Hormonal Effects on Glycogen Metabolism in the Rat Heart *in Situ*

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SUMMARY

Several hormones known to affect tissue glycogen concentration have been studied in the open-chested rat in relation to their effects on the enzymes of cardiac glycogen metabolism. Epinephrine, given by rapid intravenous injection, increased phosphorylase *a* activity and glucose-6-P concentration in both heart and skeletal muscle. In contrast, glycogen synthetase I activity was increased in heart and decreased in skeletal muscle. Continuous infusion of epinephrine (1 μg/kg min^{-1}) caused marked glycogenolysis in skeletal muscle, but no change in heart glycogen concentration. In these experiments a transient increase was seen in cardiac glycogen synthetase I activity, while the percentage synthetase I decreased in skeletal muscle. Infusion of 2.5 μg/kg min^{-1} of epinephrine decreased cardiac glycogen and produced a biphasic change in synthetase I activity, consisting of first an increase, then a decrease in heart percentage of glycogen synthetase I. All these effects of epinephrine were prevented by prior administration of the *beta* adrenergic blocking agent pronethalol. Glucagon caused marked cardiac glycogenolysis, an increase in the percentage of phosphorylase *a*, but no change in the percentage of synthetase I. These effects were also antagonized by pronethalol pretreatment. Insulin caused an increase in the percentage of synthetase I in heart without affecting phosphorylase activity. The increase in synthetase I activity was unaffected by the administration of pronethalol. Thus, in heart as in skeletal muscle, hormones affect the critical steps in glycogen synthesis and degradation, perhaps in part through a common pathway sensitive to *beta* adrenergic blockade. However, other factors such as physiological changes in cardiac muscle may be involved in the magnitude and direction of the response.

INTRODUCTION[1]

Epinephrine-induced glycogenolysis in skeletal muscle has been shown to be mediated through an increase in the fraction of glycogen phosphorylase (α-1,4-glucan: orthophosphate glucosyltransferase EC 2.4.1.1) in the *a* form (1). Recently, another factor in this process has been suggested by the data of Craig and Larner (2). They found that the addition of epinephrine to rat diaphragm promoted the conversion of the I form of glycogen synthetase (UDP-glucose: glycogen α-4-glucosyltransferase EC 2.4.1.11) which is active in the absence of glucose-6-P to synthetase D, which requires glucose-6-P for full activity. This finding is consistent with a decrease in the rate of glycogen synthesis (3). Thus, epinephrine may act in two ways to promote skeletal muscle glycogenolysis: by the acceleration of glycogen breakdown through an action on glycogen phosphorylase and by the inhibition of glycogen synthesis through an effect on glycogen synthetase.

The effect of epinephrine on cardiac glycogen is, however, not so well understood.

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Although cardiac muscle, like skeletal muscle, has been shown to respond to epinephrine with increased phosphorylase *a* activity (4, 5), cardiac glycogenolysis *in vivo* has been observed inconsistently and usually only in response to large doses of epinephrine (6).

Insulin and glucagon have also been shown to affect glycogen concentrations in various tissues. The increase in glycogen concentration seen after the administration of insulin has been attributed, in part, to an increase in the rate of glucose transport into cells (7). However, an additional factor has been implicated by the work of Villar-Palasi and Larner, which showed that insulin administration caused an increase in synthetase I activity in the rat diaphragm *in vitro* (8). Glucagon has been shown to activate phosphorylase in both heart (9) and liver (10), but to have no effect on liver glycogen synthetase activity (11).

The present communication is concerned with the effects of these hormones on glycogen synthetase and phosphorylase activities in hearts from open-chested rats and deals specifically with the basis for the observed differences in the responses of cardiac and skeletal muscle glycogen to epinephrine. Portions of this work have been reported previously (12, 13).

MATERIALS AND METHODS

All experiments were performed using male Sprague-Dawley rats weighing 175–215 g, which were given food and water ad libitum. The animals were anesthetized by intravenous injection of a mixture of α-chloralose (50 mg/kg) and sodium pentobarbital (35 mg/kg) containing atropine (1 mg/kg). A tracheal cannula was inserted and artificial respiration was begun using 96% O_2–4% CO_2 which was delivered by a Harvard rodent respirator at a rate of 90 strokes/min, the volume being determined by a parallel resistance of 15 cm H_2O. The chest was opened by a transverse incision. Carotid arterial pressure was continuously recorded on a Grass Model 5 Polygraph with a Statham P23Dc transducer. Body temperature was maintained at $37 \pm 1°$ by a thermostatically controlled heating pad. No drug was administered until at least 20 minutes after completion of surgery. Drugs were administered intravenously either by rapid injection or by continuous infusion using a Harvard syringe pump. Heart and gastrocnemius muscle were frozen *in situ* by clamping between two silver blocks cooled in liquid nitrogen by a technique similar to that described by Wollenberger *et al.* (14). Samples were stored in liquid nitrogen or at −90°.

Glycogen phosphorylase activity was measured at 30° in the direction of glucose-1-P formation by the method of Bueding *et al.* (15) modified for fluorometric assay (16). Glycogen synthetase activity was determined at 30° by UDP production according to the method of Villar-Palasi and Larner (8). The validity of this assay for heart was checked by measurement of UDP-glucose disappearance and by the uptake of radioactivity into glycogen from UDP-^{14}C-glucose-u.l. as described by Steiner (11). The activity of UDP-glucose pyrophosphorylase (UTP:α-D-glucose 1-phosphate uridyltransferase EC 2.7.7.9) was determined both in the direction of UDP-glucose production and in the direction of glucose-1-P formation. Heart samples were homogenized in 0.15 M KCl, adjusted to pH 7.4, and centrifuged at 2400 *g* for 10 min at 0°. Aliquots of the supernatant fraction were added to a reaction mixture containing 0.08 M Tris (pH 7.4), 0.0008 M UTP, 0.0008 M glucose-1-P, 0.05 M $MgCl_2$ and 0.07 M KF in a final volume of 200 μl. After 0–40 minutes of incubation at 30°, the reaction was stopped by heating 1.25 minutes at 100°, and the UDP-glucose produced was measured using UDP-glucose dehydrogenase (17). The rate of UDP-glucose production was linear over a 40-minute period. Alternatively glucose-1-P production was determined as the rate of TPNH production in the presence of excess glucose-6-P dehydrogenase and phosphoglucomutase as described by Larner and Villar-Palasi (18). This method was adapted for fluorometric assay at a tissue concentration of 400 μg/ml in a total volume of 1 ml. Glucose-6-P concentrations were measured in perchloric acid

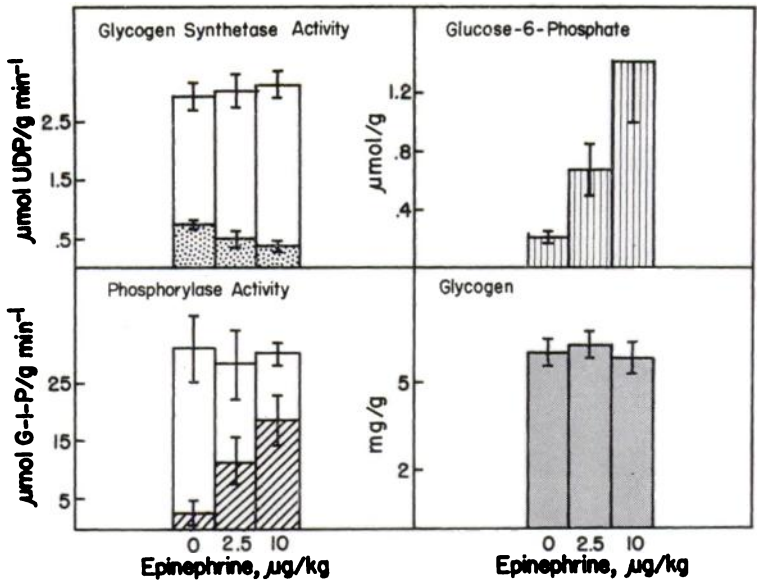

FIG. 1. *The effect of epinephrine on rat skeletal muscle*

Epinephrine was given by rapid intravenous injection and samples were frozen 15–20 sec later. The crosshatched portions of the bars represent phosphorylase activity measured without AMP and the total height activity with AMP. Glycogen synthetase activity in the absence of glucose-6-P is shown by the stippled segments, and that in the presence of glucose-6-P by the total height of the bars. Activities and concentrations are expressed per gram wet weight. Each bar represents the average of four experiments. I bars are ±1 standard error.

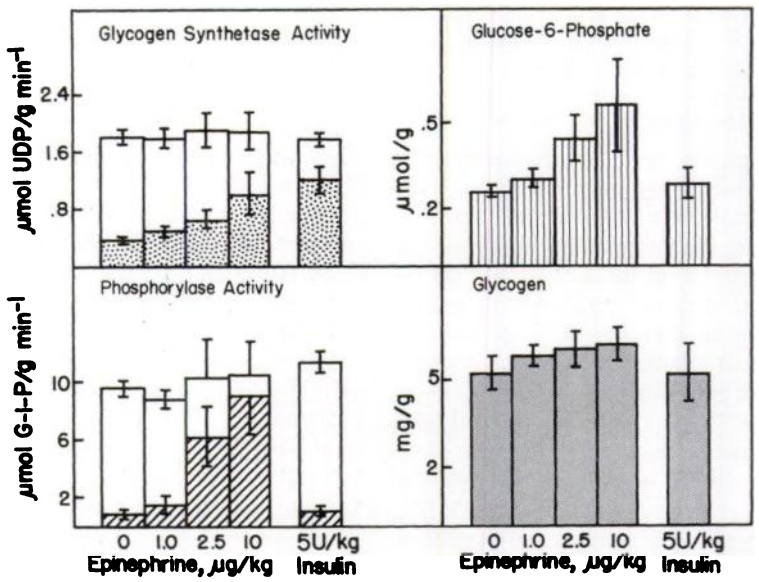

FIG. 2. *The effect of epinephrine and insulin on rat heart*

Both drugs were given by rapid intravenous injection, and hearts were frozen 15–20 sec after epinephrine or 1 min after insulin. The symbols are described in the legend of Fig. 1. Each bar represents the average of six experiments. I bars are ±1 standard error.

tissue extracts (19) using glucose-6-P dehydrogenase. The resulting TPNH was determined by the indirect fluorometric method of Lowry *et al.* (20). UDP-glucose concentrations in the same extracts were measured using UDP-glucose dehydrogenase by a fluorometric modification (17) of the method of Strominger *et al.* (21). Glycogen was extracted in KOH and precipitated with ethanol (22) and measured by an anthrone procedure (23).

All enzymes were obtained from C. F. Boehringer u. Soehne, except heart lactic acid dehydrogenase from Worthington Biochemical Corporation and UDP-glucose dehydrogenase from Sigma Chemical Company. Glucose-1-P and UDP-glucose were obtained from Sigma Chemical Company, Na_3-phosphopyruvate from Boehringer, and TPN^+, DPNH, and UTP from P-L Biochemicals. Epinephrine was used as the commercial solution Suprarenin. Glucagon and insulin were gifts from Dr. Otto Behrens of the Eli Lilly Company. Pronethalol [2-isopropylamino-1-(2-naphthyl)-ethanol] was obtained through the courtesy of Dr. J. W. Black of Imperial Chemical Industries, Ltd.

Spectrophotometric measurements were made with a Beckman monochromator equipped with a Gilford absorbance indicator and cuvette positioner. Fluorometric measurements were made with a Turner Model 111 fluorometer. For direct measurement of reduced pyridine nucleotides, the primary filter was a Corning No. 5860, and the secondary was a combination of Corning No. 4308 and 3387. When reduced pyridine nucleotides were being determined by the indirect method, the Corning No. 4308 was replaced by a Corning No. 5562.

Statistical comparisons of data were made using a t test for unpaired samples (24). A P value of <0.05 was accepted as the limit of significance.

RESULTS

Epinephrine

Fifteen seconds after the rapid intravenous injection of 10 μg/kg of epinephrine, the following effects were demonstrable in skeletal muscle: a marked activation of phosphorylase ($P < 0.05$), a significant decrease in percentage of synthetase I ($P < 0.05$), and a 6-fold increase in glucose-6-P concentration ($P < 0.05$). No change in glycogen concentration was seen in these short-term experiments (Fig. 1).

Experiments in which heart samples were frozen at the same time interval after epinephrine injection are shown in Fig. 2. Phosphorylase *a* activity and glucose-6-P concentration increased, but the latter did not reach the levels found in skeletal muscle. In contrast to the effect in skeletal muscle, the activity of the glucose-6-P independent form of glycogen synthetase was significantly increased after the administration of both 2.5 and 10.0 μg/kg of epinephrine ($P < 0.01$).

Beta adrenergic blocking agents, such as pronethalol, have been shown to antagonize the catecholamine-induced increase in heart phosphorylase *a* activity (5). Pronethalol treatment alone had no apparent direct effect, but did antagonize the effects of epinephrine on cardiac phosphorylase activity, glycogen synthetase activity, and glucose-6-P concentration (Fig. 3).

Because in these short-term experiments no glycogen breakdown was discernible, experiments were performed in which 1.0 μg/kg min^{-1} of epinephrine was infused continuously and samples of heart and gastrocnemius muscle were frozen after 0.5–10 min of infusion. In skeletal muscle the percentage of phosphorylase *a* increased from a control of 27% to 62% after 1 min and remained at a high level throughout the infusion (Fig. 4). A decrease in the percentage of synthetase I from a control of 22% to 8% was observed; this effect was also maintained throughout the infusion. Glycogen concentration was reduced after 1 min and by 10 min had decreased to approximately 50% of control values. Pretreatment of animals with 20 mg/kg of pronethalol abolished all these effects.

The effects of this infusion of epinephrine showed a different pattern in heart muscle (Fig. 4). A rapid increase in the percentage of phosphorylase *a* from 9% to 34% was followed by a slow decline to

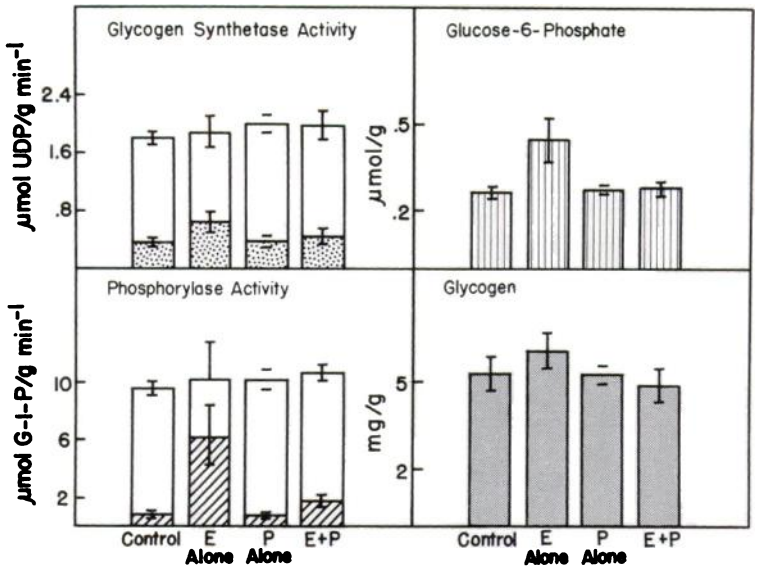

FIG. 3. *The effect of adrenergic blockade on metabolic responses of rat heart to epinephrine*

The symbols are described in the legend of Fig. 1. Six animals were given 2.5 μg/kg of epinephrine (E); two 20 mg/kg of pronethalol (P); six, pronethalol followed by epinephrine (E + P). E + P does not differ from control, but does differ from E alone ($P < 0.01$).

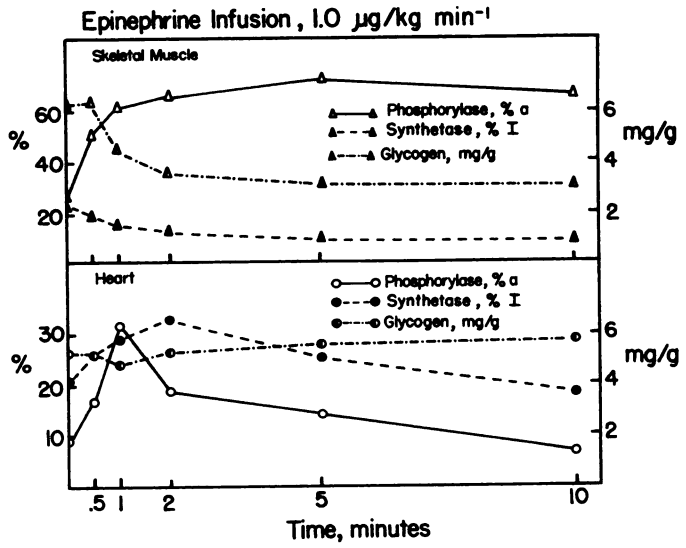

FIG. 4. *The effect of epinephrine infusion, 1 μg/kg min⁻¹*

The scale for enzyme activities is given on the left ordinate and that for glycogen on the right. Enzyme activities are expressed as:

$$\frac{\text{activity measured in the absence of activator (AMP or glucose-6-P)} \times 100}{\text{activity measured in the presence of activator}}$$

Each point represents two experiments.

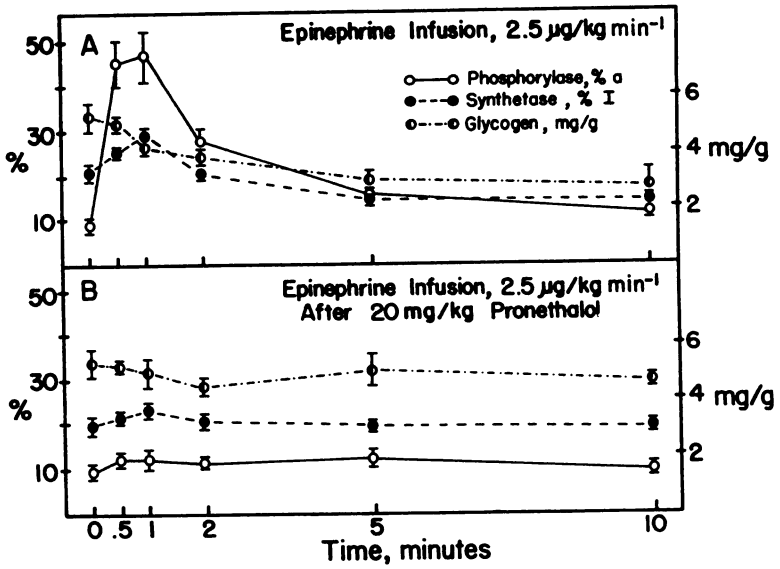

FIG. 5. *The effect of epinephrine infusions, 2.5 μg/kg min⁻¹ on phosphorylase and synthetase activities and glycogen concentration in the rat heart*

Epinephrine was administered without (A) and with (B) pronethalol pretreatment. The scale for enzyme activities is given on the left ordinate, that for glycogen concentration on the right. Each point represents the average of three to seven experiments. I bars are ±1 standard error.

control values while the infusion was still in progress. An increase was seen in the percentage of synthetase I, but this effect also had subsided after 10 min of infusion. Glycogen levels did not change.

When 2.5 μg/kg min^{-1} of epinephrine was administered by continuous infusion, cardiac glycogenolysis was observed ($P < 0.05$ at 2, 5, and 10 minutes) as shown in Fig. 5A. The pattern of the phosphorylase response was the same as that seen during the 1.0 μg/kg min^{-1} epinephrine infusion; the percentage of phosphorylase *a* increased rapidly to a peak of 47% ($P < 0.01$), then gradually decreased to near control levels. Under these conditions a biphasic change in synthetase I activity occurred; after an initial increase ($P < 0.01$), the percentage of synthetase I fell to 13%, significantly below control levels ($P < 0.02$). Pronethalol pretreatment effectively antagonized all the above effects (Fig. 5B).

Glucose-6-P and UDP-glucose concentrations were also determined in these experiments (Fig. 6A). Epinephrine infusion produced a sustained increase in glucose-6-P ($P < 0.01$) and a transient, but significant ($P < 0.01$), drop in UDP-glucose concentration. These effects can be correlated with the observed changes in enzyme activities in that the increase in phosphorylase *a* activity led to an accumulation of glucose-6-P, and the increase in synthetase I activity was accompanied by a decrease in the concentration of its substrate, UDP-glucose. The blockade by pronethalol of these changes in substrate concentrations (Fig. 6B) is consistent with blockade of the changes in enzyme activities by this same agent.

However, in the epinephrine experiments, the decrease in UDP-glucose concentration at a time when glucose-6-P concentration was elevated suggested a rate-limiting step between glucose-6-P and UDP-glucose. Of the two enzymes involved in this transformation, UDP-glucose pyrophosphorylase was the most likely candidate, although the data of Villar-Palasi and Larner (25) indicate that its activity is in excess of that of glycogen synthetase in all tissues tested, including heart. Accordingly, the activity of UDP-glucose pyrophosphorylase was de-

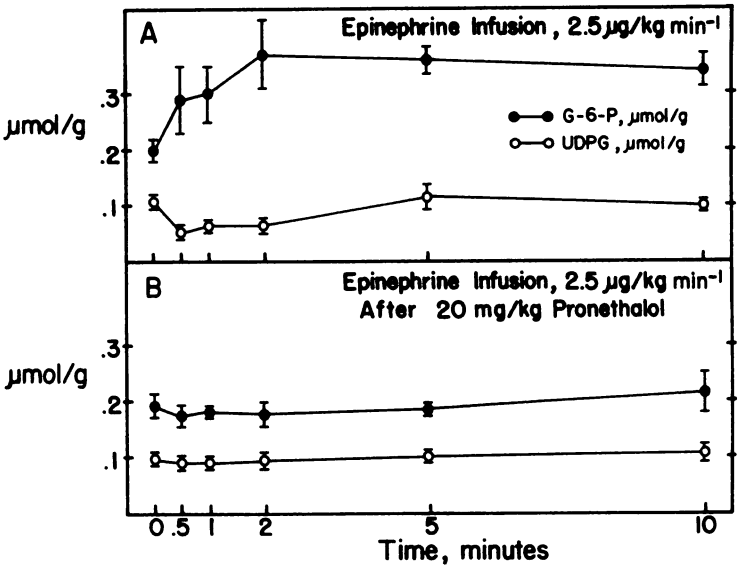

FIG. 6. *The effect of epinephrine infusion, 2.5 μg/kg min⁻¹ on substrate concentrations in the rat heart*

Concentrations are expressed in micromoles per gram wet weight. Epinephrine was given without (A) and with (B) pronethalol pretreatment. Results shown are from the same animals as shown in Fig. 5.

termined in hearts from control and epinephrine-treated rats (Table 1). When activity was measured in the direction of glucose-1-P production, this was found to be approximately twice as great as the glycogen synthetase activity, and no difference was found between the two groups of animals. The latter was also true when UDP-glucose pyrophosphorylase activity was determined in the direction of UDP-glucose synthesis, but the maximal rate of sugar nucleotide formation was essentially the same as the total glycogen synthetase activity measured in the presence of glucose-6-P. This suggests that control of glycogen synthesis may be exerted through UDP-glucose pyrophosphorylase, especially when glycogen synthetase activity is high.

Glucagon

The responses to glucagon given by intravenous infusion at the rate of 10 μg/kg min⁻¹ are shown in Table 2. Hearts were frozen after 3 min of infusion. As was expected, glucagon produced a marked increase in the percentage of phosphorylase

TABLE 1

Activities of the enzymes of glycogen synthesis in rat hearts before and after epinephrine treatment

Enzyme activities were measured as described in Materials and Methods. UDP-glucose pyrophosphorylase activities were determined in both directions on 4 control and 4 epinephrine-treated hearts. Glycogen synthetase activities are the averages of 7 control and 7 epinephrine-treated samples.

Treatment	UDP-glucose pyrophosphorylase activity		Total glycogen synthetase activity (measured with glucose-6-P), μmoles UDP/g min⁻¹ ± SE
	μmoles glucose-1-P/g min⁻¹ ± SE	μmoles UDP-glucose/g min⁻¹ ± SE	
None	3.75 ± 0.33	1.91 ± 0.18	1.97 ± 0.10
Epinephrine	4.52 ± 0.25	1.95 ± 0.38	2.17 ± 0.15

TABLE 2

Effects of insulin and glucagon on enzyme activities and substrate concentrations in the rat heart

Glucagon (10 μg/kg min^{-1}) and insulin (0.2 U/kg min^{-1}) were given by continuous intravenous infusion. Twenty minutes prior to hormone administration, pronethalol (20 mg/kg) was given by slow intravenous injection. Substrate concentrations are expressed per gram wet weight.

Treatment	N	Phosphorylase *a* (% ± SE)	Glycogen synthetase I (% ± SE)	Glucose-6-P (μmoles/g ± SE)	UDP-glucose (μmoles/g ± SE)	Glycogen (mg/g ± SE)
None	7	8.1 ± 1.3	20.7 ± 2.1	0.206 ± 0.020	0.109 ± 0.008	5.36 ± 0.51
Pronethalol	5	9.3 ± 1.2	19.8 ± 1.6	0.193 ± 0.025	0.099 ± 0.007	5.42 ± 0.52
Glucagon	5	71.4 ± 5.6[a]	23.2 ± 1.4	0.331 ± 0.049[c]	0.090 ± 0.007	2.42 ± 0.30[a]
Glucagon and pronethalol	6	12.7 ± 2.0	21.1 ± 0.9	0.104 ± 0.010[a]	0.215 ± 0.004[a]	4.98 ± 0.23
Insulin	5	6.7 ± 0.7	32.5 ± 1.3[a]	0.277 ± 0.025[d]	0.216 ± 0.022[b]	5.11 ± 0.33
Insulin and pronethalol	6	5.2 ± 1.8	31.9 ± 1.2[a]	0.307 ± 0.029[b]	0.197 ± 0.008	4.63 ± 0.50

[a] Significantly different from corresponding control $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.02$.
[d] $P < 0.05$.

a, an increase in glucose-6-P, and a decrease in glycogen concentration. No change was seen in the percentage of synthetase I or in UDP-glucose concentration. After the administration of 20 mg/kg of pronethalol, glucagon caused no change in the percentage of phosphorylase *a* or in glycogen concentration. Glucose-6-P was significantly lower and UDP-glucose concentration was increased in the samples from pronethalol-pretreated animals given glucagon when compared to controls. To determine whether this substrate pattern was produced through an effect on UDP-glucose pyrophosphorylase, the activity of this enzyme was examined. No difference could be seen between hearts from glucagon-treated animals and those given glucagon after pronethalol.

Insulin

When insulin was given intravenously in a dose of 5 U/kg, and the hearts were frozen 1 min after injection, no effect on phosphorylase activity, glucose-6-P or glycogen concentrations was observed. However, a marked increase in the fraction of synthetase in the I form was noted ($P < 0.01$) (Fig. 2). Experiments were also performed in which 0.2 U/kg min^{-1} of insulin was administered by continuous infusion. This dose decreased plasma glucose concentration by 10% or less after 5 min, at which time the hearts were frozen. No effect on phosphorylase activity or glycogen concentration was observed, but synthetase I activity and glucose-6-P and UDP-glucose concentrations were elevated (Table 2). Pronethalol pretreatment had no effect on these responses.

DISCUSSION

Epinephrine, glucagon, and insulin have all been shown to be effective in changing the concentration of tissue glycogen (9, 26). Our experiments indicate that the influence of these hormones on cardiac glycogen in the open-chested rat are mediated through effects on the enzymes of glycogen metabolism, but that they differ in efficacy and in terms of the enzymes affected.

Epinephrine promotes the conversion of phosphorylase *b* to phosphorylase *a* in both heart (4, 5) and skeletal muscle (1). In the latter tissue, such enzymic activation results in glycogenolysis, but in cardiac muscle a significant degree of glycogen breakdown is not an obligatory consequence of phosphorylase activation (19). This was confirmed by the present experiments in which 1.0 μg/kg min^{-1} of epinephrine was given

by continuous infusion. The data of Hornbrook and Brody indicate that epinephrine-induced cardiac glycogenolysis occurs only as a consequence of an increase in the activity of phosphorylase *a* and that this enzyme in heart is less sensitive to the effects of the amine than is muscle phosphorylase (27). In the present experiments, a difference in sensitivity of the two tissues was not found when the amine was injected rapidly, but was seen during epinephrine infusions. However, other factors may be of importance in explaining the observed differences in the effects of epinephrine on cardiac and skeletal muscle glycogen.

First, the increase in cardiac phosphorylase *a* activity was transient while the response in skeletal muscle was maintained throughout the infusion. Secondly, in contrast to the decrease in synthetase I activity seen in skeletal muscle, an increase in the percentage of synthetase I was seen in heart. Since the I form is apparently the more active form of the enzyme (3), the change seen in heart would be consistent with an increased rate of glycogen synthesis. Infusions of a higher dose of epinephrine caused a significant degree of glycogen breakdown. This effect was accompanied by a biphasic change in synthetase activity, consisting of an increase followed by a decrease to below control levels. A similar response was observed by Williamson in experiments using perfused rat hearts (28). In the present experiments, both phases of the synthetase response were antagonized by the *beta* adrenergic blocking agent, pronethalol. Since agents of this type have been shown to block the catecholamine-induced increase in the production of the cyclic 3′,5′-AMP (29), it is suggested that the effects of epinephrine on glycogen synthetase may be mediated through the production of the cyclic nucleotide. Cyclic AMP has been shown to promote the conversion of skeletal muscle synthetase I to the D form (30, 31), and this mechanism, if existent in cardiac muscle, could explain the decrease in synthetase I seen after the infusion of large amounts of epinephrine. The more rapidly occurring increase in synthetase I activity cannot be explained on this basis but may be secondary to physiological effects of the amine on the rat heart *in vivo*. Other workers have been unable to detect any effect of epinephrine on glycogen synthetase of the perfused nonworking rat heart (32) or of the perfused working rat heart (33). An indication that the increase in synthetase I activity may be related to the epinephrine-induced increase in cardiac work is the recent observation of Neely and Morgan (34). These investigators found that the percentage of glycogen synthetase I increased to twice the control value when a perfused rat heart was forced to work for a 30-min period. In this connection it is of interest to note that tetanic stimulation of mouse skeletal muscle, a procedure which has been shown to cause phosphorylase activation (35), increases the percentage of synthetase I (3).

Since an increase in the percentage of synthetase I has been observed after epinephrine administration to an isolated perfused heart (28), another theory of the mechanism of this epinephrine effect may be proposed. Danforth has suggested that in skeletal muscle glycogen exerts a negative feedback control over its own synthesis through an effect on glycogen synthetase (3). This investigator found that over a wide range of glycogen concentrations, the percentage of synthetase I was inversely related to the glycogen concentration. Similar observations have been made with the perfused rat heart (32). The mechanism of this feedback control is thought to be an inhibition of transferase phosphatase by glycogen (36). Since glycogen synthetase is partly bound to glycogen, a change in glycogen concentration which would be undetectable by conventional methods might be sufficient to cause deinhibition of transferase phosphatase, and a subsequent increase in the synthetase I:D ratio. If this were the case in these experiments, the increasing phase of the synthetase response could be caused through localized changes in glycogen concentration, and the decreasing phase might be a direct effect of epinephrine through the production of cyclic AMP.

The changes in glucose-6-P and UDP-glucose concentrations seen during epinephrine administration are consistent with the observed alterations in enzyme activity. The decrease in UDP-glucose concentration at a time when the percentage of synthetase I was increased indicates that in this case, synthetase D to I transformation may be a significant mechanism in the control of flux through this pathway. The substrate pattern seen during epinephrine administration also suggests that the UDP-glucose pyrophosphorylase step might be rate limiting in glycogen synthesis. No effect of epinephrine was observed on the activity of this enzyme, but the activity in the direction of UDP-glucose production was comparable to total synthetase activity, suggesting that some control at this point might be exerted through substrate availability.

The effect of insulin on heart glycogen synthetase activity is similar to that reported to occur in skeletal muscle *in vitro* (2, 8). The augmentation by epinephrine of the D to I transformation in heart is, however, exactly opposite to its action in skeletal muscle. Although the possibility exists that in the intact animal the effect of epinephrine is an indirect one, it is unlikely to be due to insulin release for several reasons: (a) the effect of epinephrine on heart synthetase occurs within 15 sec; (b) release of insulin should lead to different effects than those observed in skeletal muscle after epinephrine administration; and (c) the effects of insulin on synthetase I activity were unaffected by pronethalol pretreatment, whereas the effects of epinephrine were antagonized.

Glucagon has been shown to activate liver phosphorylase through the production of cyclic AMP (37). This hormone has also been shown to increase cardiac phosphorylase *a*, presumably through the production of cyclic AMP (9) and to increase the rate of spontaneously beating mammalian atria (38). Our experiments have demonstrated an activation of cardiac phosphorylase which is blocked by pronethalol, which again suggests an involvement of cyclic AMP in the response. Glucagon, however, had no effect on cardiac glycogen synthetase. It must be noted, though, that while the effects of epinephrine were observed throughout a 10-min infusion, the response to glucagon was tested at only one time interval. It is possible that an effect of glucagon on synthetase activity might have been revealed if the full time course of the infusion had been examined.

The mechanisms involved in the conversion of the I to D form of skeletal muscle glycogen synthetase appear to be similar to those which regulate muscle phosphorylase *b* kinase (30). This may not be true in heart where glycogen synthetase and phosphorylase may be interrelated as part of a glycogen sparing rather than a glycogenolytic system. However, the effects on both heart enzymes were blocked by pronethalol, which has been shown to prevent the catecholamine-induced synthesis of cyclic 3′,5′-AMP in heart. Thus the mechanisms which control glycogen synthetase and phosphorylase *b* kinase in heart may be basically similar to those in skeletal muscle. They may, however, differ in their sensitivity to epinephrine and may be influenced by physiological activity of the muscle. The observations that glucagon and insulin can influence the activity of one enzyme without affecting the other indicate that although the interconversion mechanisms may be basically similar, other factors may be involved.

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