Induced Synthesis of Hepatic Uridine Diphosphate Glucose-Glycogen Glucosyltransferase after Administration of Insulin to Alloxan-diabetic Rats*

DONALD F. STEINER† AND JUDITH KING

From the Department of Biochemistry, University of Chicago School of Medicine, Chicago 37, Illinois

(Received for publication, August 23, 1963)

It was reported previously that administration of insulin to severely diabetic rats results in massive glycogen deposition in the liver and increased activity of the enzyme glycogen synthetase (uridine diphosphate glucose-glycogen glucosyltransferase, EC 2.4.1.11) in homogenates of liver (1, 2). This paper describes refinements in the assay procedure for glycogen synthetase which have enabled us to establish that the activity of this enzyme begins to rise sharply about 40 minutes after insulin administration. The magnitude of the increase in synthetase is considerably greater than was reported by us previously. Investigations of the mechanism of this hepatic effect of insulin are presented which suggest that the synthesis of the enzyme protein is accelerated by insulin administration.

EXPERIMENTAL PROCEDURE

Glucagon-free bovine insulin was supplied by Novo Terapeutis, Copenhagen. Puromycin dihydrochloride obtained from Nutritional Biochemicals Corporation was dissolved in water at a concentration of 20 mg per ml and neutralized with HCl to pH 7.0 before injection. Actinomycin D, provided by Dr. Clement A. Stone of the Merck Institute, was dissolved in 0.9% NaCl solution at a concentration of 500 μg per ml for injection. UDP-glucose was obtained from Pabst Laboratories, and glucose-6-P and glucose-6-P dehydrogenase from the California Corporation for Biochemical Research. Enzymatic preparation of UDP-glucose-14C was facilitated by the addition of yeast crystalline inorganic pyrophosphatase during the second incubation step of the procedure described previously (2), thereby increasing yields to 80 to 90%. Residual α-amylase activity in glycogen extracted from rat livers by cold trichloroacetic acid (2) was eliminated by heating a concentrated neutral solution of the glycogen to 100° for 5 minutes and filtering off any precipitate formed before the final precipitation. The methods for estimation of blood glucose, liver glycogen, and protein have been described (2).

Preparation of Animals—Female Sprague-Dawley rats weighing 120 to 140 g were given a single intravenous injection of alloxan (35 mg per kg) after deprivation of food for 48 hours, and were not used for experiments until 3 or more weeks after alloxan injection. Severity of the diabetes was classified arbitrarily to the time necessary for incorporation of 10% of the substrate, and were expressed as micromoles per hour per unit of tissue. Presentation of the results in this form permitted comparisons with estimated rates in vivo.

Glucokinase activity of liver supernatant fractions was measured by following the rate of reduction of TPN at 340 με in a Beckman DU spectrophotometer in a reaction mixture containing 200 μmoles of Tris-HCl (pH 7.8), 0.2 μmole of TPN, 5.0 μmoles of MgCl₂, 300 μmoles of glucose, 1.25 μg of purified glucose-6-P dehydrogenase (Boehringer), 0.2 ml of supernatant fraction, and 1 Composition, approximately 40% α- and 60% β-glycerophosphate (Mann Chemical Company).
3.0 μmoles of ATP (added last to the mixture) in a final volume of 3.0 ml. 6-Phosphogluconate dehydrogenase activity was measured by omitting glucose, ATP, and glucose-6-P dehydrogenase from the above mixture and initiating the reaction by adding 0.05 to 0.10 μmole of 6-phosphogluconate (sodium). Glucokinase estimates were then corrected for the small reduction of TPN stemming from further oxidation of glucose-6-P at this step. The glucose-6-P dehydrogenase activity of the supernatant fraction was measured essentially as described by Glock and McLean (4), except that magnesium ion was omitted from the reaction mixture. Appropriate corrections were made for 6-phosphogluconate oxidation as with the glucokinase assays. The results were expressed as micromoles of TPN reduced per hour per g of supernatant protein.

RESULTS

Conditions for Assay of Glycogen Synthetase—Detailed studies of the properties of hepatic glycogen synthetase will be published later, but it seems appropriate to call attention here to some considerations in the selection of pH, buffer, and ionic composition, which significantly affect the accuracy of the results when crude preparations are assayed. The marked instability of hepatic glycogen synthetase at 37° in the absence of glucose-6-P or UDP has been reported (5, 6). We have found that the unprotected enzyme is inactivated more rapidly at 37° as the pH is raised above 7.4. It is therefore important to establish that the conditions chosen for assay are appropriate for measuring activity in the absence of glucose-6-P as well as in its presence, since it may function not only as a stimulator of the reaction, but also as a stabilizer for the enzyme protein.

Several buffer combinations were tested over the pH range from 7.0 to 8.6. These included Tris-HCl, Tris-sulfate, Tris-maleate, and potassium glycero-phosphate. The best results were obtained with potassium glycero-phosphate buffer, which permitted high levels of incorporation with a broad maximum at pH 7.4 both with and without glucose-6-P. In contrast, relatively sharp pH optima near pH 8.0 were observed with the three amino buffers, as reported by Leloir and Goldemberg (5). The more favorable results obtained with glycero-phosphate may be attributed in part to the protective effect which is exerted on glycogen synthetase and many other proteins by polyols such as sucrose and glycero. The phosphate ester of glycero apparently retains this protective property, but our data show that it does not stimulate the glycogen synthetase reaction by the specific mechanism which is shared only by glucose-6-P and a few structurally related aldohexose phosphates.

With regard to the ionic composition of the reaction mixture, the importance of preventing interference from α-amylase by careful exclusion of chloride ion has already been mentioned. We have also avoided adding magnesium ion, although it has been reported to stimulate glycogen synthetase activity (7-10). Its effect on the assay of glycogen synthetase in liver homogenates, however, seems to be due chiefly to complex effects on several interfering reactions2 (see also Leloir and Goldemberg (5)).

FIG. 1. Effect of insulin administration for 48 hours upon blood glucose and liver glycogen concentration, glycogen synthetase activity, and the activities of several enzymes of the supernatant fraction in severely diabetic rats. The animals received intraperitoneal injections of 5.0 units of glucagon-free insulin and subcutaneous injections of 4 units of protamine zinc insulin (Lilly) at zero time. The rats to be killed at 48 hours were given another injection of 4 units of protamine zinc insulin subcutaneously at 24 hours. The animals were killed at the times indicated, and assays were carried out as described in “Experimental Procedure.” Each point represents the average of four determinations, and brackets indicate the standard error of the mean.

Effect of Insulin on Glycogen Synthetase Activity—Changes in glycogen synthetase activity were measured throughout the period of metabolic transition initiated by insulin administration to severely diabetic animals. The results of a typical experiment are presented in Fig. 1, in which changes in activity of hepatic glycogen synthetase and several other enzymes of carbohydrate metabolism were followed during 48 hours of insulin administration. As we had observed previously (1), glycogen was rapidly deposited at a rate corresponding to about 60 to 80 μmoles of glucose units per g of liver per hour. The glycogen concentration reached peak levels of 10 to 12% by about 12 hours. The activity of glycogen synthetase increased approximately 4-fold during the first 6 hours, but then declined rapidly so that by 24

lease of inorganic phosphate, which permits rapid phosphorolysis of labeled glucose from glycogen by glycogen phosphorylase.
little while glycogen synthetase rose rapidly to peak levels. During the first 6 hours after insulin administration showed that phosphate shunt responded more slowly, both showing a steeper rise during the period from 24 to 48 hours. Measurements made after this early period, significant glycogen deposition was found only when enzyme elevation was also observed. Moreover, the increment in enzyme activity after insulin was more than sufficient to account for the rate of glycogen deposition in vivo. Since increased levels of glucose-6-P do not occur in the liver of diabetic rats at this time after insulin administration (1, 2), it seems reasonable to relate the deposition of glycogen directly to the increased amounts of the synthetic enzyme.

Mechanism of Glycogen Synthetase Response—Considerable evidence is now available which indicates that the antibiotic puromycin is a specific and powerful inhibitor of protein synthesis (15, 16), and of induction by hormones of several enzymes in liver (17, 18). It exerts this action at the ribosome, where it combines with and detaches the growing polypeptide chain from its site of assembly (19). Several experiments with puromycin are summarized in Table I. When puromycin was injected 20 minutes after insulin, it significantly inhibited incorporation of leucine-1-14C into liver protein and completely inhibited the rise of glycogen synthetase after insulin injection. It did not significantly affect the control levels of enzyme in the short period of the experiments. Puromycin inhibited the small increment in glycogen concentration due to insulin in both trials, but in Experiment A it also lowered the level of glycogen in the control group. Puromycin, however, did not inhibit the hypoglycemic action of insulin.

Further evidence for the involvement of protein synthesis in the glycogen synthetase response was obtained from experiments with the amino acid analogue, ethionine (20). Ethionine has been shown to inhibit partially the increase of tyrosine transaminase in liver after cortisone administration (21). In the experiment presented in Table II, ethionine inhibited the synthesis of glycogen synthetase after insulin about 50%. The hypoglycemic response to insulin was not impaired in the ethionine-treated group, again pointing to the independence of these two effects of insulin. The mechanism of the inhibition of protein synthesis by ethionine is not known. Low liver ATP concentration in rats after ethionine injection has recently been reported (22), and ethionine may also interfere with many transmethylation reactions involving S-adenosylmethionine. Inhibition of protein synthesis can hardly be considered to be a specific effect of the antimetabolite.

Actinomycin D has been shown to be a highly specific inhibitor of DNA-dependent synthesis of cellular RNA by RNA polymerase (23–25). Experiments with this antibiotic provide indirect evidence for an obligate transcription process which may occur during the period after insulin administration before increased glycogen synthetase activity appears. The results of experiments with actinomycin D are presented in Table III. In Experiments A and B, actinomycin was administered to diabetic rats 45 minutes before insulin in amounts corresponding to doses 2. There was a definite delay of about 40 minutes preceding the response. Between 40 and 60 minutes, significant elevation of synthetase activity and glycogen deposition first appeared. No delay in hypoglycemia was observed with the relatively large dose of glucagon-free insulin given in these experiments. However, the hypoglycemia was always less marked in severely diabetic rats than in mildly diabetic or normal animals. Since the greatest enzyme increments were always observed in the most severely diabetic animals, there was a somewhat inverse correlation between the magnitude of the enzyme response and the hypoglycemic response to insulin. In all our measurements during this early period, significant glycogen deposition was found only when enzyme elevation was also observed. Moreover, the increment in enzyme activity after insulin was more than sufficient to account for the rate of glycogen deposition in vivo. Since increased levels of glucose-6-P do not occur in the liver of diabetic rats at this time after insulin administration (1, 2), it seems reasonable to relate the deposition of glycogen directly to the increased amounts of the synthetic enzyme.

Mechanism of Glycogen Synthetase Response—Considerable evidence is now available which indicates that the antibiotic puromycin is a specific and powerful inhibitor of protein synthesis (15, 16), and of induction by hormones of several enzymes in liver (17, 18). It exerts this action at the ribosome, where it combines with and detaches the growing polypeptide chain from its site of assembly (19). Several experiments with puromycin are summarized in Table I. When puromycin was injected 20 minutes after insulin, it significantly inhibited incorporation of leucine-1-14C into liver protein and completely inhibited the rise of glycogen synthetase after insulin injection. It did not significantly affect the control levels of enzyme in the short period of the experiments. Puromycin inhibited the small increment in glycogen concentration due to insulin in both trials, but in Experiment A it also lowered the level of glycogen in the control group. Puromycin, however, did not inhibit the hypoglycemic action of insulin.

Further evidence for the involvement of protein synthesis in the glycogen synthetase response was obtained from experiments with the amino acid analogue, ethionine (20). Ethionine has been shown to inhibit partially the increase of tyrosine transaminase in liver after cortisone administration (21). In the experiment presented in Table II, ethionine inhibited the synthesis of glycogen synthetase after insulin about 50%. The hypoglycemic response to insulin was not impaired in the ethionine-treated group, again pointing to the independence of these two effects of insulin. The mechanism of the inhibition of protein synthesis by ethionine is not known. Low liver ATP concentration in rats after ethionine injection has recently been reported (22), and ethionine may also interfere with many transmethylation reactions involving S-adenosylmethionine. Inhibition of protein synthesis can hardly be considered to be a specific effect of the antimetabolite.

Actinomycin D has been shown to be a highly specific inhibitor of DNA-dependent synthesis of cellular RNA by RNA polymerase (23–25). Experiments with this antibiotic provide indirect evidence for an obligate transcription process which may occur during the period after insulin administration before increased glycogen synthetase activity appears. The results of experiments with actinomycin D are presented in Table III. In Experiments A and B, actinomycin was administered to diabetic rats 45 minutes before insulin in amounts corresponding to doses
Diabetic rats were given intraperitoneal injections of 5.0 units of Novo insulin or 0.9% NaCl solution. After 20 minutes, the animals also were given puromycin (10 mg per 100 g, isotonic in NaCl) or an equivalent volume of 0.9% NaCl solution intraperitoneally. In Experiment A, the animals were killed 80 minutes after insulin injection. In Experiment B, all animals received 2.0 µc of leucine-1-¹⁴C intraperitoneally 10 minutes after puromycin injection and were killed 1 hour later, i.e. 90 minutes after insulin injection. Preparation of homogenates and assay conditions were as described in “Experimental Procedure.” To estimate radioactivity incorporated into liver protein, aliquots of homogenates were precipitated and washed with cold 5% trichloroacetic acid, extracted with ethanol and ether, transferred to weighed aluminum cups, dried, and counted in a windowless gas flow counter. Plasma aliquots were dried and counted similarly.

---

**Table I**

Effect of puromycin on response to insulin of hepatic glycogen synthetase activity, hepatic glycogen concentration, and blood glucose level of severely diabetic rats

Diabetic rats were given intraperitoneal injections of 5.0 units of Novo insulin or 0.9% NaCl solution. After 15 minutes, the animals also were given puromycin (dissolved in 4 ml of water and adjusted to pH 7.0) or an equivalent volume of 0.9% NaCl solution. The animals were killed 80 minutes after insulin injection. Preparation of liver homogenates and assay conditions were as described in “Experimental Procedure.” To estimate radioactivity incorporated into liver protein, aliquots of homogenates were precipitated and washed with cold 5% trichloroacetic acid, extracted with ethanol and ether, transferred to weighed aluminum cups, dried, and counted in a windowless gas flow counter. Plasma aliquots were dried and counted similarly.

<table>
<thead>
<tr>
<th>Injections</th>
<th>No. of animals</th>
<th>Glycogen synthetase activity</th>
<th>Liver glycogen</th>
<th>Blood glucose</th>
<th>Trichloroacetic acid-insoluble radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µmoles glucose/g liver/hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>−Glucose-6-P</td>
<td>+Glucose-6-P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>6.2 + 1.8*</td>
<td>90 + 5.3*</td>
<td>18.2 + 2.7*</td>
<td>607</td>
</tr>
<tr>
<td>Insulin only</td>
<td>2</td>
<td>54.8 + 9.2</td>
<td>146 + 15.0</td>
<td>22.1 + 0.8</td>
<td>308</td>
</tr>
<tr>
<td>Puromycin only</td>
<td>4</td>
<td>7.1 + 1.7</td>
<td>79 + 6.2</td>
<td>11.3 + 1.7</td>
<td>505</td>
</tr>
<tr>
<td>Insulin and puromycin</td>
<td>4</td>
<td>4.9 + 0.6</td>
<td>74 + 11.8</td>
<td>13.1 + 0.8</td>
<td>292</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>5.8 + 9.5</td>
<td>50 + 9.7</td>
<td>17.3 + 1.6</td>
<td>470</td>
</tr>
<tr>
<td>Insulin only</td>
<td>3</td>
<td>26.9 + 4.9</td>
<td>183 + 7.5</td>
<td>22.8 + 2.6</td>
<td>204</td>
</tr>
<tr>
<td>Puromycin only</td>
<td>3</td>
<td>6.2 + 0.9</td>
<td>59 + 9.8</td>
<td>19.4 + 4.7</td>
<td>475</td>
</tr>
<tr>
<td>Insulin and puromycin</td>
<td>3</td>
<td>3.6 + 0.7</td>
<td>57 + 8.0</td>
<td>19.4 + 2.1</td>
<td>274</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

---

**Table II**

Effect of DL-ethionine on response to insulin of hepatic glycogen synthetase activity, hepatic glycogen concentration, and blood glucose level of severely diabetic rats

Diabetic rats were given intraperitoneal injections of 5.0 units of Novo insulin or 0.9% NaCl solution. After 15 minutes, the animals also were given intraperitoneal injections of 100 µg of DL-ethionine (dissolved in 4 ml of water and adjusted to pH 7.0) or an equivalent volume of 0.9% NaCl solution. The animals were killed 20 minutes after insulin injection. Preparation of liver homogenates and assay conditions were as described in “Experimental Procedure.”

<table>
<thead>
<tr>
<th>Injections</th>
<th>No. of animals</th>
<th>Glycogen synthetase activity</th>
<th>Liver glycogen</th>
<th>Blood glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µmoles glucose/g liver/hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>−Glucose-6-P</td>
<td>+Glucose-6-P</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>4.6 + 0.1*</td>
<td>65 + 5.3*</td>
<td>23.7 + 5.0*</td>
</tr>
<tr>
<td>Insulin only</td>
<td>3</td>
<td>40.0 + 5.2</td>
<td>166 + 11.1</td>
<td>29.2 + 0.4</td>
</tr>
<tr>
<td>Ethionine only</td>
<td>3</td>
<td>4.4 + 0.8</td>
<td>59 + 4.1</td>
<td>14.8 + 4.5</td>
</tr>
<tr>
<td>Insulin and ethionine</td>
<td>3</td>
<td>16.6 + 2.3</td>
<td>91 + 4.7</td>
<td>18.0 + 2.2</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

---

reported to inhibit RNA synthesis and induced formation of enzymes in rat liver 5 hours after cortisone administration (28). The increase of glycogen synthetase was not inhibited completely by this procedure. In experiments C and D, the dose of actinomycin was increased and the antibiotic was injected 2 hours before the insulin. The rise of glycogen synthetase was now suppressed completely and the deposition of glycogen in the liver was inhibited. As with puromycin and ethionine, there was no inhibition of the hypoglycemic response to insulin. In fact, in experiments C and D, the hypoglycemic effect of insulin was enhanced in the actinomycin-treated animals. In Experiment C, incorporation of radioactivity into total liver protein after injection of leucine-1-¹⁴C was not significantly depressed by actinomycin. In the experiments reported by Greengard, Smith, and Acs (18, 26), a dose of actinomycin of 70 µg per 100 g of body weight inhibited enzyme induction and RNA synthesis in liver but likewise did not affect incorporation of radioactivity into liver proteins. Higher doses of actinomycin, as were required to completely suppress the synthetase response in our experiments, have been shown to inhibit the synthesis of all RNA in rat liver (27). Significant depression of protein synthesis then results within 6 to 12 hours after actinomycin administration (28). The results of our experiments with puromycin, ethionine, and actinomycin suggest that both RNA and protein synthesis are integral parts of the glycogen synthetase response.

Effect of Hypophysectomy on Glycogen Synthetase Response—When insulin was administered to hypophysectomized, severely diabetic rats, an increase of glycogen synthetase was obtained which was equal in magnitude to the increase obtained in equivalent nonhypophysectomized diabetic rats. These experiments
DISCUSSION

All the evidence presented here suggests that the increase in glycogen synthetase activity in liver after administration of insulin to alloxan-diabetic rats is due to accelerated synthesis of the enzyme protein. The complete inhibition of the increase in glycogen synthetase by specific inhibitors of protein or RNA synthesis, such as puromycin and actinomycin D, provides strong evidence to support this conclusion. Alternative mechanisms could be suggested, such as the conversion of an inactive precursor of glycogen synthetase to an active form, or some kind of modification of the existing enzyme that results in a marked increase in its catalytic power. The evidence obtained thus far, however, does not support either of these interpretations.

The data in Tables I and III regarding the incorporation of leucine-1-14C into the total protein fraction of liver 90 and 120 minutes after insulin administration do not show a stimulation of protein synthesis. Such a result is not unexpected, since the effect of insulin is presumably highly selective, involving only a tiny fraction of the total enzyme complement of the liver.

These results cannot be readily related to the findings of Villar-Palasi and Larner (29), who have observed activation of glycogen synthetase in rat diaphragm after exposure to insulin. In their experiments, only the fraction of synthetase activity measured without glucose-6-P added to the assay mixture increased after insulin treatment. Friedman and Larner (30) have presented evidence that this change is the result of interconversion of two forms of glycogen synthetase in muscle by reversible phosphorylation of the enzyme protein. Such an activation process, however, would not account for the large increases in activity measured with glucose-6-P added to the assay mixture which occur in the liver after insulin administration. We have observed that hepatic glycogen synthetase of several species differs greatly in thermal stability from muscle synthetase, even after extensive purification, and it is probable that these two enzymes differ chemically. Accordingly, observations on the one tissue should not be extended to the other without experimental validation.

The effect of insulin upon glycogen synthesis is the earliest
Although conclusive evidence that insulin can enter the cells of the liver has been shown in Fig. 1 implies that the information-bearing RNA for glycogen synthetase in liver slices in vitro is rapidly degraded by enzymes present there (32). Our previous experiments and these reported here appear to rule out the participation of other known hormones in the glycogen synthetase response. However, our attempts to produce a response with peritoneal injections of 2.0 ml of 5% glucose solution only, and the other control group received 2.0 ml of 5% glucose solution containing 0.5 unit of Novo insulin. One control group received the 2.0 ml of 5% glucose solution only, and the other control group received 1.0 ml of 0.9% NaCl solution. The animals were killed at the times indicated. Preparation of homogenates and assay conditions were as described in "Experimental Procedure."

<table>
<thead>
<tr>
<th>Injections</th>
<th>Time of death</th>
<th>No. of animals</th>
<th>Glycogen synthetase activity</th>
<th>Blood glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td></td>
<td>-Glucose-6-P</td>
<td>+Glucose-6-P</td>
</tr>
<tr>
<td>Experiment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
<td>4</td>
<td>3.3 ± 0.4*</td>
<td>84 ± 12*</td>
</tr>
<tr>
<td>Glucose and insulin</td>
<td>100</td>
<td>4</td>
<td>23.0 ± 7.1</td>
<td>165 ± 12</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>60</td>
<td>3</td>
<td>6.0 ± 0.6</td>
<td>88 ± 16</td>
</tr>
<tr>
<td>Glucose</td>
<td>60</td>
<td>4</td>
<td>8.6 ± 2.3</td>
<td>74 ± 4.0</td>
</tr>
<tr>
<td>Glucose and insulin</td>
<td>60</td>
<td>4</td>
<td>20.5 ± 6.0</td>
<td>144 ± 20</td>
</tr>
<tr>
<td>Glucose and insulin</td>
<td>120</td>
<td>4</td>
<td>49.8 ± 14</td>
<td>214 ± 28</td>
</tr>
</tbody>
</table>

* Standard error of the mean

The effect of the hormone detected thus far in the liver (1, 2, 31). Although conclusive evidence that insulin can enter the cells of the liver is lacking, it seems likely that it may do so, since it is rapidly degraded by enzymes present there (32). Our previous experiments and these reported here appear to rule out the participation of other known hormones in the glycogen synthetase response. However, our attempts to produce a response with liver slices in vitro have failed. We believe that the failure of experiments with slices only indicates the technical inadequacies of the slice procedure (cf. Goldstein, Stellas, and Knox (33)).

These results indicate that the rate of synthesis of glycogen synthetase in liver is subject to regulation by a mechanism which can be affected in some manner by insulin. Since the acceleration of enzyme synthesis by insulin is inhibited by actinomycin D, it is likely that the formation of information-bearing or "messenger" RNA is required. The synthesis of this fraction of cellular RNA has been shown to be highly susceptible to inhibition by actinomycin D (27). The relative rapidity of the increase and subsequent decay of glycogen synthetase activity shown in Fig. 1 implies that the information-bearing RNA for this protein may have a relatively short half-life as does bacterial messenger RNA (34). A reasonable mechanism for the acceleration of synthesis of glycogen synthetase after insulin can be postulated in which the structural gene for the enzyme protein directs the synthesis of increased amounts of messenger RNA. Such a mechanism would imply that glycogen synthetase activity is normally regulated at the level of expression of its structural gene. In terms of the hypothesis of Jacob and Monod (35), the role of insulin here would be analogous to the role of an inducer in bacterial systems.

If insulin does act directly as an inducer, however, it cannot be the only substance which affects the level of glycogen synthetase, for even with the marked deficiency of insulin which is present in severe alloxan diabetes (36), the activity of hepatic glycogen synthetase is actually elevated slightly (2). This increased amount of enzyme might be interpreted as an indication of the accumulation in the liver in severe diabetes of a metabolite which can stimulate enzyme formation, i.e. an inducer substance, or conversely as an indication of the relative lack of some metabolite which can repress enzyme synthesis, i.e. a repressor substance. The effect of insulin on enzyme synthesis could then be explained in terms of an effect upon the concentration of this unknown inducer or repressor, thus indirectly producing a temporary increase in the rate of enzyme synthesis. On the other hand, insulin could as well act to augment or supplement the effect of this metabolite directly at the specific site where the synthesis of the appropriate messenger RNA is initiated.

It is clear that the extent to which insulin can affect enzyme synthesis is related to the degree to which the organism has been deprived of the hormone. Our data indicate that the increased synthesis of enzyme after insulin is greatest in the most severely diabetic rats, and is less marked in animals with milder diabetes. Preliminary experiments with normal rats fasted for 20 hours indicate that, in this instance, enzyme synthesis contributes very little to the deposition of glycogen when glucose is administered.

Glucokinase and the dehydrogenases of the hexose monophosphate shunt in the liver supernatant fraction increase much more slowly than does glycogen synthetase after insulin (see Fig. 1). These changes are also inhibited by puromycin, and are probably also the result of induced enzyme synthesis (14). The singular characteristics of the time course of response of glycogen synthetase, glucokinase, and the two dehydrogenases seem to rule out the possibility that all these enzymes could be assigned to a single unit of genetic expression or "operon" (35). Moreover, it seems likely that the levels of these more slowly responding enzymes are controlled by changes in the concentration of certain key metabolites, which serve as inducers or repressors.

The various metabolic adaptations which occur in the liver after insulin administration to diabetic animals, or after refeeding fasted normal animals, seem to be conditioned by the severity of the state of diabetes or deprivation of food to which the organism has been subjected (1, 13, 14). Insulin deprivation is clearly an essential feature of both of these states. The enormous quantities of food consumed by the severely diabetic animal will not serve to initiate or sustain metabolic adaptations in the liver unless insulin is also present. Our findings with respect to glycogen synthetase suggest that in the liver the crucial importance of insulin may lie in an ability to affect the rates of synthesis of one or more key enzymes and thus to initiate changes in the concentrations of certain important metabolites within the hepatic cells. The relative amounts of these critical metabolites may in turn influence the over-all pattern of hepatic metabolism through such diverse mechanisms as induction and repression, mass action, and feedback inhibition. Such a close interplay of insulin and metabolites upon regulatory mechanisms could explain why...
a source of nutrition is required with the hormone for full expression of its effects.\(^4\)

**SUMMARY**

The administration of insulin to alloxan-diabetic rats resulted in a several fold increase in hepatic glycogen synthetase activity and rapid deposition of glycogen during the first 6 hours of treatment. Enzyme activity subsequently declined, but glycogen continued to rise to peak levels of 10 to 12% at about 12 hours. By 24 hours, glycogen synthetase activity had returned essentially to control levels. The activities of glucokinase and the dehydrogenases of the hexose monophosphate shunt in liver also increased after insulin, but followed slower and distinctly different courses with peak levels at 48 hours or later.

Experiments are presented which suggest that this increase in glycogen synthetase activity is due to an increase in the amount of the enzyme protein. The increase of synthetase after insulin administration was delayed for about 40 minutes, during which time it could be inhibited completely by puromycin and partially by ethionine. Actinomycin D also inhibited the increase of glycogen synthetase in liver, while we have not done this. However, our data on the time course of the increase in glucokinase activity in alloxan-diabetic rats is in essential agreement with their findings, especially with respect to the important point that very little increase in glucokinase activity occurs in diabetic liver until at least 6 hours have elapsed after insulin administration. The induced synthesis of glycogen synthetase by the insulin response to insulin thus distinctly precede any synthesis of glucokinase. The small decrease in hepatic glucose-6-P concentration which we have found early after insulin administration is consistent with these observations, in that the increase of glucokinase would not significantly supplement the cellular glucose-6-P pool until about 6 to 12 hours after insulin was first given.

**REFERENCES**


4 Note Added in Proof—Since this paper was submitted for publication, a report by Salas, Víñuela, and Sols has appeared (37) concerning the insulin-dependent synthesis of hepatic glucokinase in fasted normal and alloxan-diabetic rats. These workers have corrected their measurements of glucokinase activity by subtracting glucose phosphorylation due to the static low K\(_s\) hexokinase of liver, while we have not done this. However, our data on the time course of the increase in glucokinase activity in alloxan-diabetic rats are in essential agreement with their findings, especially with respect to the important point that very little increase in glucokinase activity occurs in diabetic liver until at least 6 hours have elapsed after insulin administration. The induced synthesis of glycogen synthetase by the insulin response to insulin thus distinctly precede any synthesis of glucokinase. The small decrease in hepatic glucose-6-P concentration which we have found early after insulin administration (1) is consistent with these observations, in that the increase of glucokinase would not significantly supplement the cellular glucose-6-P pool until about 6 to 12 hours after insulin was first given.


Induced Synthesis of Hepatic Uridine Diphosphate Glucose-Glycogen Glucosyltransferase after Administration of Insulin to Alloxan-diabetic Rats
Donald F. Steiner and Judith King


Access the most updated version of this article at http://www.jbc.org/content/239/5/1292.citation

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/239/5/1292.citation.full.html#ref-list-1