Glucose-6-phosphatase Overexpression Lowers Glucose 6-Phosphate and Inhibits Glycogen Synthesis and Glycolysis in Hepatocytes without Affecting Glucokinase Translocation

EVIDENCE AGAINST FEEDBACK INHIBITION OF GLUCOKINASE*

(Received for publication, February 18, 1999, and in revised form, May 11, 1999)

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In hepatocytes glucokinase (GK) and glucose-6-phosphatase (Glc-6-Pase) have converse effects on glucose 6-phosphate (and fructose 6-phosphate) levels. To establish whether hexose 6-phosphate regulates GK binding to its regulatory protein, we determined the effects of Glc-6-Pase overexpression on glucose metabolism and GK compartmentation. Glc-6-Pase overexpression (4-fold) decreased glucose 6-phosphate levels by 50% and inhibited glycogen synthesis and glycolysis with a greater negative control coefficient on glycogen synthesis than on glycolysis, but it did not affect the response coefficients of glycogen synthesis or glycolysis to glucose, and it did not increase the control coefficient of GK or cause dissociation of GK from its regulatory protein, indicating that in hepatocytes fructose 6-phosphate does not regulate GK translocation by feedback inhibition. GK overexpression increases glycolysis and glycogen synthesis with a greater control coefficient on glycogen synthesis than on glycolysis. On the basis of the similar relative control coefficients of GK and Glc-6-Pase on glycogen synthesis compared with glycolysis, and the lack of effect of Glc-6-Pase overexpression on GK translocation or the control coefficient of GK, it is concluded that the main regulatory function of Glc-6-Pase is to buffer the glucose 6-phosphate concentration. This is consistent with recent findings that hyperglycemia stimulates Glc-6-Pase gene transcription.

The relative activities of hepatic glucokinase and glucose-6-phosphatase, which catalyze the first and last steps in glucose utilization and production, respectively, are thought to have a major role in regulating blood glucose homeostasis (1–5). The activities of these two enzymes change in a converse manner during fasting and refeeding or during insulin deficiency and insulin treatment. Fasting and insulin deficiency are associated with inhibition of glucokinase transcription and with a gradual decline in total glucokinase activity, whereas refeeding or insulin treatment restores glucokinase activity by induction of glucokinase transcription (4, 5). Conversely, the transcription of glucose-6-phosphatase is negatively regulated by insulin, and the activity of glucose-6-phosphatase is markedly increased in fasted or insulin-deficient diabetic states (6, 7). In addition to changes in total enzyme concentration by regulation of gene transcription, glucokinase activity is also regulated acutely by a translocation mechanism (8–10). This involves the sequestration of glucokinase in an inactive state in the nucleolus (10) bound to a 68-kDa regulatory protein at low concentrations of extracellular glucose (8, 9). A rise in extracellular glucose or low concentrations of fructose or sorbitol cause the rapid dissociation of glucokinase from its regulatory protein and the translocation of the enzyme to the cytoplasm. This translocation mechanism results in a large increase in glucokinase activity in the cytoplasm within minutes of a rise in extracellular glucose concentration (8, 10). The binding properties of glucokinase to its 68-kDa regulatory protein have been extensively characterized from studies on the purified proteins (11–15). Binding of glucokinase to the regulatory protein is enhanced by fructose 6-phosphate, and this effect is antagonized by fructose 1-phosphate. Both ligands bind to the same site on the regulatory protein and alter its affinity for glucokinase. It has been proposed that the rise in fructose 6-phosphate in hepatocytes during active glycogenolysis and gluconeogenesis causes increased binding of glucokinase to the regulatory protein and decreased glucose phosphorylation (15–17), whereas the increase in fructose 1-phosphate that results from metabolism of fructose or sorbitol causes dissociation of glucokinase from the regulatory protein (9, 18). Although the role of fructose 1-phosphate in explaining the translocation of glucokinase by fructose and sorbitol is now firmly established (18), the role of changes in fructose 6-phosphate in regulating the binding of glucokinase to its regulatory protein in the intact cell remains a contentious issue, with arguments both for (15–17) and against (19) a physiological role for changes in fructose 6-phosphate in the intact cell. Adenovirus-mediated glucose-6-phosphatase overexpression markedly suppresses the hepatocyte glucose 6-phosphate content (20) and thereby fructose 6-phosphate which is in equilibrium with glucose 6-phosphate (16) and thus provides a powerful tool to unequivocally test the physiological role of changes in hexose 6-phosphate in the intact cell.

In order to evaluate the regulatory function of glucose-6-phosphatase in the hepatocyte, this study had three aims. The first was to determine the role of changes in hepatocyte hexose 6-phosphate content in regulating glucokinase translocation.

* This work was supported by Grant195002 from the Juvenile Diabetes Foundation International (to L. A.), Grant NIH1P50H2598801 from the National Institutes of Health (to C. B. N.), and a Canadian Diabetes Foundation International (to L. A.), Grant NIH1P50H2598801

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† The abbreviation used is: Glc-6-Pase, glucose-6-phosphatase.
The second was to determine the control strengths (control coefficients) of glucose-6-phosphatase and glucokinase on glycolysis and glycogen synthesis. The third was to determine whether glucose-6-phosphatase overexpression alters the control strength of glucokinase on glycolysis or glycogen synthesis or the response coefficients of these pathways to glucose in the hepatocyte. The results support a hypothesis that the primary regulatory function of glucose-6-phosphatase in the hepatocyte is to buffer the glucose 6-phosphate concentration. This hypothesis is consistent with recent apparently paradoxical findings on glucose-6-phosphatase activity in experimental and pathological states.

**EXPERIMENTAL PROCEDURES**

**Materials**—Glucose dehydrogenase (Bacillus) was from Calbiochem. Glucose dehydrogenase (Thermoplasma) and all other enzymes were from Sigma. [U-14C]Glucose, [2-3H]glucose, and [3-14H]glucose were from NEN Life Science Products. Sources of other reagents were as described previously (21).

**Preparation of Recombinant Adenoviruses**—Recombinant adenoviruses containing the cDNA encoding either the catalytic subunit of rat glucose-6-phosphatase (AdCMV-G6P), Escherichia coli β-galactosidase (AdCMV-βGal), or rat liver glucokinase (AdCMV-GKL) under the control of the cytomegalovirus promoter were prepared as described previously (22–24).

**Hepatocyte Isolation and Culture**—Hepatocytes were isolated by collagenase perfusion of the liver of male Wistar rats (body weight 180–250 g) obtained from Bantin & Kingman (Hull, UK) and fed ad libitum (25). The hepatocytes were suspended in minimum essential medium supplemented with 7% (v/v) neonatal calf serum and inoculated in 24-well plates at a density of 4 × 10⁴ cells/cm². After cell attachment (2–3 h), the medium was replaced with serum-free medium containing the adenoviruses (21).

**Treatment of the Hepatocytes with Adenoviruses**—After amplification in 293 cells AdCMV-G6Pase and AdCMV-βGal were purified by CsCl density gradient centrifugation (24) and diluted to the same absorbance (280 nm) and stored at −70 °C. Dilutions of the AdCMV-G6Pase stock were determined that resulted in glucose-6-phosphatase overexpression between 4- and 6-fold above endogenous activity (25 ± 2 milliunits/mg, n = 11). For all experiments with AdCMV-G6Pase, controls with equivalent titers of AdCMV-βGal (based on the absorbance at 280 nm) were used. The highest AdCMV-βGal titer resulted in expression of 150–200 milliunits/μg β-galactosidase/mg of protein.

AdCMV-GKL was amplified in 293 cells, and aliquots of medium lysate were used. For experiments determining the control strength of glucose-6-phosphatase or glucokinase on glycolysis or glycogen synthesis, 5 titers of adenovirus at progressive 2-fold dilutions were used. For experiments examining a range of substrate concentrations, two adenovirus titers were used that resulted in enzyme overexpression of 2 ± 0.1- or 4.6 ± 0.5-fold, (means ± S.E., n = 5) above endogenous levels. After cell attachment the hepatocyte monolayers were incubated for 90 min in serum-free minimum essential medium containing the adenovirus. The medium was then replaced with serum-free minimum essential medium containing 10 mM dexamethasone, and the hepatocyte monolayers were cultured for 18 h. Incubations for metabolic studies were in fresh medium containing 2 μM cycloheximide (21).

**Metabolic Studies**—Incubations were for 3 h in minimum essential medium containing the substrates indicated and either [U-14C]glucose (2 μCi/ml) for determination of glycolysis synthesis or [2-3H]glucose or [3-14H]glucose (2 μCi/ml) for determination of glucose phosphorylation or glycolysis, respectively. On termination of the incubations, the medium from experiments with [3H]glucose was collected into 0.1 M HCl for determination of H₂O (see Ref. 19). Results are expressed as nanomoles of glucose detritiated for 3 h per mg of cell protein which was determined by an automated Lowry method (26). Glucose-6-phosphatase, β-galactosidase and glucokinase activities, were determined on the termination of the incubations with [3H]glucose as described below. For determination of glycolysis synthesis hepatocyte monolayers were washed 3 times with 150 mM NaCl and extracted in 0.1 M NaOH. Extracts were deproteinized with trichloroacetic acid (10%, w/v) containing glycerol carrier, and radioactivity incorporated into glycerol was determined as described in Ref. 25. Glycoly synthesis was expressed as nanomoles of glucose incorporated into glycerol for 3 h per mg of cell protein. For determination of glucose 6-phosphate hepatocyte monolayers were washed once in 150 mM NaCl and snap-frozen in liquid N₂. They were then extracted in 3% (w/v) HCO₃ and glucose 6-phosphate was determined in the neutralized perchlorate extracts (19). Loss of H₂O from [2-3H]glucose in hepatocytes is generally assumed to occur after phosphorylation of glucose to glucose 6-phosphate and equilibration of glucose 6-phosphate with fructose 6-phosphate via phosphoglucomutase. However, an exchange reaction between glucose and glucose 6-phosphate is catalyzed by glucose-6-phosphatase (catalyzed by glucose-6-phosphatase) also contributes to loss of 2-tritium from glucose (16). This exchange reaction was estimated by incubation of hepatocyte monolayers in medium containing 130 mM KCl, 3 mM HEPES, 20 mM KHCO₃, 0.5 mM EDTA, 0.05 mg/ml digitonin, 25 mM [2-3H]glucose, 0.5 mM glucose 6-phosphate, pH 7.2, for 60 min. On termination of the incubation medium was collected in 0.1 M NaOH, and assayed for 2-tritium. Rates of formation of H₂O were linear with time, and there was no loss of glucose-6-phosphatase activity from the permeabilized cells during the incubation.

**Enzyme Activity Determination**—For determination of glucose-6-phosphatase and β-galactosidase (total activity), the hepatocyte monolayers were washed 3 times with 150 mM NaCl to completely remove medium glucose. They were then extracted by brief sonication (~5 s) in buffer containing 150 mM KCl, 3 mM HEPES, 2 mM dithiothreitol, 0.05 mg/ml digitonin, pH 7,2, and assayed immediately. For glucose-6-phosphatase the assay based on Ref. 27 contained 27 mM glucose 6-phosphate, 50 mM imidazole, 1 mM EDTA, 2.5 mM NAD, 0.6 units/ml mutarotase, 6 units/ml glucose dehydrogenase (Bacillus), pH 6.5. For β-galactosidase the assay contained 34 mM lactose, 80 mM potassium phosphate, 1 mM MgSO₄, 2 mM CaCl₂, 0.6 units/ml glucose-6-phosphatase (Thermoplasma), pH 7.0. Glucokinase translocation was determined from the distribution of activity between free and bound fractions during permeabilization of the hepatocyte monolayers with digitonin in the presence of 5 mM Mg²⁺ as in Ref. 21, and the free glucokinase activity representing the cytoplasmic fraction (28) is expressed as a percentage of total activity (21). The total activity of glucokinase in this study was 13.7 ± 1.5 (n = 11) milliunits/mg protein.

**Determination of Control Coefficients**—The control strengths or control coefficients (Cₑ) of glucose-6-phosphatase or glucokinase were each determined for glycosynthesis or glycolysis. The control coefficient is defined as the fractional change (+ increase, − decrease) in metabolic flux (J) that results from a fractional change in enzyme activity (e) as shown in Equation 1.

\[
C_e = \frac{\Delta J}{\Delta e_0} = \frac{\Delta J}{\Delta e_0} e_0
\]

Where J is the flux through the pathway, and e is the activity of the enzyme, and can be determined from the slope of log J (glycolysis or glycogen synthesis) against log e (glucokinase or glucose-6-phosphatase) (29, 30). Unless indicated otherwise, control coefficients were determined from the initial slope of double logarithmic plots of flux (J) against total enzyme activity (e).

In experiments involving one or two levels of enzyme overexpression over conditions, control coefficients were determined by the Taylor expansion of Equation 1 (see Equation 2) for levels of enzyme overexpression of ±2-fold above endogenous activity.

\[
C_e = \frac{\frac{J - J_0}{J}}{\frac{e - e_0}{e_0}} J_0
\]

For determination of the effects of glucose-6-phosphatase overexpression on the control coefficient of glucokinase on glycolysis or glycosynthesis, hepatocytes treated with either AdCMV-G6Pase (2 titers that resulted in enzyme overexpression of either 2- or 4.6-fold) or with equivalent titers of AdCMV-βGal were incubated with varying concentrations of sorbitol (5–200 μM) to induce varying degrees of translocation of glucokinase. The control coefficient of glucokinase was determined from the slope of plots of log flux against log free glucokinase activity. The validity of this method rests on two assumptions as follows: the effect of sorbitol on glycosynthesis or glycolysis is exclusively due to glucokinase translocation, and the free (bound) glucokinase activity is a measure of the active glucokinase in the cytoplasm (28).

The response coefficient of glycosynthesis or glycolysis to extracellular glucose was determined from the slopes of plots of log flux against log glucose concentration (31) in cells treated with AdCMV-G6Pase or AdCMV-βGal. The response coefficient, R₉Gluc, is a measure of the sensitivity of flux to glucose (see Equation 3).
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**RESULTS**

**Effects of Graded Glucose-6-phosphatase Overexpression on Glucose Metabolism**—The effects of glucose-6-phosphatase overexpression on glucose metabolism were initially determined in incubations containing 25 mM glucose. Hepatocytes were treated with 5 titers of AdCMV-G6Pase at sequential 2-fold dilutions such that the highest titer resulted in approximately 4-fold overexpression of glucose-6-phosphatase activity for the untreated and AdCMV-G6Pase-treated cells. Standard errors for glucose-6-phosphatase activity were <15% of the means (x axis, not shown). The cells that were treated with AdCMV-βGal had the same glucose-6-phosphatase activity as untreated cells (21.5 ± 2.4 milliunits/mg) but are plotted on the x axis against the corresponding titers of AdCMV-G6Pase. Values are means ± S.E. for 4 (A) or 6 (B) cultures. *p < 0.05; **p < 0.005 AdCMV-G6Pase versus corresponding titer of AdCMV-βGal.

The response coefficient is a function of the control coefficient ($C_{\text{G6Pase}}^{\text{G6Pase}}$) and the elasticity ($e$) of the enzyme with respect to the parameter (glucose) and thus provides a measure of whether a metabolic perturbation (e.g. glucose-6-phosphatase overexpression) alters the sensitivity of glucokinase to glucose (its elasticity) (see Equation 4).

$$R_{\text{G6Pase}} = C_{\text{G6Pase}}^{\text{G6Pase}} e$$

(Eq. 4)

The elasticity of glucokinase ($GK$) with respect to glucose is a function of the intrinsic kinetics of glucokinase and of the binding of glucokinase to its regulatory protein. Thus the determination of the response coefficient is an additional approach to determine whether glucose-6-phosphatase overexpression alters binding of glucokinase to its regulatory protein (without measurement of glucokinase compartmentation).

Expression of Results—Results are expressed as means ± S.E. for the number of cell cultures indicated. Control or response coefficients were determined from individual experiments (linear regression of double logarithmic plots or Taylor expansion for a single enzyme overexpression), and means and S.E. were determined. Statistical analysis was by the Student’s paired $t$ test.

**Glucose-6-phosphatase Overexpression Does Not Affect Glucokinase Translocation in Response to Either Glucose or Sorbitol**—Glucose and precursors of fructose 1-phosphate cause translocation of glucokinase from a bound state in the nucleus (10) to the cytoplasm which can be determined by a digitonin-permeabilization assay (8, 21). Changes in the hepatocyte hexose 6-phosphate content are thought to have a physiological role in regulating the binding of glucokinase to its regulatory protein (15–17). Since glucose-6-phosphatase overexpression lowers the hepatocyte glucose 6-phosphate content by 50% (Fig. 1), we determined whether this lowering of glucose 6-phosphate and fructose 6-phosphate (16) interferes with glucokinase translocation in response to varying glucose concentration or to sorbitol, a precursor of fructose 1-phosphate. Overexpression of AdCMV-G6Pase by more than 3-fold did not affect in permeabilized cells (see “Experimental Procedures”) accounted for 1.7% of the rate of detritiation in intact cells, and in hepatocytes overexpressing glucose-6-phosphatase by 5-fold above endogenous activity, the exchange reaction accounted for 7.9% of the rate of detritiation in intact cells. Thus the exchange reaction appears to account for only a small proportion of the rate of detritiation of [2-$^3$H]glucose.

Glucose-6-phosphatase overexpression inhibited glycolysis, determined from the detritiation of [3-$^3$H]glucose, by up to 15% and glycogen synthesis by 50% at 4-fold glucose-6-phosphatase overexpression (Fig. 2). Treatment with AdCMV-βGal had no effect on glycolysis but caused a small inhibition of glycogen synthesis (15%) at the highest viral titers (150–200 milliunits/mg β-galactosidase). Throughout this study the effects of glucose-6-phosphatase overexpression on rates of metabolic flux or on control coefficients or response coefficients were determined relative to AdCMV-βGal-treated controls.

The control coefficient of glucose-6-phosphatase on glycolysis or glycogen synthesis was determined from the slope of double logarithmic plots of metabolic flux ($J$) against glucose-6-phosphatase activity ($e$). Representative plots are shown as insets in Fig. 2. The control coefficient of glucose-6-phosphatase on both glycogen synthesis and glycolysis is negative since enzyme overexpression inhibits metabolic flux. The control coefficient on glycolysis synthesis ($C_{\text{G6Pase}}^{\text{G6Pase}} = -0.45 ± 0.08$) was 2-fold greater ($p < 0.007$) than on glycogen synthesis ($C_{\text{G6Pase}}^{\text{G6Pase}} = -0.19 ± 0.04$) indicating that a fractional change in glucose-6-phosphatase (G6Pase) activity causes a 2-fold greater fractional inhibition of glycogen synthesis compared with glycolysis at 25 mM glucose.

**Glucose-6-phosphatase Overexpression Does Not Affect Glucokinase Translocation in Response to Either Glucose or Sorbitol**—Glucose and precursors of fructose 1-phosphate cause translocation of glucokinase from a bound state in the nucleus (10) to the cytoplasm which can be determined by a digitonin-permeabilization assay (8, 21). Changes in the hepatocyte hexose 6-phosphate content are thought to have a physiological role in regulating the binding of glucokinase to its regulatory protein (15–17). Since glucose-6-phosphatase overexpression lowers the hepatocyte glucose 6-phosphate content by 50% (Fig. 1), we determined whether this lowering of glucose 6-phosphate and fructose 6-phosphate (16) interferes with glucokinase translocation in response to varying glucose concentration or to sorbitol, a precursor of fructose 1-phosphate. Overexpression of AdCMV-G6Pase by more than 3-fold did not affect...
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FIG. 2. Glucose-6-phosphatase has a greater negative control coefficient on glycogen synthesis than on glycolysis. Hepatocytes were either untreated or treated with 5 titers of AdCMV-G6Pase (G6Pase, ○) or AdCMV-βGal (β-Gal, ●) as in Fig. 1. They were incubated for 3 h in medium containing 25 mM glucose and either [3-14C]glucose for determination of glycolysis (A) or [U-14C]glucose for determination of glycogen synthesis (B). Rates of glycolysis (detritiation of [3-14C]glucose, nmol/h per mg) or glycogen synthesis (incorporation of [U-14C]glucose into glycogen, nmol/h per mg) are plotted against the glucose-6-phosphatase activity of the untreated or AdCMV-G6Pase-treated cells. Cells treated with AdCMV-βGal are plotted on the same region of the x axis as the corresponding titers of AdCMV-G6Pase-treated cells. Results are means ± S.E. for six cultures. Insets show representative double logarithmic plots from which the control coefficients (C_G6Pase) were determined from the initial slope: C^GS_G6Pase = 0.45 ± 0.08; C^Gly_G6Pase = 0.19 ± 0.04, n = 5.

glucokinase translocation in response to either glucose or sorbitol (Fig. 3, A and B), and it also did not affect the detritiation of [2-3H]glucose at any substrate concentration (Fig. 3, C and D).

Effects of Glucose-6-phosphatase Overexpression on Glycolysis and Glycogen Synthesis in Different Substrate Conditions—In experimental conditions associated with varying degrees of glucokinase translocation by glucose or sorbitol, glucose-6-phosphatase overexpression (>3-fold) significantly inhibited glycolysis at all substrate concentrations and glycogen synthesis at all concentrations above 10 mM glucose or in the presence of sorbitol (Fig. 4).

The control coefficients of glucose-6-phosphatase on glycogen synthesis or glycolysis were determined by the Taylor expansion for the substrate conditions in Fig. 4, for experiments where the increment of glucose-6-phosphatase overexpression was ≥2-fold above endogenous levels. The Taylor expansion is an approximation, based on a single increment in enzyme expression and is a measure of the control coefficient at the overexpressed rather than endogenous level (31). Since control coefficients generally decrease with increasing enzyme concentration, values obtained by this method may be lower than by linear regression of double logarithmic plots.

For the incubations at varying glucose concentrations the control coefficient for glycogen synthesis (C^GS_G6Pase) was between −0.29 and −0.35 and for glycolysis (C^Gly_G6Pase) between −0.03 and −0.22. For the experiments at varying sorbitol concentrations, C^Gly_G6Pase was between −0.29 and −0.36 and C^GS_G6Pase between −0.11 and −0.13 (Table I). In most cases the control coefficients for glycogen synthesis were higher than for glycolysis by ≥2-fold. There was little or no variation in the control coefficient on glycogen synthesis at varying substrate conditions. Our results do not allow us to determine whether the variation in control coefficient on glycolysis is significant.

Effects of Glucose-6-phosphatase Overexpression on the Response Coefficients of Glycolysis and Glycogen Synthesis to Glucose—To evaluate whether glucose-6-phosphatase overexpression alters the response of glucose metabolism to glucose concentration changes in glucokinase activity, we determined the response coefficients of glycolysis and glycogen synthesis to glucose for AdCMV-G6Pase-treated and AdCMV-βGal-treated cells from the data in Fig. 4, A and C. Response coefficients were determined from the double logarithmic plots (log flux against log glucose) for individual experiments. The response coefficients for glycolysis (R^Gly) were similar for AdCMV-G6Pase-treated and AdCMV-βGal-treated cells (1.56 ± 0.07 and 1.18 ± 0.07, n = 6), and the response coefficients for glycogen synthesis (R^GS) were lower (p < 0.03) for AdCMV-G6Pase-treated than for AdCMV-βGal-treated cells (2.07 ± 0.08 and 2.26 ± 0.08, n = 6). Since glucose-6-phosphatase does not increase the response coefficient to glucose of either glycolysis or glycogen synthesis, this is further evidence against decreased binding of glucokinase to its regulatory protein in response to a lowering of hexose 6-phosphate by glucose-6-phosphatase overexpression.

The Control Coefficient of Glucokinase on Glycolysis Is Lower Than on Glycogen Synthesis—We have shown previously that glucokinase has a very high control coefficient on glycogen synthesis (21). To investigate the underlying mechanism for the greater negative control coefficient of glucose-6-phosphatase on glycogen synthesis compared with glycolysis, we determined the control coefficient of glucokinase on glycolysis and glycogen synthesis at varying glucose concentrations (5–35 mM) in hepatocytes treated with 5 titers of AdCMV-GKL to achieve varying degrees of glucokinase overexpression. Control coefficients were determined from the initial slope of plots of log flux versus log total glucokinase activity. A representative experiment is shown in Fig. 5, and the results of replicate experiments plotted individually are summarized in Table II. The control coefficient of glucokinase on both glycolysis and glycogen synthesis decreased with increasing glucose concentration, and in these substrate conditions the control coefficient for glycogen synthesis was greater than for glycolysis (Table II).

Effects of Glucose-6-phosphatase Overexpression on the Control Coefficient of Glucokinase on Glycogen Synthesis—Since glucokinase and glucose-6-phosphatase have positive and negative control coefficients, respectively, on glycogen synthesis and glycolysis, we determined whether glucose-6-phosphatase overexpression alters the control coefficient of endogenous glucokinase. This was determined from the experiments with varying sorbitol concentration (Fig. 4D) from double logarithmic plots of glycogen synthesis against free glucokinase activity (see “Experimental Procedures”). Overexpression of glucose-6-phosphatase by 2-fold above endogenous levels inhibited glycogen synthesis but did not affect the control coefficient (Table III and Fig. 6), indicating that the sensitivity of glycogen synthesis to an incremental increase in glucokinase activity is unaf-
fected. However, at higher levels of glucose-6-phosphatase overexpression (4.6 ± 0.5-fold above endogenous levels), the control coefficient on glycogen synthesis was decreased by 35%, whereas that on glycolysis was unaffected (Table III).

DISCUSSION

Glucokinase (hexokinase IV) differs from the other hexokinase isoenzymes (I–III) in that it is not inhibited by physiological concentrations of glucose 6-phosphate, the product of the reaction (4). Since binding of purified glucokinase to the regulatory (inhibitory) protein is enhanced by fructose 6-phosphate (11, 13), it has been proposed that fructose 6-phosphate is a substitute for end product inhibition by glucose 6-phosphate because fructose 6-phosphate and glucose 6-phosphate are maintained in equilibrium by phosphoglucomutase (19). This hypothesis was tested using mannitol which is metabo-
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Fig. 5. Effects of adenovirus-mediated glucokinase overexpression on glycogen synthesis and glucose-6-phosphatase activity at varying glucose concentration. Hepatocytes were either untreated or treated with 5 titers of AdCMV-GKL to achieve varying degrees of glucokinase overexpression. After 16 h culture, they were incubated for 3 h in medium containing the glucose concentrations (5–35 mM) indicated and either [3-3H]glucose for determination of glycogen synthesis (A) or [U-14C]glucose for determination of glycogen synthesis (B). Results are plotted as log flux (nmol/3 h per mg) against log total glucokinase activity (milliunits/mg protein) and are a representative experiment out of four. Control coefficients determined from the individual experiments are summarized in Table II.

TABLE II
Control coefficients of glucokinase on glycogen synthesis

Hepatocytes were treated with varying titers of AdCMV-GKL as in Fig. 5 and after 16 h culture were incubated for 3 h in fresh medium with the glucose concentrations indicated and either [3-3H]glucose for determination of glycogen synthesis (C^glycogen) or [U-14C]glucose for determination of glycogen synthesis (C^glycogen). Glycogen synthesis (C^glycogen) were determined from the initial slope of double logarithmic plots of flux against enzyme activity (see Fig. 5) determined by linear regression for the first 2 titers of enzyme overexpression. Results are means ± S.E. for four cultures.

| [Glucose] | Glycogen synthesis C^glycogen | Glycogen synthesis C^glycogen |
|-----------|------------------------------|------------------------------|
| 5         | 1.3 ± 0.3                    | 1.8 ± 0.3                    |
| 10        | 0.5 ± 0.1                    | 1.5 ± 0.2                    |
| 20        | 0.2 ± 0.1                    | 1.0 ± 0.3                    |
| 35        | 0.3 ± 0.1                    | 0.7 ± 0.2                    |

*p < 0.05; **p < 0.001 glycogen synthesis versus glycolysis.

Fig. 6. Effects of glucose-6-phosphatase overexpression on the control coefficient of glucokinase on glycogen synthesis. For experimental details see legends to Figs. 3 and 4. Hepatocytes treated with either AdCMV-G6Pase (G6Pase, ○, □) or AdCMV-βGal (β-Gal, ●) were incubated with the concentrations of sorbitol shown in Fig. 3B in parallel incubations without or with [U-14C]glucose for determination of glycogen synthesis and glucose-6-phosphatase activity, respectively. Double logarithmic plots of glycogen synthesis (nmol/3 h per mg) against free glucokinase activity (milliunits/mg protein) are means of five experiments. Control coefficients determined from the slopes of individual experiments are summarized in Table III. Glycose-6-phosphatase activities were 2.0 ± 0.1-fold (C) or 4.6 ± 0.5-fold (D) above endogenous activity.

Effects of glucose-6-phosphatase overexpression on the control coefficient of glucokinase on glycogen synthesis. Experimental conditions were as described in Fig. 4. Hepatocytes were treated with 2 titers of AdCMV-G6Pase that resulted in enzyme overexpression of either 2.0 ± 0.11 (n = 5) or 4.6 ± 0.5 (n = 5) fold increase above endogenous activity or with equivalent titers of AdCMV-βGal. They were then incubated with varying sorbitol concentrations (5, 10, 50, 200 μM) for determination of glycogen synthesis and glycogen translocation. The control coefficients of glucokinase on glycogen synthesis (C^glc) or glycogen synthesis (C^glycogen) were determined from the slope of double logarithmic plots of flux against free glucokinase activity (see Fig. 6). Results are means ± S.E. for five cultures.

| Treatment | Glycolysis C^glycogen | Glycogen synthesis C^glycogen |
|-----------|-----------------------|-----------------------------|
| AdCMV-βGal | 0.97 ± 0.10           | 2.73 ± 0.26                 |
| AdCMV-G6Pase (2.0-fold) | 1.10 ± 0.08 | 2.66 ± 0.24                 |
| AdCMV-βGal | 1.17 ± 0.11           | 2.84 ± 0.26                 |
| AdCMV-G6Pase (4.6-fold) | 0.94 ± 0.09 | 1.84 ± 0.17                 |

*p < 0.05 AdCMV-G6Pase versus respective AdCMV-βGal.

The first aim of this study was to determine whether lowering the hexose 6-phosphate content of hepatocytes by glucose-6-phosphatase overexpression causes dissociation of glucokinase from its regulatory protein as assessed from the distribution of glucokinase between free and bound states and from the response coefficients of glycogen synthesis and glycogenesis to glucose. If changes in the hepatocyte hexose 6-phosphate content have a physiological role in regulating the bind-
Glucose-6-phosphatase and Glucokinase in Hepatocytes

The present results show first that glucose-6-phosphatase overexpression inhibits glycogen synthesis and glycolysis from glucose without affecting glucokinase translocation. Second, they show that both glucokinase and glucose-6-phosphatase have a greater control coefficient (positive and negative, respectively) on glycogen synthesis than on glycolysis. Third, they show that the control coefficient of glucokinase but not that of glucose-6-phosphatase is dependent on glucose concentration. Fourth, they show that the control coefficient of glucokinase on glycogen synthesis is greater than the control coefficient of glucose-6-phosphatase. Finally, glucose-6-phosphatase overexpression does not increase the control coefficient of glucokinase. The latter finding does not support a regulatory role for glucose-6-phosphatase in increasing the sensitivity of regulation of glucose metabolism. The findings that the control coefficient of glucokinase but not that of glucose-6-phosphatase is dependent on glucose concentration and that glucokinase has a greater control coefficient than glucose-6-phosphatase, particularly at low glucose, are consistent with our previous hypothesis that the compartmentation of glucokinase in the hepatocyte is a major contributing factor to both the high control strength at low glucose concentration and to the glucose dependence of the control strength (21, 28).

Glucokinase and glucose-6-phosphatase have converse effects on the hepatocyte glucose 6-phosphate content (20, 34). The present study shows converse effects on glycolysis and glycogen synthesis with both glucokinase and glucose-6-phosphatase having a greater control strength on glycogen synthesis than on glycolysis. Based on these findings that overexpression of glucose-6-phosphatase has no effect on glucokinase translocation and that it also does not increase the control strength of glucokinase, we propose that the main regulatory function of glucose-6-phosphatase is to buffer the glucose 6-phosphate concentration in the hepatocyte. It is noteworthy that in the intact cell the buffering role of the glucose-6-phosphatase system would be determined not only by changes in expression of the catalytic subunit of glucose-6-phosphatase but also by the glucose 6-phosphate transporter which determines the kinetics of entry of glucose 6-phosphate into the endoplasmic reticulum.

The role of glucose 6-phosphate in regulating both glycolysis and glycogen synthesis in the hepatocyte is well established. Glucose 6-phosphate regulates glycolysis by increasing the concentration of fructose 2,6-bisphosphate, a potent allosteric activator of phosphofructokinase-1 (35, 36). Substrate-induced
translocation of glucokinase is associated with a marked increase in glucose 6-phosphate and fructose 2,6-bisphosphate in hepatocytes, and the latter explains the stimulation of glycolysis by sorbitol (19, 28). Glucose 6-phosphate increases glycogen synthesis both by acting as an allosteric activator of glycogen synthase and by increasing the dephosphorylation state of glycogen synthase by rendering the enzyme a better substrate for synthase phosphatase (37). The latter effect has been established from studies demonstrating a correlation between the activation state of glycogen synthase and glucose 6-phosphate in hepatocytes overexpressing glucokinase (34).

The present hypothesis that the primary regulatory function of glucose-6-phosphatase is to buffer the concentration of glucose 6-phosphate, a pivotal regulator of glycogen synthesis and glycogenolysis, is consistent with various recent apparently paradoxical findings on glucose-6-phosphatase. First, studies both in vivo (38) and in vitro on isolated hepatocytes or hepatoma cell lines (6, 39, 40) have shown that high glucose concentrations induce glucose-6-phosphatase gene transcription. If the primary regulatory role of glucose-6-phosphatase in the liver cell was to control gluconeogenesis then induction of transcription by glucose is unexpected. Accordingly, the transcription of hepatic phosphoenolpyruvate carboxykinase, which unlike glucose-6-phosphatase has an exclusive role in gluconeogenesis, is repressed by glucose metabolism (41). However, if the primary regulatory function of glucose-6-phosphatase were to buffer the glucose 6-phosphate concentration then induction by hyperglycemia is not a pathological consequence of uncontrolled diabetes (38) but a compensatory mechanism. We have shown recently that glucose-6-phosphatase overexpression in vivo increases blood glucose levels in the fed state and when fasted rats are challenged with an oral glucose tolerance test but paradoxically not in the fasted state (42). This finding can also be explained by the primary regulatory role of glucose-6-phosphatase in buffering glucose 6-phosphate levels in the absorptive state when glucokinase is in the cytoplasmic compartment. Glycogenolysis and gluconeogenesis are the two sources of glucose 6-phosphate for hepatic glucose production during fasting. The rate of glycogenolysis is determined by the activity of phosphorylase. The regulation of gluconeogenesis has been extensively studied by Groen and co-workers (43) who determined the control coefficients of the gluconeogenic enzymes in hepatocytes from starved rats and demonstrated the importance of regulation at pyruvate kinase and pyruvate carboxylase. The flux control coefficient of glucose-6-phosphatase on gluconeogenesis was found to be remarkably low (−0.02), and the glucose 6-phosphate concentration was far below the $K_m$ (43). Accordingly the “flux-generating steps” of hepatic glucose production are phosphorylase and pyruvate metabolism at the phosphoenolpyruvate branchpoint. This would explain why glucose-6-phosphatase overexpression does not elevate fasting glycaemia (42). It does, however, markedly elevate glucose levels in the fed state and particularly the response to an oral glucose tolerance test (42), consistent with the present hypothesis of a role of glucose-6-phosphatase in buffering the glucose 6-phosphate concentration of the hepatocyte.

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