The Effects of Streptozotocin-induced Diabetes and Insulin Supplementation on Expression of the Glycogen Phosphorylase Gene in Rat Liver*

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We have previously observed that the chronic effects of streptozotocin-induced diabetes cause a decrease in the total hepatic glycogen phosphorylase activity with a corresponding reduction in the phosphorylase protein levels. These effects were normalized by insulin administration to diabetic rats. There was no change in the total glycogen synthase activity as a result of diabetes or insulin supplementation. These results are extended to examine the effects of diabetes and insulin administration to diabetic animals on the expression of phosphorylase and glycogen synthase enzymes. The expression (i.e. mRNA levels) of phosphorylase was down-regulated (45% of normal levels) in diabetic livers, and this was normalized by insulin supplementation to diabetic animals. Diabetes or insulin supplementation to diabetic rats showed no effect on the transcription rate of phosphorylase. As expected, diabetes (or insulin administration to diabetic animals) did not cause any alteration in the mRNA levels or in the transcription rate of hepatic glycogen synthase. The stability of phosphorylase mRNA was then examined using hepatocytes prepared from normal and diabetic rats. Diabetes caused a decrease in the half-life of phosphorylase mRNA from 14 h in normal hepatocytes to 6.5 h in diabetic hepatocytes. Insulin supplementation to the medium of diabetic hepatocytes increased the half-life of phosphorylase mRNA to a level comparable with normal values. This study indicates that the chronic effect of insulin on the activation of the total hepatic phosphorylase activity (and protein) is mediated through the stabilization of its mRNA levels.

It is now well established that reversible phosphorylation is one of the major mechanisms by which hormones exert their effects on the regulation of metabolic pathways. Glycogen metabolism is one of the well studied metabolic processes regulated by such a mechanism involving phosphorylation-dephosphorylation of many proteins (or enzymes) (1–5). Glycogen phosphorylase and glycogen synthase are the two key regulatory enzymes that catalyze the rate-limiting steps of glycogen degradation and synthesis, respectively. Both of these enzymes exist in two interconvertible phosphorylated and dephosphorylated forms. The level of the active phosphorylated form of phosphorylase (a-form) is balanced by the opposing actions of phosphorylase phosphatase and phosphorylase kinase. Unlike phosphorylase, the dephosphorylated form of glycogen synthase (a-form) is active, and its levels are regulated by several protein kinases (including protein kinase A, protein kinase C, glycogen synthase kinase-3, casein kinase II, and phosphorylase kinase) and synthase phosphatase (protein phosphatase type 1).

The liver plays a central role in the maintenance of blood glucose homeostasis. Glycogen metabolism in hepatic tissue is one of the major metabolic processes involved in such a homeostasis. It has been well established that the balance between levels of insulin and glucagon is vital for this regulation. Insulin is involved in both the acute and the chronic regulation of glycogen synthase and phosphorylase, the key regulatory enzymes of glycogen metabolism. Both the acute and the chronic effects of insulin are determined mainly with the use of type 1 insulin-dependent diabetes mellitus animals produced with an injection of streptozotocin (STZ) or alloxan (6–10). The acute effects of insulin are exerted through decreases in the activities of protein kinase A (with or without changes in the levels of cyclic AMP) (6, 7), glycogen synthase kinase-3 (11), and protein kinase C (12), and an activation of protein phosphatase 1 (13, 14). All of these changes result in an increase in the activity of glycogen synthase and a decrease in the activity of phosphorylase involving only the active forms of these enzymes. The net long term chronic effects of insulin are similar to its acute effects on the activities of glycogen synthase and phosphorylase; however, insulin also causes a decrease in the total activity of phosphorylase (8–10, 15, 16). A similar decrease in total phosphorylase activity has been observed in cultured hepatocytes prepared from alloxan-induced diabetic rats (17). The decrease in the total glycogen phosphorylase activity in diabetic liver is due to a reduction in the enzymic protein level (10, 18), and it is probably due to a decrease in its synthesis (18). The reason for the decreased synthesis of phosphorylase and whether insulin plays any role in this process or in regulation of the phosphorylase gene are not known. Phosphorylase protein levels are not altered in the fed/fasted state, which indicates that the phosphorylase gene is not under nutritional regulation (19). The abnormal expression of the phosphorylase gene has been reported in muscle tissue in altered physiological conditions such as regenerating muscles in rats (20) and dystrophic muscles in mice (21).

Because the total activity of glycogen phosphorylase is altered as a result of chronic insulin effects, it was of interest to determine whether insulin plays any role in the expression of the glycogen phosphorylase at the gene expression or the

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1 The abbreviations used are: STZ, streptozotocin; DRB, 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole.
mRNA stability levels. We have also included studies on glycogen synthase for comparative purposes. In the present work, we have therefore studied the expression of glycogen synthase and phosphorylase genes in the livers of normal, streptozotocin-induced diabetic (i.e. insulin-deficient condition), and insulin-treated streptozotocin-induced diabetic rats. There were no differences in the mRNA levels or in the transcription rates of glycogen syntheses among three groups of animals. The expression (i.e. mRNA levels and total phosphorylase activity) of the phosphorylase gene was, however, regulated by insulin through the control of its mRNA half-life.

EXPERIMENTAL PROCEDURES

Materials—TRIzol, fetal calf serum, Dulbecco's modified Eagle's medium, and William's E media were purchased from Life Technologies, Inc. Random primer labeling kit, salmon sperm DNA, and Nuc-trap push columns for radiolabeled probe purification were supplied by Stratagene. Oligo(dT)-cellulose spin columns were from 5 Prime — 3 Prime, Inc (Boulder, CO), whereas (a-32P)dCTP and (s-35)UTP were obtained from DuPont. Insulin was obtained from Eli Lilly Co. RNA synthesis inhibitor DRB, creatine phosphokinase, creatinine phos- phate, CTP, UTP, tRNA, streptozocin, Swinn's 5-77 medium, and all other chemicals used in this investigation were purchased from Sigma. Sustained-release insulin implants (Implant) were purchased from a Linshin Canada, Inc DNA probes for glycogen synthase and phos- phorylase were generously provided by Dr. E. Y. C. Lee (University of Linshin Canada, Inc) (23), respectively. Dr. W. J. Roesler of this Department provided us with the cDNA probe for phosphoenolpyruvate carboxykinase.

Animals—Male Sprague-Dawley rats (body weight, 200–250 g) were divided into three groups of seven animals each: normal control, STZ-induced diabetic, and insulin-treated STZ-induced diabetic. The latter two groups of rats were made diabetic by a single intraperitoneal injection of streptozocin (60 mg/kg of body weight) dissolved in 100 mM citrate, pH 4.5, containing 150 mM NaCl (8, 10, 16). Control rats were injected with citrate buffer alone. Diabetic rats were divided into two groups 1 week after the STZ injection, and insulin implants were subcutaneously inserted in one group of diabetic rats. Normal control and diabetic animals were also similarly sham treated. After 3 weeks, the rats of all three groups were killed between 0900 and 1000 h in fed condition. Blood samples collected by orbital sinus bleeding were cen-

trifuged at 3000 for 10 min, and the plasma samples were stored at −80°C for glucose and insulin estimation. A part of liver samples were stored at −80°C for glycogen synthase and phosphorylase assays, and the remaining portion of liver samples were processed immediately for total RNA and nuclei isolation.

Assay of Glycogen Synthase and Phosphorylase—The activities of glycogen synthase and phosphorylase in liver samples were carried out as described previously (8, 24–26). Glycogen synthase activity was determined by the method of Thomas et al. (25) by measuring the incorporation of [14C]glucose from UDP-[14C]glucose into glycogen. Phosphorylase activity was measured in the direction of glycogen syntheses from [14C]glucose-1-phosphate by the method of Tan and Nuttall (26). One unit of glycogen synthase and phosphorylase is the amount of enzyme that incorporates 1 mmol of [14C]glucose from their respective substrates into glycogen. The active forms of these enzymes were cal-

culated as the percentage of the total enzyme activity and were expressed as the activity ratios.

Isolation of Total RNA and Northern Blot Analysis—Total RNA was isolated from liver insulin and phosphorylase in liver samples using TRIzol reagent (27). Poly(A)+ RNA was sepa-

rated from total RNA by using oligo(dT)-cellulose spin columns. Polya+ RNA (2 μg) was fractionated in 1% agarose gel containing 2.2 M formaldehyde. After electrophoresis, the gel was soaked in 10 × SSC (1.5 M NaCl and 0.15 M sodium citrate) for 4 h and was blotted onto a Hybond N+ membrane. The filter was then baked for 2 h at 80°C in a vacuum oven. Hybridization was performed for 6 h at 42°C in 50% formamide, 5 × Denhardt's solution (0.1% each of Ficol, polyvinylpyr-

rolidone, and bovine serum albumin), 5 × SSPE (0.75 M NaCl, 50 mM NaH2PO4, and 50 mM EDTA), 0.1% SDS, and 200 μg/ml sonicated salmon sperm DNA. Glycogen synthase and phosphorylase probes were labeled with [α-32P]dCTP using a random labeling kit. Rat liver glyco-

gen synthase cDNA insert of 1.6 kilobase pairs in pGEM-3z vector (reconstituted with human liver glycogen phosphorylase cDNA insert of 2.56 kilobase pairs in pKK 233-2 vector (digested with Ncol and Xba, 2.0-kilobase pair fragment), and chicken brain β-actin cDNA (2-kilobase pair Pst fragment) were used for probing. Hybridization was carried out at 42°C for 16 h. The membranes were subsequently washed three times for 10 min each time in 200 ml of 2 × SSC and 0.1% SDS at 28°C followed by two times for 30 min each in 200 ml of 0.1 × SSC and 0.1% SDS at 50°C. The hybridized filters were exposed to Kodak X-OMAT x-ray film with an intensifying screen at −80°C. Quanti-

fication of mRNA was accomplished by densitometric scanning of autoradiograms with the use of an LKB 2202 Ultrascan laser densitomer.

Nuclear Transcription Assay—Nuclei were isolated from liver tissue essentially as described by Chauhan and Dakshinamurti (28) with the following minor modifications. 1 ml of homogenate was mixed with 2 ml of 2.3 M sucrose in 50 mM Tris/HCl, pH 7.5, containing 25 mM KCl, 2 mM MgCl2, 0.5 mM dithiothreitol, 10 mM EGTA, 0.14 mM spermidine, and 0.1 mM phenylmethylsulfonyl fluoride by injection in a Beckman Ti 50 centrifuge tube. The mixture was then underlaid by 1.0 ml of 2.3 M sucrose in the above buffer with a syringe. After centrifugation for 30 min at 39,000 rpm in a Beckman Ti 50 rotor at 4°C, the nuclear pellet was taken up in the storage buffer containing 50 mM Tris/HCl, pH 8.3, 5 mM MgCl2, 0.1 mM EDTA, 50% glycerol, and 0.1 mM phenylmethyl-

sulfonyl fluoride and stored at −80°C. The nuclear run-on assay was carried out according to Chauhan and Dakshinamurti (28) except that the incubation temperature was 30°C. Labeled transcripts were iso-

lated from the use of TRIzol reagent as described by Chomczynski and Sacchi (27). After adding 50 μg of RNA as carrier RNA, the samples were heated to 95°C for 5 min. TRIzol reagents were passed through 22 gauge needle several times. RNA was precipitated with isopropl alcohol, and the pellet RNA was solubilized in hybridization solution.

Plasmid DNA (4 μg) was applied onto a Hybond N+ membrane using a slot blot manifold and baked for 2 h at 80°C. The membranes were prehybridized for 6 h at 50°C in 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 0.2% SDS, and 250 μg/ml RNA. Hybridization was carried out with the corresponding transcripts in the same prehy-

bridization solution (final volume, 1.0 ml) at 45°C for 72 h. The membranes were washed twice for 30 min each time in 2 × SSC and 0.1% S DS at 30°C, washed thrice in 0.1 × SSC and 0.1% SDS at 55°C for 20 min each, and exposed to Kodak X-Omat x-ray film with intensifying screen at −80°C. Quantitation of autoradiograms was carried out by densitometry. Scanning was carried out with the use of an LKB 2202 Ultrascan laser densitomer. The densitometric values of the experimental groups were expressed as a percentage of the normal group. The amounts of radioactivity in bands were further confirmed by cutting out the appropriate membrane bands and counting in a scintillation coun-

ter. The results by densitometry and scintillation counting showed identical patterns (results not shown). Nonspecific hybridization of the respective probes to pGEM-3z, pKK 233-2, or pBR 322 DNA was negligible.

Cell Culture and Stability of mRNA—The procedures for isolation of hepatocytes from normal and diabetic rats and the maintenance of monolayer cultures were as described previously (29, 30). Briefly, cell suspensions obtained by perfusing livers with collagenase were washed three times with William's medium, and the final cell pellets were resuspended in Dulbecco's modified Eagle's medium containing 10% new born calf serum. Cells (5 × 105) were cultured in collagen-coated dishes (diameter, 100 mm) in the same medium. After 4 h, the medium was changed to serum-free Dulbecco's modified Eagle's medium contain-

ing 5.5 mM glucose, 0.2% bovine serum albumin, and 100 μM de-

amethasone. After 16 h, the medium was replaced with fresh serum-

free medium containing glucose, bovine serum albumin, and deamethasone with RNA synthesis inhibitor DRB (50 μM/ml) in the presence and absence of insulin (100 μU). Isolation of total RNA, poly(A)+ RNA, and Northern blot analysis were carried out as men-

tioned earlier.

Plasma Analysis—Plasma glucose was determined according to the method described by Raabo and Terkildsen (31). Plasma insulin was measured by the double-antibody method (32). Statistical analysis was carried out by Student's t test.

RESULTS

Plasma glucose and insulin levels and the activities of glycogen synthase and phosphorylase are summarized in Tables I and II. The diabetic rats were characterized by a 4–5-fold increase in plasma glucose and significant decreases in plasma insulin levels (Table I). The values for insulin-treated diabetic animals were similar to those for normal control rats. As ex-

pected, diabetes caused a decrease in the activity of the active
were no changes in the expression of the glycogen phosphorylase mRNA levels (i.e., no significant correlation between the total phosphorylase activity and glycogen synthase levels that were slightly higher (127%) than those in diabetic rats). Insulin treatment of diabetic rats increased phosphorylase activity by 45%, as compared with normal animals (Fig. 1A). However, phosphorylase mRNA levels were decreased in diabetic animals (Fig. 1B). The results on the expression of glycogen synthase and phosphorylase among the three groups of animals show that insulin treatment of diabetic animals recovered enzyme activities to levels comparable with normal values.

The data on the expression of glycogen synthase and phosphorylase in normal, streptozotocin-induced diabetic, and insulin-treated diabetic rats are shown in Table I. There were no differences in mRNA levels for glycogen synthase among the three groups of animals. However, phosphorylase mRNA levels were decreased in diabetic rats by 45%, as compared with normal animals (Fig. 1B). Insulin treatment of diabetic rats increased phosphorylase mRNA levels to levels that were slightly higher (127%) than those in normal control animals. As shown in Fig. 2, there was a significant correlation between the total phosphorylase activity and phosphorylase mRNA levels ($r = 0.67$ at $p < 0.001$). There were no changes in the expression of the glycogen synthase mRNA levels in the three groups of rats.

The increase in phosphorylase mRNA levels in insulin-treated diabetic rats suggests the possible involvement of this hormone at either the transcription or the mRNA stability level. The results on the rate of transcription for phosphorylase, glycogen synthase, phosphoenolpyruvate carboxykinase, and $\beta$-actin genes are presented in Table II. The transcription of the phosphoenolpyruvate carboxykinase gene was used as an internal standard because of its well-established negative regulation by insulin in rat liver (33). The transcription of the $\beta$-actin gene was included as an internal control, because it is not influenced by the diabetic state and insulin administration. As expected, there were no changes in the transcription rates of the glycogen synthase and the $\beta$-actin genes among the three groups of animals (Fig. 3). The transcription of the phosphoenolpyruvate carboxykinase gene increased in diabetic animals and was normalized after the insulin treatment. It was surprising that there was no alteration in the rate of transcription of the $eta$-actin gene as a result of diabetes or insulin treatment of diabetic animals. Nonspecific hybridization of the respective probes to pGEM-3z, pKK 233–2, or pBR 322 DNA was negligible (data not shown).

The results presented in Fig. 3 indicate that insulin plays a role at the post-transcriptional level of phosphorylase gene expression. Therefore, we examined the stability of phosphorylase mRNA in isolated hepatocytes from normal and diabetic rats. Hepatocytes, kept in serum-free medium for 16 h, were incubated in the presence of DRB (RNA synthesis inhibitor), and samples were withdrawn at various times for mRNA measurements. Insulin was also added to one set of the diabetic hepatocytes along with DRB to compare mRNA stability in

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**Table I**

| Animals       | Body weight | Plasma glucose | Plasma insulin |
|---------------|-------------|----------------|----------------|
| Normal        | 372 ± 8     | 7.2 ± 0.4      | 441 ± 37       |
| Diabetic      | 390 ± 13    | 30.9 ± 1.3     | 81 ± 6         |
| Insulin-treated diabetic | 362 ± 8     | 5.8 ± 0.5      | 571 ± 39       |

$p < 0.001$.

**Table II**

| Enzymes        | Normal | Diabetic | Insulin-treated diabetic |
|----------------|-------|---------|-------------------------|
| Phosphorylase a| 71.4 ± 4.1 | 38.6 ± 1.8 | 77.5 ± 5.7             |
| Phosphorylase total | 181.0 ± 5.3 | 97.9 ± 4.9 | 212.7 ± 14.5           |
| Activity ratio | 0.40 ± 0.02 | 0.39 ± 0.01 | 0.36 ± 0.01            |
| Glycogen synthase a | 0.51 ± 0.05 | 0.16 ± 0.02 | 0.52 ± 0.03            |
| Glycogen synthase total | 1.24 ± 0.08 | 1.20 ± 0.07 | 1.22 ± 0.05            |
| Activity ratio | 0.41 ± 0.02 | 0.13 ± 0.01 | 0.42 ± 0.02            |

$p < 0.0001$.

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**Fig. 1.** Northern blot analysis (A) and quantitation (B) of the relative levels of glycogen synthase and phosphorylase mRNA in livers of normal (N), streptozotocin-induced diabetic (D), and insulin-treated diabetic (I) rats. 2 $\mu$/g of poly(A)$^+$ RNA isolated from total RNA was fractionated, transferred onto a Hybond N$^+$ membrane, and then hybridized with glycogen synthase and phosphorylase cDNA probes as described under "Experimental Procedures." A, two representative autoradiograms of Northern blots of liver preparations from different animal groups. B, the densitometric analysis of glycogen synthase and phosphorylase mRNA are presented as the means ± S.E. of seven rats in each group. Quantitation was carried out with respect to equal amounts of $\beta$-actin in each group. Comparisons were made by Student's t test. *, $p < 0.01$ versus normal rats. **, $p < 0.005$ versus diabetic rats.
diabetic hepatocytes in the absence and the presence of insulin. Phosphorylase mRNA levels were measured at the indicated times by Northern blot analysis of poly(A)$^1$RNA. As shown in Fig. 4, the rate of degradation of phosphorylase mRNA was faster in diabetic hepatocytes as compared with the normal hepatocytes. The half-lives of phosphorylase mRNA in normal and diabetic hepatocytes were $14$ and $6.5$ h, respectively. The presence of insulin in the medium of diabetic hepatocytes increased the phosphorylase mRNA stability by increasing its half-life from $6.5$ h to $13.5$ h, which is comparable with normal hepatocytes.

**DISCUSSION**

The primary objective of this study was to examine the chronic effects of diabetes and insulin supplementation on phosphorylase gene expression in intact animals. Previous studies in our laboratory (8, 10, 16) and others (9, 18) have demonstrated that the activities of phosphorylase and glycogen synthase are altered in the livers of animal models of type 1 insulin-dependent diabetes mellitus. In streptozotocin- or alloxan-induced diabetic animals, the activities of both the active form and the total (i.e. active plus inactive) phosphorylase are decreased in the livers of diabetic animals. On the other hand, the activity of the active form of glycogen synthase is decreased with no change in the total enzyme activity in liver tissue. It was of interest, therefore, to know whether changes in enzyme activities are reflected through changes in gene expression for these enzymes.

We have previously demonstrated that the decrease in total hepatic phosphorylase activities in streptozotocin-induced diabetic rats is due to a parallel reduction in enzymic protein and that these levels are normalized by insulin (10). The decrease in total hepatic phosphorylase activity in diabetic rats has also been observed by other investigators (9, 15, 18, 34). Rulfs et al. (18) have further reported that the decrease in total phosphorylase activity is related to the reduction in phosphorylase protein as a result of the decreased synthesis of this protein. In contrast to these results, Bahnak and Gold (9) have observed that the decrease in total phosphorylase protein is due to a more pronounced increase in the degradation rate of phosphorylase. As suggested by Rulfs et al. (18), the discrepancy between the data of these two studies could be due to differences in the purity of the preparations used and the time course examined. A positive correlation between changes in hepatic total phosphorylase activity and enzymic protein content has also been observed in mice as a result of diurnal rhythm and during their development (35, 36). In the present study, we have observed decreased phosphorylase mRNA levels in diabetic liver and normalization of these levels after insulin treatment (Fig. 1). The decrease in phosphorylase mRNA levels in diabetic liver is parallel to the decrease in total phosphorylase activity (Fig. 2). These data support the view that the decrease in total phosphorylase activity in diabetic livers is due to decreased protein synthesis and are in agreement with the pre-
Phosphorylase Gene Expression in Diabetes

After incubation of normal and diabetic hepatocytes in serum-free medium for 16 h, cells were incubated in the presence of RNA synthesis inhibitor 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole. The diabetic hepatocytes were incubated, in addition to the RNA synthesis inhibitor, in the absence or the presence of 100 nM insulin. Cells were withdrawn at indicated times, and phosphorylase mRNA levels were determined by Northern blots as described under "Experimental Procedures." The value at zero time in each group was taken as 100%. Each point is the mean ± S.E. of three hepatocyte preparations in each group. The phosphorylase mRNA levels in diabetic hepatocytes (incubated without insulin) were significantly lower (p < 0.01) at all time points between 3 and 12 h as compared with the mRNA levels in normal and diabetic (incubated in the presence of insulin) hepatocytes.

Insulin plays a major role in the regulation of hepatic phosphorylase mRNA levels by decreasing its stability. These hepatoma cell results are not in agreement with the results of Bahnak and Gold (9) and our findings (8, 16, Fig. 1) in rat diabetic livers. It is possible that the regulation of glycogen synthase both at the mRNA and the protein levels by insulin is different in these cancerous cells as compared with normal hepatic tissue. It is well known that there are marked differences in the response of certain genes in liver tissue and in hepatoma cell lines (39). Moreover, the regulation of glycogen synthase is a complex one. The enzyme is phosphorylated at many sites by several kinases (40) and therefore probably possesses many intermediate activities that are not similarly activated by activator glucose 6-phosphate (41). In addition, synthase phosphatase (protein phosphatase type 1) plays a significant role in determining glycogen synthase activity, and the activity of synthase phosphatase decreases in type 1 diabetes (5, 8, 42). The activity of this enzyme is also affected by the microenvironment of the glycogen particle, such as protein factors and allosteric effectors. These factors will influence the measured glycogen synthase activity in liver extracts.

The nuclear run-on analyses carried out with nuclei isolated from livers of normal, STZ diabetic, and insulin-treated diabetic rats indicate that the transcription rate of glycogen synthase was unaltered, and these data support the observation of unchanged glycogen synthase mRNA levels in these three groups of rats (Fig. 3). Surprisingly, there was also no change in the transcription rate of the phospholamban gene in diabetic rats as compared with normal or insulin-treated diabetic rats. This indicates that the decrease in phosphorylase mRNA (Fig. 1) is probably due to the decreased stability of phosphorylase mRNA in STZ diabetic rats. The stability of phosphorylase mRNA was therefore studied in hepatocytes isolated from normal and diabetic rats. The stability of phosphorylase mRNA as indicated by the measurement of its half-life is reduced in diabetic hepatocytes as compared with normal hepatocytes, and the stability is increased with the addition of insulin to hepatocytes derived from diabetic animals (Fig. 4). These results indicate that the decreased levels of phosphorylase mRNA in diabetic rat liver are due to their reduced stability and that they could be normalized by insulin.

In the present study, we have observed no change in the expression of the glycogen synthase gene (i.e. mRNA levels) and no alteration in the total glycogen synthase activity in diabetic livers (Fig. 1). In addition, insulin administration to diabetic animals also had no effect on glycogen synthase gene expression. Miller (34) also found that total glycogen synthase activities in whole homogenates were similar in normal and diabetic livers. Bahnak and Gold (9), however, reported a 2-fold increase in the total glycogen synthase activity in alloxan-induced diabetic animals and normalization of these values after insulin administration to diabetic animals. The increase in the amount of enzymic protein is due to an increased rate of protein synthesis. The reason for the discrepancy between our results and those of Bahnak and Gold (9) is unclear except for the fact that we measured enzyme activity 3 weeks after the induction of diabetes, whereas they determined enzyme activity 8 days after an alloxan injection. Okubo et al. (38) observed that insulin caused an increase in the total glycogen synthase activity involving protein synthesis in rat hepatoma H4 cells. However, in contrast, insulin caused the decrease in glycogen synthase mRNA levels by decreasing its stability. These hepatoma cell results are not in agreement with the results of Bahnak and Gold (9) and our findings (8, 16, Fig. 1) in rat diabetic livers. It is possible that the regulation of glycogen synthase both at the mRNA and the protein levels by insulin is different in these cancerous cells as compared with normal hepatic tissue. It is well known that there are marked differences in the response of certain genes in liver tissue and in hepatoma cell lines (39). Moreover, the regulation of glycogen synthase is a complex one. The enzyme is phosphorylated at many sites by several kinases (40) and therefore probably possesses many intermediate activities that are not similarly activated by activator glucose 6-phosphate (41). In addition, synthase phosphatase (protein phosphatase type 1) plays a significant role in determining glycogen synthase activity, and the activity of synthase phosphatase decreases in type 1 diabetes (5, 8, 42). The activity of this enzyme is also affected by the microenvironment of the glycogen particle, such as protein factors and allosteric effectors. These factors will influence the measured glycogen synthase activity in liver extracts.

The nuclear run-on analyses carried out with nuclei isolated from livers of normal, STZ diabetic, and insulin-treated diabetic rats indicate that the transcription rate of glycogen synthase was unaltered, and these data support the observation of unchanged glycogen synthase mRNA levels in these three groups of rats (Fig. 3). Surprisingly, there was also no change in the transcription rate of the phospholamban gene in diabetic rats as compared with normal or insulin-treated diabetic rats. This indicates that the decrease in phosphorylase mRNA (Fig. 1) is probably due to the decreased stability of phosphorylase mRNA in STZ diabetic rats. The stability of phosphorylase mRNA was therefore studied in hepatocytes isolated from normal and diabetic rats. The stability of phosphorylase mRNA as indicated by the measurement of its half-life is reduced in diabetic hepatocytes as compared with normal hepatocytes, and the stability is increased with the addition of insulin to hepatocytes derived from diabetic animals (Fig. 4). These results indicate that the decreased levels of phosphorylase mRNA in diabetic rat liver are due to their reduced stability and that they could be normalized by insulin.

It is now obvious that insulin is involved in both acute and chronic regulation of phosphorylase activity in liver tissue. The acute effect of this hormone is to regulate the enzyme activity by decreasing the active form of phosphorylase through covalent modification of hepatic glycogenolytic enzymes. On the other hand, the chronic effect of insulin is a positive regulation at the gene level, as observed in this investigation, by increasing stability of phosphorylase mRNA, thereby increasing the phosphorylase protein levels and total activity. Both mechanisms of regulation may therefore act simultaneously to control the steady-state physiological levels of active phosphorylase in vivo.

The diabetic condition that leads to decreased circulating insulin levels also results in significant increase in glucagon levels (10, 16). Insulin replacement in diabetic rats that produces normalization of the total phosphorylase activity and phosphorylase mRNA levels also causes a decrease in glucagon levels. It is therefore reasonable to think that glucagon might also contribute to phosphorylase gene expression. However, insulin caused an increase in phosphorylase mRNA stability in hepatocyte culture in which no manipulation of glucagon levels was carried out. This suggests that insulin is primarily responsible for this metabolic response. The exact mechanism of how insulin regulates the stability of phosphorylase mRNA has yet to be determined.

Insulin plays an important role in the regulation of hepatic
gene expression by control at the transcription and/or posttranscription level (43–47). The rate of mRNA degradation plays an important role in controlling the level of gene expression (48), and it is very well known that the stabilities of many mRNAs are altered in response to a variety of physiological and pharmacological stimuli. Specific sequences within the translated, 5' non-translated, and 3' non-translated regions seem to function as cis-acting elements in regulating mRNA stability. These cis-acting sequences are recognized by trans-acting cytoplasmic proteins that bind to molecules and change the secondary structures of the mRNA molecules, thereby increasing its accessibility for nuclease attack. Although it is known that type 1 diabetes produces a lower plasma insulin level and impairs the expression of hepatic genes under insulin regulation, the exact mechanism by which insulin regulates the translatability and stability of mRNA needs to be examined by future studies.

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