase mRNA from liver (2.4 kilobases) and...
The intracellular concentration of hepatic Glc-6-P (mM/liter of intracellular water) was estimated from the tissue concentration (μM/kg wet weight) assuming that 70% of liver weight is water and 30% of liver weight is extracellular. All data are presented as mean ± S.E.

### Table II

<table>
<thead>
<tr>
<th>Glc-6-Pase/GK ratio</th>
<th>C+I</th>
<th>D</th>
<th>D+I</th>
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<tbody>
<tr>
<td>Glucose (μM)</td>
<td>7.9 ± 0.2</td>
<td>18.1 ± 0.2*</td>
<td>8.0 ± 0.2</td>
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<tr>
<td>Glucose (μM/g of liver/min)</td>
<td>4.6 ± 0.4</td>
<td>2.8 ± 0.3*</td>
<td>3.5 ± 0.4</td>
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<tr>
<td>Glucose-6-Pase (μM)</td>
<td>0.51 ± 0.08*</td>
<td>0.70 ± 0.05*</td>
<td>0.49 ± 0.08*</td>
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<tr>
<td>Glucose-6-Pase/GK ratio</td>
<td>1.7 ± 0.4</td>
<td>0.8 ± 0.1*</td>
<td>3.5 ± 1.3*</td>
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</table>

*p < 0.01 versus C.

### Table III

<table>
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<tr>
<th>Flux</th>
<th>C</th>
<th>C+I</th>
<th>D</th>
<th>D+I</th>
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<tbody>
<tr>
<td>HGP (μM/g of liver/min)</td>
<td>1.53 ± 0.15</td>
<td>0.30 ± 0.07*</td>
<td>2.53 ± 0.13*</td>
<td>0.35 ± 0.18*</td>
</tr>
<tr>
<td>TGO (μM/g of liver/min)</td>
<td>1.96 ± 0.08</td>
<td>0.45 ± 0.08*</td>
<td>3.88 ± 0.22*</td>
<td>0.92 ± 0.23*</td>
</tr>
<tr>
<td>GC (μM/g of liver/min)</td>
<td>0.46 ± 0.06</td>
<td>0.15 ± 0.03*</td>
<td>1.35 ± 0.15*</td>
<td>0.17 ± 0.09*</td>
</tr>
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</table>

*p < 0.01 versus C and D.

*p < 0.01 versus C.

### Fig. 5

Glc-6-Pase/GK ratio for V_max, Glc-6-Pase/GK ratio for activity at the hepatic substrate concentrations measured in vivo, and hepatic glucose production (HGP), presented in each of the four groups (C, n = 10; C+I, n = 11; D, n = 8; D+I, n = 7), as percent of control (C = 100%). The intracellular concentration of hepatic Glc-6-Pase (μM/liter of intracellular water) was estimated from the tissue concentration (μM/kg wet weight) assuming that 70% of liver weight is water and 30% of liver weight is extracellular.

### Fig. 6

Correlations between the ratios of Glc-6-Pase/GK V_max values (A; r² = 0.634, p < 0.01) and activities (B; r² = 0.819, p < 0.01) and HGP in the C and D groups.

Previous studies examining the chronic regulation of hepatic GK and Glc-6-Pase by insulin have employed markedly insulinopenic animal models of diabetes. These studies have demonstrated a severe reduction (to ~10% of control level) in GK activity and mRNA (2-4, 10-12). The 90% partially pancreatectomized rat is only moderately insulinopenic and in this respect is more similar to non-insulin-dependent diabetes mellitus and insulin-treated insulin-dependent diabetes mellitus patients than previously studied models (2-4, 10-12). This may explain why the GK mRNA levels and GK activity were only 40% lower than control values. Taken together with the absence of significant changes in the K_m of GK, these findings support the leading role of decreased GK gene expression in the down-regulation of GK in diabetes. In response to hyperinsulinemia, an ~2-fold increase in GK mRNA was paralleled by an ~2-fold stimulation in GK V_max in the diabetic group. Thus, it is tempting to speculate that in the most common form of diabetes in
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humans, i.e. non-insulin-dependent diabetes mellitus, the concomitant hyperinsulinemia may compensate for defective GK gene expression and lead to near-normal GK mRNA and V\text{GK}. Interestingly, although mRNA increased by –2-fold after hyperinsulinemia in control rats, GK V\text{GK} did not change. Furthermore, high insulin concentrations in controls caused a mild decrease in GK K\text{m}, suggesting further regulation of GK enzyme. These observations suggest that the short-term regulation of hepatic GK by insulin may differ in control and diabetic rats. In the latter, there is a more rapid coupling between the effects of insulin on GK transcription and V\text{GK}, but the regulation of the enzyme affinity is lost or impaired. In contrast, in control animals there is a dissociation between the apparent prompt stimulation of GK gene transcription and the actual microsomal pH which may be increased, GC may limit Glc-6-P utilization.

In diabetes, the presence of sorbitol 6-phosphate (37), and Glc-6-Pase was inhibited by che-

As a result, the latter was –50% of GK activity. It is possible that in diabetes, the enzymes in their setting up the assays for glucokinase and glucose-6-phosphatase. We also thank Meizihu Hu, Gary Sebel, and Kathleen Howard for excellent technical assistance.

Acknowledgments—We thank Drs. Jack Erlichman, Norman Fleischer, and Harry Shamoan for critical reading of the manuscript and Drs. Shimon Efrat, Alex Lange, and Simon Pilkis for in vitro studies in which liver extracts were incubated with insulin, or with insulin mediators, demonstrated a significant reduction in Glc-6-Pase activity (39, 40). Hepatic Glc-6-Pase activity was suppressed by carbohydrate feeding in one study; however, because insulin levels were not reported, their role could not be evaluated (41).

Although the insulin-induced changes in Glc-6-Pase and in the ratio of Glc-6-Pase/GK activities paralleled those in TGO and HGP, the latter were much larger. Thus, it is unlikely that TGO and HGP were controlled exclusively through changes in Glc-6-Pase and GK activities. This suggests that other mechanisms are involved in the acute regulation of HGP in response to insulin, and short-term changes in GK or Glc-6-Pase activities can account for a small portion of these metabolic effects. In conclusion, GK and Glc-6-Pase are important long-term regulators of fasting HGP in diabetes. However, the decrease in HGP following acute hyperinsulinemia does not correlate with these changes in Glc-6-Pase and GK activities, suggesting additional mechanism(s) for acute regulation of HGP by insulin.

REFERENCES


Insulin may influence in vitro hepatic glucose fluxes by changes in hepatic enzymes (V\text{max} or K\text{m}) and by changes in hepatic substrate concentrations. Our results indicate that high concentrations of insulin (in the presence of normoglycemia) lowered the K\text{m} of GK in control rats. Thus, it is possible that insulin induces both rapid stimulation of GK transcription, as suggested by increased mRNA, and GK enzyme activation. Glc-6-Pase V\text{max} and activity were decreased by high insulin concentrations. These observations have not been previously reported in vivo, probably due to the concomitant presence of hypoglycemia and counter-regulation. However, in vitro studies in which liver extracts were incubated with insulin, or with insulin mediators, demonstrated a significant reduction in Glc-6-Pase activity (39, 40). Hepatic Glc-6-Pase activity was suppressed by carbohydrate feeding in one study; however, because insulin levels were not reported, their role could not be evaluated (41).
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