A Dual Role for Insulin in the Regulation of Cardiac Glycogen Synthase*

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Thomas B. Miller, Jr.
From the Department of Biochemistry, University of Massachusetts Medical School, Worcester, Massachusetts 01605

The effects of insulin on enzymes involved in regulation of glycogen synthesis were investigated in the perfused rat heart. In hearts from normal fed rats, perfusion with insulin (10^{-8} M) for 5, 10, and 15 min resulted in a 100% increase in the activity of the I (glucose 6-phosphate-independent) form of glycogen synthase. Glycogen synthase activity was completely unresponsive to insulin when hearts from fed alloxan-diabetic rats were perfused under exactly the same conditions. The effect of insulin to activate glycogen synthase in hearts from normal rats was apparent using either glucose (5 mM) or palmitate (0.5 mM) as substrates, showing synthase activation to be independent of glucose transport. While the acute effect of insulin was associated with a 50% increase in total glycogen after 15 and 30 min in normal hearts, neither insulin nor diabetes produced any apparent changes in the activities of protein kinase or glycogen phosphorylase or tissue concentrations of adenosine 3':5'-monophosphate (cyclic AMP).

The impairment of synthase activation in diabetic hearts was coincident with a 60 to 70% decrease in total synthase phosphatase activity. The acute effect of insulin to activate synthase in perfused diabetic hearts could be restored by treatment of diabetic rats, in vivo, with 4 units of insulin 1 to 6 hr prior to heart perfusion. This restoration of the synthase activation was accompanied by restoration of total synthase phosphatase activity. Restoration of both synthase activation and total phosphatase activity by treatment, in vivo, of diabetic rats with insulin could be blocked by simultaneous treatment with 10 mg of cycloheximide. Although perfusion of hearts with insulin was not found to alter synthase phosphatase activity, these data suggest that the regulation of synthase phosphatase activity may be involved in insulin-mediated glycogen synthase activation. Further, these data demonstrate a dual role for insulin in regulation of cardiac glycogen synthase. One effect involves protein synthesis and maintenance of the enzymatic machinery responsible for acute regulation of glycogen synthase activity. The other effect is a rapid effector activation of glycogen synthase through an as yet undetermined mechanism.

An effect of insulin to promote the activation of glycogen synthase (conversion of synthase from a glucose 6-phosphate-dependent to a glucose 6-phosphate-independent form) in the heart has been well documented in vivo (1–4) along with a single report in vitro (6) although the actual mechanism of this effect remains undetermined. Chain et al. (6) demonstrated a deficiency in incorporation of [14C]glucose into glycogen in isolated perfused hearts from either streptozotocin or anti-insulin serum-treated rats, suggesting a diabetes-related defect in the pathway of cardiac glycogen synthesis. Such a defect might easily be explained by the effect of diabetes to inhibit both glucose transport and phosphorylation (7). A report by Das (8) showing a decreased percentage of glycogen synthase I in hearts from diabetic rats suggested that the decrease might be due to a diabetes-related deficiency in glycogen synthase phosphatase. Recently, Nuttall et al. (4) demonstrated that the in vivo administration of insulin to fed rats, which resulted in a rapid (5 min) increase in cardiac glycogen synthase activity, was ineffective as an activator of synthase in diabetic rats. In the same report, it was shown that synthase phosphatase activity in the diabetic rat was less than half of that found in hearts of normal fed rats and that when insulin was given 30 min before the diabetic rats were killed, synthase phosphatase activities returned to normal. Although this study (4) was carefully controlled, interpretation of the data obtained using the in vivo technique is extremely difficult due to system and hormone interactions which control minute-to-minute metabolism in the whole animal.

In the present study, we have determined the effect of insulin and alloxan diabetes on enzymes involved in the regulation of glycogen metabolism using the isolated perfused rat heart. The purpose of this study was to determine in an in vitro system if insulin activates glycogen synthase in hearts from diabetic rats, and if not, to determine the cause of the defect in synthase activation. Although insulin does directly activate synthase in hearts from normal rats, and if not, to determine the cause of the defect in synthase activation. Although insulin does directly activate synthase in hearts from normal rats, and if not, to determine the cause of the defect in synthase activation. Although insulin does directly activate synthase in hearts from normal rats, and if not, to determine the cause of the defect in synthase activation.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain (Charles River Breeding Laboratories) weighing 200 to 250 g were maintained on Purina laboratory chow and water ad libitum. Diabetes was induced after an overnight fast by injection of alloxan (60 mg/kg) into the tail vein and diagnosed 2 days later by a maximum positive urine glucose test using Lilly Tes-Tape. Diabetic animals were used 3 to 4 days after alloxan injection. Heart perfusion of the Langendorff type was carried out on an apparatus essentially the same as described by Morgan et al. (9). The perfusion medium was Krebs-Henseleit bicarbonate buffer.
containing 2.5 mM calcium and 0.5 mM dithiothreitol/EDTA and was gassed with O₂:CO (95%:5%). After removal from decapitated rats, hearts were immediately chilled in ice cold buffer containing heparin (1 unit/ml) and then mounted on the aortic cannula. Gravity flow perfusion at 37°C from a reservoir 60 cm above the heart was immediately begun and allowed to continue for 5 min. Then the perfusions were aliquoted into 20 µl of the above perfusion buffer and a constant aortic pressure of 60 mm Hg was maintained using a roller pump. After 30 min of recirculation perfusion using the appropriate substrate, saline (0.9% NaCl solution) or insulin (final concentration of 10⁻⁸ M) was introduced into the recirculation reservoir and perfusion was continued for 5, 10, 15, or 30 min as indicated. Perfusions were terminated at the times indicated by freeing the hearts between Wollenberger clamps cooled in liquid nitrogen. Frozen hearts were prepared for analyses by pulverization in a percussion instrument at liquid nitrogen temperature and stored at -70°C until the analyses were performed (10).

Tissue glycogen determinations were carried out on frozen heart powder as previously described (11) and data are expressed as micromoles of glucose/g of heart. Tissue adenosine 3′:5′-monophosphate synthesis was performed on frozen heart powder according to Gilman (12) and data are reported as picomoles/mg of heart. Glycogen synthase activity was extracted with 100 mM KF, 10 mM EDTA, pH 7.8 from frozen heart powder (100 mg of tissue/ml) at two-thirds speed using a Polytron homogenizer. After centrifugation at 8000 x g for 10 min at 2°C, supernatant synthase activity was determined using the [γ-32P]ATP histone phosphorylation system. Enzyme data is expressed as micromoles of UDP-Glc incorporated into glycogen/min/g of heart. For protein kinase analyses, frozen heart powder was homogenized with 150 mM KF, 5 mM potassium phosphate, and 2 mM EDTA (100 mg of tissue/4 ml), pH 6.8, using the Polytron as described above. After centrifugation in the cold at 8000 x g for 10 min, aliquots of the supernatant were assayed in the absence and presence of added cyclic AMP for protein kinase activity using the [γ-32P]ATP histone phosphorylation system. Phosphorylation data are expressed as nanomoles of [γ-32P]ATP incorporated into histone/min/g of heart.

For protein kinase analyses, frozen heart powder was homogenized with 150 mM KF, 5 mM potassium phosphate, and 2 mM EDTA (100 mg of tissue/4 ml), pH 6.8, using a Polytron homogenizer. After centrifugation at 8000 x g for 10 min at 2°C, supernatant fractions were placed in an ice bath. For the zero time phosphatase value, synthase activity was determined on 30-µl aliquots of the supernatant fractions. Then, tubes containing 200 µl of the supernatant fractions were transferred to a 30°C water bath, incubated for 10 min, and then the incubation was stopped by transferring 30-µl aliquots directly into the synthase assay mix for synthase activity determination. Synthase activity at zero time was subtracted from synthase activity after the 10-min incubation to determine synthase D to I conversion with respect to time. Under these conditions, total synthase activity was unaffected by the incubation and synthase D to I conversion was linear with respect to time for at least 10 min. Phosphatase activity is expressed as nanomoles of glucose incorporated into glycogen from uridine diphosphoglucose/min/g of heart.

Palmitic acid was formed into a complex with albumin by dissolving 2 mmol of the acid in 3 ml of hot ethanol which was then added to 100 ml of 50 mM K₂CO₃ at 50°C. After boiling for 10 min to drive off the ethanol, the fatty acid solution was added to 100 ml of a 24% solution of albumin at 50°C. After dialysis, the fatty acid albumin complex was diluted in the perfusion buffer to the desired concentration.

γ-labeled [32P]ATP was prepared from carrier-free [32P]P phosphorite as described by Schultz et al. (17). All radioisotopes and the decapitated rat were produced by Lilly and that used for perfusion (monocomponent insulin) was a gift from NOVO Research Institute. Fraction V albumin (fatty acid poor) was purchased from Miles Laboratories. New England Nuclear and counted in a model 3330 Packard Tri Carb Liquid Scintillation Counter. Type Ia calf thymus histone, alloxan, palmitic acid, and rabbit liver glycogen were purchased from Sigma Chemical Co. Insulin used for treatment of animals was produced by Lilly and that used for perfusion (monocomponent insulin) was a gift from NOVO Research Institute. Fraction V albumin (fatty acid poor) was purchased from Miles Laboratories.

All data are expressed as mean ± standard error of the mean (S.E.) and statistical significance determined by Student's t test. Bars on either side of the mean represent 1.3 E.

RESULTS

The first set of experiments was run to determine the effects of alloxan diabetes on the activation of cardiac glycogen synthase by insulin. Using 5 mM glucose as substrate, hearts from normal and diabetic rats were perfused for the initial 5-min gravity flow and 30-min recirculation period as described under "Materials and Methods." Then, saline or insulin (at a final concentration of 10⁻⁸ M) was added to the recirculating medium and perfusion continued for a further 5, 10, 15, or 30 min prior to the termination of the experiments. As can be seen in Fig. 1, perfusion of hearts from normal rats with insulin for 5, 10, or 15 min resulted in a doubling of synthase I activity, whereas the effect was no longer present after 30 min, in complete agreement with an earlier report by Adolfsson et al. (5). In contrast, hearts from diabetic rats were not responsive to this acute effect of perfusion with insulin at any of the times tested. While the percentage of synthase I was found to be lower in diabetic than in normal hearts as previously reported (4, 5), total glycogen synthase activity (measured in the presence of Gc-6-P) were the same in diabetic as in normal hearts (data not shown). Therefore, the data in Fig. 1 demonstrated a defect in the direct activation of glycogen synthase by insulin in hearts from diabetic rats.

Fig. 2 shows the effects of perfusion of hearts with insulin for 15 min on the tissue activities of glycogen phosphorylase and protein kinase and concentrations of cyclic AMP and glycogen. Neither total phosphorylase activities nor phosphorylase a levels were altered by the diabetic state or acute insulin exposure. Although the data are not shown, activities were the same in normal and diabetic hearts perfused for 5, 10, or 30 min without and with insulin. Again, the data for normal hearts are in agreement with those reported earlier (5). While there was a slight decrease in total protein kinase activity measured in hearts from diabetic rats (Fig. 2), perfusion with insulin had no effect on the cyclic AMP-independent
form of the kinase in hearts from normal or diabetic rats. Again, although not shown, the data on protein kinase remained the same for normal and diabetic hearts perfused for 5, 10, and 30 min plus or minus insulin. Tissue cyclic AMP concentrations were essentially unchanged by diabetes or insulin perfusion (Fig. 2) and although the data are not shown, the length of perfusion with and without insulin had no effect. Although total glycogen levels in hearts from normal rats were not increased statistically by insulin at either 5 or 10 min, perfusion with insulin for 15 min (Fig. 2) and 30 min resulted in 50 and 52% increases in tissue glycogen, respectively. Except for the glycogen changes which occurred in the normal hearts in response to 15- or 30-min insulin exposure, Fig. 2 could be essentially duplicated for the 5-, 10-, and 30-min perfusions without or with insulin. These data show that neither the diabetic state nor acute insulin has any appreciable effect on phosphorylase, cyclic AMP-dependent protein kinase, or cyclic AMP.

The next group of hearts were perfused in order to determine if insulin-mediated synthase activation was dependent on glucose transport and whether the defect in insulin activation of synthase in diabetic hearts could be due to the well known defect in insulin stimulation of glucose uptake (7). Heart perfusions were the same as described for Figs. 1 and 2 except that perfusion with insulin lasted for only 10 min and fatty acid replaced glucose (0.5 mM palmitate bound to 0.6% albumin) as the substrate for the entire perfusion. Fig. 3 shows that replacement of glucose with fatty acid had no effect on the acute activation of synthase by insulin in normal hearts. Again, synthase activity in the diabetic hearts was unchanged by the 10-min exposure to insulin. Since no glucose was present during the total 45 min of perfusion, these data clearly demonstrated that the acute effect of insulin to activate synthase in normal hearts and the defect in this insulin action in diabetic hearts were both independent of glucose transport and phosphorylation.

Also shown in Fig. 3 are the data obtained on the same hearts for phosphorylase, protein kinase, and glycogen. In agreement with Fig. 2, neither phosphorylase nor protein kinase activities were affected by acute insulin perfusion or the diabetic state. Insulin perfusion did not result in increased glycogen levels in normal or diabetic hearts as was expected in the absence of glucose in the normal and in the absence of an insulin response in the diabetic. Levels of tissue glycogen in hearts from diabetic rats were 2- to 3-fold higher than those in hearts from normal rats, a well known phenomenon (18).

Since alloxan diabetes resulted in a defect in acute (5 to 30 min) activation of glycogen synthase by insulin in the perfused hearts, the next group of experiments was designed to determine if normal regulation of synthase could be restored by treating diabetic rats with insulin, in vivo. Alloxan-diabetic rats were injected intramuscularly with 4 units of insulin (2 units of regular + 2 units of Lente) 1, 2, 3, 5, and 6 h prior to heart perfusion. Perfusions were carried out with 5 mM glucose as substrate as described under “Materials and Methods” in

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**Fig. 2.** Effect of insulin on phosphorylase, protein kinase, cyclic AMP, and glycogen. The procedures and techniques used for heart perfusion and the analyses were the same as described under “Materials and Methods” and in the text. Adjoining bars represent phosphorylase activities measured in the absence and presence, respectively, of added 5'-AMP or protein kinase activities assayed in the absence and presence, respectively, of added cyclic AMP. Plus INS represents hearts perfused with insulin for 15 min. Percentage figures over the bars depict phosphorylase a or cyclic AMP-independent protein kinase as percentage of total activities. Open bars represent data obtained from normal hearts while crosshatched bars represent data from diabetic hearts. Each mean represents data for 8 to 12 hearts. *p < 0.01 versus normal control.

**Fig. 3.** Insulin action using fatty acid as substrate. The procedures and techniques for heart perfusion and analyses were described under “Materials and Methods” and in the text. Adjoining bars in the Synthase panel represent activity assayed in the absence and presence of added Glc-6-P, respectively, and percentage figures depict the synthase I data as a percentage of total activity. All other data and symbols are the same as described for Fig. 2. Each bar represents the mean of data for 12 hearts. *p < 0.01 versus normal control.
absence or presence of $10^{-8}$ M insulin for the final 10 min. It can be seen that treatment of the whole animal with insulin for as short a time as 1 h resulted in restoration of the acute effect of insulin to activate glycogen synthase in the perfused heart (Fig. 4). Since perfusion with insulin for less than 15 min did not result in statistical increases in heart glycogen as stated earlier, data on the same hearts shown in Fig. 4 perfused without and with insulin were pooled. The mean values for glycogen in micromoles of glucose/gram of heart for 0, 1, 2, 3, 5, and 6 h of insulin treatment were $15.0 \pm 1.4, 16.0 \pm 1.7, 17.3 \pm 2.1, 17.2 \pm 0.7, 14.0 \pm 1.5$, and $13.1 \pm 1.4$, respectively. Therefore, the acute effect of insulin to activate glycogen synthase could not be correlated with glycogen concentrations but was dependent on the maintenance of a regulatory system which required longer periods of insulin exposure.

The final series of experiments was designed to determine if the in vivo restoration of synthase activation by insulin could be altered by blocking protein synthesis, and if glycogen synthase phosphatase activity might be involved. Where indicated, rats were injected with 4 units of insulin (intramuscularly) or 10 mg of cycloheximide (intraperitoneally), or both, 2 h prior to heart perfusions. Perfusions were carried out as described under "Materials and Methods," using 5 mM glucose as substrate in the absence or presence of $10^{-8}$ M insulin for the final 10 min. Fig. 5 shows the results of that study. Perfusion of hearts from normal rats with insulin resulted in activation of synthase to $36\%$ from a control level of $28\%$. Hearts from diabetic rats did not respond to perfusion with insulin unless the rats were treated with insulin, in vivo, for 2 h prior to perfusion. While treatment of normal rats with cycloheximide had no effect on insulin activation of synthase in these perfused hearts, simultaneous treatment of diabetic rats with cycloheximide and insulin abolished the in vivo restoration by insulin. When glycogen synthase phosphatase activities were measured in these same hearts, diabetes decreased the activity by $60\%$; in vivo treatment of diabetic rats with insulin restored the activities to the normal level; cycloheximide prevented insulin restoration of the phosphatase; and cycloheximide alone in the normal rats had no effect. Mean glycogen concentrations in untreated normal and diabetic hearts were $9.0 \pm 0.7$ and $15.0 \pm 1.4$ micromoles of glucose/gram of heart, respectively. Treatment of animals with either insulin or cycloheximide, or both, under these conditions had no significant effect on cardiac glycogen levels. Therefore, restoration of the acute regulation of synthase by insulin in the diabetic appears to be independent of the glycogen concentrations and probably involves protein synthesis. These data are consistent with the possible involvement of the synthase

![Figure 4](http://www.jbc.org/)

**Fig. 4.** In vivo restoration of in vitro synthase activation. The procedures and techniques for heart perfusion and analyses were described under "Materials and Methods" and in the text. Adjoining bars represent percentage of synthase I when hearts were perfused without (−) or with (+) insulin for the final 10 min. Times under bars represent hours of insulin treatment, in vivo, prior to heart perfusion. Each bar represents the mean of data obtained for six hearts. *$p < 0.01$ versus proper control perfused without insulin.

![Figure 5](http://www.jbc.org/)

**Fig. 5.** Effect of insulin and cycloheximide on synthase and synthase phosphatase. The procedures and techniques for heart perfusion and analyses were described under "Materials and Methods" and in the text. Normal and diabetic rats were treated with insulin or cycloheximide, or both, 2 h prior to heart perfusion as described in the text. In the Synthase panel, percentages above the bars depict percentage of synthase I while adjoining bars represent synthase activity assayed minus and plus added Glc-6-P. Perfusion with insulin for the final 10 min is indicated by INS below adjoining bars. In vivo treatment of rats is indicated under adjoining bars (N, normal; D, diabetic; DI, diabetic treated with insulin; DIC, diabetic treated with insulin and cycloheximide; NC, normal treated with cycloheximide). Each bar represents the mean of data obtained for 8 to 12 rats. *$p < 0.01$ versus proper control perfused without insulin for the Synthase panel or versus normal for the Synthase Phosphatase panel.
phosphatase activity as an important factor in the acute regulation of glycogen synthase by insulin.

**DISCUSSION**

Adolfsen et al. (5) were the first to demonstrate an acute and direct effect of insulin to activate glycogen synthase in the isolated perfused rat heart preparation. This effect of insulin was associated with an increase in glucose 6-phosphate, total glycogen, and ["H]glucose incorporation into glycogen although the mechanism of activation was unexplained. Recently, Nuttall et al. (4) confirmed earlier observations (1-9) showing that insulin administration to whole rats resulted in a rapid activation of cardiac glycogen synthase and extended those studies to show that insulin administration to diabetic rats for up to 15 min did not result in cardiac synthase activation. They suggested that insulin was acting in fasted rats after 15 min by producing a stable modification of the synthase phosphatase or synthase D. While a 30-min treatment of diabetic rats with insulin was shown to normalize synthase D phosphatase activity (4), their report did not indicate that this treatment resulted in a statistically significant increase in synthase I activity or whether the effect could be blocked by an inhibitor of protein synthesis.

In the present report, the acute action of insulin on the enzymes controlling glycogen synthase has been studied using isolated perfused hearts from normal and alloxan-diabetic rats. This in vitro system offers the advantages of being hormonally responsive and free from the hormonal interactions which must always be considered when interpreting whole animal data. This study agrees with and extends the earlier work of Adolfsson et al. (5) showing that perfusion of hearts from normal fed rats with insulin for 5, 10 and 15 min resulted in glycogen synthase activation. Since that study (5) was carried out using an extremely high concentration of glucose (14 mM) in the perfusion medium, it could be argued that the effect was due to the direct effect of glucose or glucose 6-phosphate on activation of synthase (19-23) secondary to the insulin-mediated increase in glucose uptake. The present study shows this not to be the case since we demonstrated that insulin effectively activated synthase in perfused hearts from normal rats using physiological concentrations of either glucose (5 mM) or palmitate (0.5 mM). Therefore, this acute effect of insulin is direct and independent of glucose or glucose transport.

Perfusion of hearts from diabetic rats with insulin from 5 to 30 min did not result in glycogen synthase activation. These results are in agreement with those obtained in vivo by Nuttall et al. (4) but extend their observation by showing that the diabetes-related defect is in the heart muscle itself rather than being due to hormonal or systems interactions, in vivo. Therefore, the present study clearly demonstrate that insulin regulation of cardiac glycogen synthase is defective in hearts from alloxan-diabetic rats.

The present report also included a study of cyclic AMP, protein kinase, synthase phosphatase, and phosphorylase to determine if one or all of these might be involved in either the mechanism of insulin action or the site of the diabetes-related defect in synthase activation. Tissue cyclic AMP concentrations were apparently unaltered by either insulin or diabetes. Therefore, to hypothesize that alterations in tissue concentrations of cyclic AMP were involved in the mechanism of insulin action or the diabetes-related defect, one would have to invoke a cyclic AMP pool theory which is impossible to prove using current technical procedures. Although occasional decreases were observed in total protein kinase activities in diabetic hearts (Fig. 2), we did not observe any change in kinase activity assayed in the absence of added cyclic AMP. It should be pointed out, however, that the assay used in the present study determined histone phosphorylation rather than synthase inactivation as an end point. Several previous studies have suggested that at least one of the actions of insulin might involve decreases in synthase kinase activity (24-27). Perhaps the effects of insulin or diabetes on kinase activities might have proved different if a synthase inactivation assay had been used. Further, the recent work of several groups (28-32) have led to the speculation that insulin might be working through a cyclic AMP-independent protein kinase. Obviously, only further study can answer these questions.

The acute effect of insulin to activate glycogen synthase in perfused hearts from diabetic rats could be restored if the rats were injected with insulin from 1 to 6 h prior to perfusion. Shorter times were not studied. The acute effect of insulin to activate synthase exhibited a near perfect correlation with total synthase phosphatase activity. While diabetes resulted in a 60% decrease in total phosphatase (Fig. 5), in vivo treatment of diabetic rats with insulin restored both phosphatase activity and synthase activation. That the effect somehow involved protein synthesis was apparent since simultaneous treatment with insulin and cycloheximide, in vivo, blocked restoration of phosphatase activity and acute synthase activation. Another important consideration was that cycloheximide treatment of normal rats did not alter the synthase activation capability or phosphatase activity. These results indicate that one of the roles of insulin is to control the level of synthase phosphatase activity.

Since the correlation between total phosphatase activity and acute synthase activation was in such close agreement, it seemed appropriate to speculate that the acute effect of insulin to activate synthase might also involve some change in the phosphatase. However, we were unable to measure an acute change in phosphatase activity in normal hearts perfused with insulin for 5, 10, 15, or 30 min. Although Nuttall et al. (4) were able to show synthase changes in response to insulin administration to the whole animal as early as 5 min, the earliest effect that they reported on phosphatase was at 15 min in normal fasted rats and 30 min in diabetic rats. They were unable to show any effect of insulin on phosphatase in normal fasted rats. Although the data were not shown, from our data, we saw no changes in total synthase phosphatase activity when hearts from diabetic rats were perfused with insulin for as long as 30 min. Therefore, it would seem at least possible that the effect of insulin which they observed on the phosphatase (4) might involve some final phase of protein synthesis rather than a stable phosphatase modification. Although the time (15 to 30 min) is admittedly short for an effect on protein synthesis, the question can only be answered by injecting a protein synthesis inhibitor along with or just prior to insulin. If insulin is acting through covalent modification of a phosphatase, it must be a specific synthase phosphatase separate from phosphorylase phosphatase since the present report along with others (4, 5) shows synthase activation without phosphorylase inactivation. Also, an insulin-mediated alteration in the synthase D molecule itself, making it a better substrate for the phosphatase-catalyzed reaction, has not yet been ruled out.

The present data are inconsistent with the diabetes-related defect in synthase activation and decrease in synthase phosphatase activity being due to the elevated tissue glycogen concentrations found in diabetic hearts. Although insulin treatment, in vivo, for 1 to 6 h restored the acute effect of insulin to activate synthase, the high tissue glycogen levels were virtually unaltered by the treatment. Further, while insulin treatment and insulin plus cycloheximide treatment were shown to markedly alter synthase activation and synthase phosphatase activity in diabetic hearts, tissue glycogen would...
concentrations were unchanged by these treatments. Although the present study clearly demonstrates that insulin activation of synthase can occur even in the presence of the elevated glycogen concentrations found in hearts from diabetic rats, it remains to be determined whether an acute effect of insulin might be to alter the sensitivity of synthase phosphatase to inhibition by glyco-

In conclusion, the present study demonstrates that the acute effect of insulin to activate glycogen synthase in the isolated perfused rat heart is defective in hearts from alloxan-diabetic rats. Further, treatment of these diabetic rats with insulin, in vivo, results in restoration of the acute effect of insulin to activate glycogen synthase in the perfused heart. Finally, the acute effect of insulin to activate cardiac synthase is correlated with maintenance of total synthase phosphatase activity. Therefore, insulin plays a dual role in regulation of cardiac glycogen synthesis. There is a chronic effect to maintain the enzymatic machinery necessary for effector activation of synthase and an acute effect to directly activate synthase through an as yet unknown mechanism.

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A dual role for insulin in the regulation of cardiac glycogen synthase.

T B Miller, Jr