A Hypersensitivity of Glycogen Phosphorylase Activation in Hearts of Diabetic Rats*

(Received for publication, August 25, 1980, and in revised form, October 20, 1980)

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This study was initiated to determine whether glycogen phosphorylase activation was defective in hearts of alloxan diabetic rats. When hearts were perfused by gravity flow for 1 to 10 min with various concentrations of epinephrine, activation of glycogen phosphorylase in the diabetic was significantly greater at every time and epinephrine concentration than that seen in the normal. Cyclic AMP accumulation and protein kinase activation by epinephrine in the diabetic were not appreciably different or were lower than the normal responses to the hormone. The effects of epinephrine on cyclic AMP and protein kinase were blocked in both normal and diabetic hearts by propranolol. While the β blocker prevented phosphorylase activation in the normal hearts, it did not block phosphorylase activation by epinephrine in the diabetic hearts. Likewise, the α agonist phenylephrine activated phosphorylase in the diabetic but not in the normal hearts. While glucagon produced the same phosphorylase hypersensitivity in diabetic hearts, the cyclic AMP and protein kinase responses were not altered by diabetes. Phosphorylase phosphatase activity was found to be unaltered by either epinephrine or diabetes, whereas phosphorylase kinase activation by epinephrine in the diabetic was double the normal response. These data are consistent with a diabetes-related unmasking of an α effect on cardiac phosphorylase activation and an unexplained increase in the sensitivity of phosphorylase kinase activation by protein kinase.

The role of catecholamines in cardiac function has been well documented (for review see Ref. 1). Robison et al. (2) in 1965 reported that epinephrine caused a rapid rise in cyclic AMP, an enhanced contractile force, and the conversion of glycogen phosphorylase to the a form in the perfused rat heart. The catecholamine-induced activation of cardiac phosphorylase was shown to be the result of an increase in phosphorylase kinase activity (3–6) which was itself the result of cyclic AMP-mediated activation of protein kinase (7, 8). Most, if not all, of the stimulatory effects of epinephrine on phosphorylase activation are thought to be mediated through activation of β receptors (1).

Recently, it was reported that hearts from streptozotocin diabetic rats have a deficiency in the number of ventricular β adrenergic receptors which may be associated with the diabetes-related negative chronotropic effect (9). The purpose of the present study was to determine whether the normal response of the isolated perfused rat heart to epinephrine might be decreased by a diabetic state. While the present study shows that diabetes may decrease epinephrine-mediated increases in cyclic AMP and protein kinase activation, phosphorylase b to a conversion in the diabetic is hypersensitive to epinephrine. A comparison of the effects of different types of stimulatory hormones on phosphorylase activation offers some insight into the possible mechanism of this hypersensitivity.

EXPERIMENTAL PROCEDURES

Heart Perfusion—Fed male rats of the Sprague-Dawley strain (Charles River Breeding Laboratories) weighing 200 to 250 g and maintained on Purina laboratory chow and water ad libitum were used for these studies. Diabetes was induced by injection of alloxan (90 mg/kg) into the tail vein and diagnosed on the day of perfusion by a maximum positive urine glucose test and a positive urine ketone test using Ames Keto-Diastix. Diabetic animals were used 3 to 4 days after alloxan injection. After quick removal from decapitated rats, hearts were immediately chilled in ice-cold Krebs-Henseleit bicarbonate buffer (gassed with 95% O2 and 5% CO2) containing 2.5 mM calcium, 5.0 mM glucose, 0.5 mM disodium EDTA, and 1 unit/ml of sodium heparin. Hearts were then mounted on the aortic cannula and retrograde gravity flow perfusion was begun at 37°C from a reservoir 70 cm above the heart with the same buffer without heparin. All hearts were equilibrated in this manner for 10 min and then switched to a second reservoir containing the same buffer without or with added hormones. After 1 to 10 min of additional perfusion, hearts were frozen, while beating, 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, Glc-6-P, ATP, and Cyclic AMP Analyses—Tissue glycogen determinations were carried out on frozen heart powder by the method of Cornblath et al. (11) and are expressed as micromoles of glucose/g of wet heart. Tissue glucose-6-phosphate and ATP were extracted as previously described (12) and assayed enzymatically using published procedures (13, 14). Results are expressed as micromoles/g of wet heart. Tissue cyclic AMP analysis was performed on frozen heart powder by the method of Gilman (15) and data are expressed as picomoles/mg of wet heart. Although not shown, dry weights were unaffected by either diabetes or hormonal intervention.

Glycogen Synthase and Phosphorylase Assays—Soluble glycogen synthase activity was extracted from frozen heart powder (100 mg of tissue/ml) as previously described (16) and determined using the ϵ-Glc-6-P filter paper assay of Thomas et al. (17). Data are presented as micromoles of glucose incorporated into glycogen from UDP-Gl/ g of wet heart/min and/or as per cent synthase I (I/total × 100). Soluble glycogen phosphorylase activity was extracted from frozen heart powder (20 mg of tissue/ml) as previously described (16) and determined using the ϵ-AMP filter paper assay of Gilboe et al. (18). Data are presented as micromoles of glucose incorporated into glycogen from Glc-1-P/g of wet heart/min and/or as per cent phosphorylase a (a/total × 100).

Protein Kinase Assay—Frozen heart powder was homogenized with a buffer containing 150 mM KF, 5 mM magnesium phosphate, and 2 mM EDTA (25 mg of tissue/ml) at pH 6.8 using a 15-g burst at two-thirds speed with a Polytron homogenizer, all at 4°C. After centrifugation in the cold at 8000 × g for 10 min, 20-μl aliquots were added to the

* This research was supported by National Institutes of Health Research Career Development Award AM00375.

† Recipient of National Institutes of Health Research Grant HL20476. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tubes containing 50 μl of 17 mM potassium phosphate, pH 6.8, 4500 μg of type IIA calf thymus histone, 5 mM magnesium acetate, 1.2 μM cAMP. Assays were carried out at 30°C for 10 min using the filter paper assay as described by Corbin and Reimann (19). Assays were linear with time and proportional to protein added under these conditions. Protein kinase activity was expressed as picomoles of 32P incorporated into histone/g of heart weight/min, and cAMP-dependent protein kinase in the active form (cAMP independent/total protein kinase x 100). Phosphorylation of endogenous substrates was subtracted from total phosphorylation to calculate histone phosphorylation.

Glycogen Phosphorylase Kinase Assay—Soluble phosphorylase kinase was assayed by measuring 32P incorporation from γ-labeled [32P]ATP into phosphorylase with slight modifications of the procedures described by McCullough and Walsh (20) and Hayes et al. (21). Frozen heart powder (100 mg) was homogenized with a buffer (1 ml) containing 30 mM Tris base, 30 mM KCl, 5 mM EDTA, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, and 1 mg/ml of crystalline alubumin at pH 7.5 at 2°C using the Polytron homogenizer as previously described. After centrifugation at 10,000 x g for 10 min at 2°C, aliquots were diluted a further 5-fold (for assay at pH 6.8) and 10-fold (for assay at pH 8.2). For the assay, 40 μl of the X50 and X100 diluents were added to tubes containing 30°C containing 200 μM of 60 mM Tris (pH 6.8 for X50 dilution and pH 8.6 from X100 dilution), 60 mM β-glycerol phosphate (pH 6.8 and 8.6, respectively), 4.5 mM γ-labeled [32P]ATP (60 cpml/mmol), 14.5 mg/ml crystalline phosphorylase b, 5 mM EDTA, 1.4 mg of phosphorylase b, and 0.4 mg of crystalline phosphorylase a. After 5 and 35 min, 50 μl aliquots were withdrawn from the tubes, spotted in a 2x2 Whatman ET-31 filter paper, and washed in ice-cold containing 10% trichloroacetic acid with 8% pyrophosphate. After all samples were placed in the beaker, the papers were washed a further 15 min and the wash discarded. Papers were washed 2 more times for 15 min each with 10% trichloroacetic acid, then 5 min in ethanol, and 5 min in ether. After drying, the papers were placed in a scintillation vial containing 10 ml of National Diagnostic Betafluor and counted. The rate of 32P incorporation into phosphorylase was linear between 5 and 40 min under these conditions. Phosphorylase kinase activity was expressed as the ratio of activity at pH 6.8 to that measured at pH 8.2.

Glycogen Phosphorylase Phosphatase—Soluble phosphorylase phosphatase activity was determined by measuring the ability of a tissue extract to convert phosphorylase a to the b form. Frozen heart powder (200 mg) was homogenized with buffer (1 ml) containing 10 mM 2-[N-morpholino]ethane sulfonic acid, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 21.5 mM mercaptoethanol, and 50 mM NaF. At 5 and 35 min, 50 μl aliquots were withdrawn from the tubes, spotted in a 2x2 Whatman ET-31 filter paper, and washed in ice-cold containing 10% trichloroacetic acid with 8% pyrophosphate. After all samples were placed in the beaker, the papers were washed a further 15 min and the wash discarded. Papers were washed 2 more times for 15 min each with 10% trichloroacetic acid, then 5 min in ethanol, and 5 min in ether. After drying, the papers were placed in a scintillation vial containing 10 ml of National Diagnostic Betafluor and counted. The rate of 32P incorporation into phosphorylase was linear between 5 and 40 min under these conditions. Phosphorylase phosphatase activity was expressed as the ratio of activity at pH 6.8 to that measured at pH 8.2.

Materials—Nucleotides, type II AS histone, phosphorylase a, phosphorylase b, alloxan, epinephrine, phenylephrine, and propranolol were obtained from Sigma Chemical Co. Crystalline glucagon was a gift from the Eli Lilly Co. γ-labeled [32P]ATP, [14C]Gl-1-P, [3H]UDP-Glc, and [1H]AMP were purchased from New England Nuclear.

Data Computation and Expression—All data are expressed as means ± S.E. and statistical significance determined by Student's t test. Bars on either side of the mean represent one S. E.

RESULTS

Since Keely et al. (7) demonstrated maximal activation of glycogen phosphorylase in perfused hearts of normal rats during the 2nd to 5th min of continuous perfusion with epinephrine, the first set of experiments was designed to determine whether the responsiveness of the glycogen phosphorylase activation cascade to various concentrations of epinephrine was altered by insulin deficiency. After the 10-min equilibration perfusion, hearts from normal and diabetic rats were perfused for a further 5 min without epinephrine or in the presence of increasing concentrations of epinephrine (0.014, 0.028, 0.14, 0.28 and 1.0 μM). Fig. 1 shows the results of these perfusions. In Panel A, cAMP levels were the same in normal and diabetic hearts perfused without epinephrine, whereas cAMP accumulation in response to epinephrine was significantly lower in the diabetics at all concentrations tested (p < 0.01). In Panel B, the activation of cAMP-dependent protein kinase by epinephrine appeared to be unaltered by the diabetic state regardless of the epinephrine concentration used. In Panel C, glycogen phosphorylase activation by all concentrations of epinephrine was much greater in diabetics than in normal hearts (p < 0.01). In Panel D, glycogen synthase activity was decreased in the normal hearts by increasing epinephrine concentrations, whereas synthase in the diabetic hearts, already at a basal level, was unaffected by epinephrine. Fig. 2 shows the data on ATP, Glc-6-P, and glycogen for the three hearts represented in Fig. 1. ATP levels (Fig. 2A) in normal hearts appeared to be increased by the lower concentrations of epinephrine, whereas the higher concentrations had no such effect. ATP concentrations were significantly less (p < 0.01) in the diabetic hearts perfused without epinephrine as previously demonstrated (12) and at the lower epinephrine concentrations, but the diabetic and normal values were not different at the higher epinephrine concentrations. Whereas glucose 6-phosphate (Fig. 2B) was unaffected by epinephrine in the normal hearts, glucose 6-phosphate levels in the diabetic hearts were higher initially (22) and increasing concentrations of epinephrine caused increasing levels of the hexose phosphate. Glycogen levels (Panel C) were appreciably higher in diabetics in agreement with earlier studies (22) and were relatively unaffected by epinephrine during the 3-min hormone perfusion of normal and diabetic hearts. The lack of effect on glycogen was probably due to the short term exposure to epinephrine.
min without added hormone and then for 1 to 10 additional min in the presence of 0.28 μM epinephrine. Figs. 3 and 4 show the results of these perfusions on the enzymatic and metabolite profiles. In Fig. 3 (Panel A), cAMP accumulation in the diabetics remained the same as or lower than the normals between 1 and 10 min. In Panel B (Fig. 3), there were no differences between normal and diabetic hearts except at the 3-min time period, in agreement with the premise that protein kinase activation in the diabetic heart is not hypersensitive to epinephrine. Phosphorylase activation by epinephrine in normal hearts peaked at 1 min and remained active for the 10-min period (Fig. 3C). The response of phosphorylase in diabetic hearts was double that found for normal hearts at 1 min and remained statistically elevated at 2 through 10 min (p < 0.01), in agreement with Fig. 1 showing a hypersensitivity to epinephrine in the diabetic. In Panel D (Fig. 3), glycogen synthase in the normals was decreased by 1 to 2 min and remained unchanged during the following 8 min. As previously shown, glycogen synthase activity in the diabetics was already extremely low after the 10-min equilibration perfusion (16) and, therefore, was unaffected by epinephrine during the 10-min perfusion (Panel D).

Fig. 4 shows the data on ATP, Glc-6-P, and glycogen in the same hearts represented in Fig. 3. In Panel A (Fig. 4), ATP levels were initially lower in the diabetic hearts prior to epinephrine perfusion (zero time), but perfusion with 0.28 μM epinephrine for 1 to 10 min resulted in no differences between normal and diabetic hearts. In agreement with Fig. 2, Panel B (Fig. 4) shows that Glc-6-P levels were initially higher in the diabetics and that epinephrine caused a significant increase in the diabetics over the normals at 1 through 10 min (p < 0.01). Glycogen levels (Fig. 4C), already low in the normal hearts, were unchanged by this concentration of epinephrine over the 10-min perfusion, whereas glycogen levels in the diabetic

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**Fig. 2.** Concentration effects of epinephrine on ATP, Glc-6-P, and glycogen. The data presented here are for the same hearts represented in Fig. 1. Heart perfusions and protocols are the same as given for Fig. 1. Each mean represents the data from at least 12 hearts. The symbols are the same as for Fig. 1.

**Fig. 3.** Time-dependent effects of epinephrine in insulin-deficient hearts. Hearts from normal and alloxan diabetic rats were perfused for 10 min without added hormone and then for 1 to 10 min with 0.28 μM epinephrine as described under “Experimental Procedures” and in the text. Each mean represents the data from at least 12 hearts. The symbols are the same as for Fig. 1.

**Fig. 4.** Time-related effects of epinephrine on ATP, Glc-6-P, and glycogen. The data presented here are for the same hearts represented in Fig. 3. Each mean represents the data from at least 12 hearts. The symbols are the same as for Fig. 1.
hearts were statistically decreased by epinephrine after 4 min of perfusion. Therefore, Figs. 1 and 2 are in agreement with Figs. 3 and 4 showing that hearts from diabetic rats are hypersensitive to phosphorylase activation by epinephrine although cAMP accumulation and protein kinase activation are either the same or lower than in the normals.

It was next necessary to determine whether the diabetes-related hypersensitivity of phosphorylase activation to epinephrine was due to an α or a β receptor effect. Fig. 5 shows the results when hearts from normal and diabetic rats were perfused for the final 90 s with dl-propranolol (0.6 μM) without and with epinephrine (0.28 μM). In Panel A (Fig. 5), the β blocker was effective in preventing the action of epinephrine to increase cAMP in normal and diabetic hearts. In this series, cAMP levels were lower in the diabetics (p < 0.001). Protein kinase (Panel B) was slightly (p < 0.05) activated in the normal hearts at this concentration of epinephrine in the presence of propranolol, as was reported by Keely et al. (23). Protein kinase activity in the diabetics was unchanged in response to epinephrine. Panel C (Fig. 5) shows that phosphorylase activation by epinephrine was blocked by propranolol in the normal, whereas the β blocker had no apparent effect on the hypersensitivity in the diabetic (p < 0.001 in diabetic control versus diabetic with epinephrine). In Panel D, synthase activity was unchanged by epinephrine in normals but significantly decreased by epinephrine in the diabetics (p < 0.025).

In an attempt to further elucidate whether the α or β component was involved, another series of perfusion was carried out in which hearts from normal and diabetic rats were perfused for the final 90 s with 0.28 μM phenylephrine. Panels A and B (Fig. 6) show that the α agonist has no effect on cAMP or protein kinase in normal and diabetic hearts and no effects on phosphorylase and synthase in normal hearts (Panels C and D). However, phenylephrine did effectively activate phosphorylase (p < 0.005) and inactivate synthase (p < 0.005) in hearts from diabetic rats. Therefore, the data in Figs. 5 and 6 appear to show that there is an α component related to phosphorylase activation which shows up in diabetic but not normal hearts.

The question of whether the hypersensitivity was solely due to an α effect was investigated by perfusion of hearts with glucagon, a hormone which supposedly acts through neither α nor β receptor sites. Fig. 7 represents hearts from normal and diabetic rats which were perfused for the final 90 s with 0.1 μM glucagon. Panels A and B show the expected responses of cAMP accumulation and protein kinase activation in both normal and diabetic hearts. Again, however, the diabetics were hypersensitive with regards to phosphorylase activation (p < 0.01 versus normal with glucagon). Synthase was inactivated in normal hearts by glucagon and unaffected in diabetic hearts.

**Fig. 5.** Effect of β blockade on epinephrine action. Hearts from normal and alloxan diabetic rats were perfused for 10 min with hormone-free buffer and then for the final 90 s with 0.6 μM dl-propranolol without and with 0.28 μM epinephrine. Heart perfusions and protocols were described under "Experimental Procedures" and in the text. Each mean represents the data from 10 hearts. The cross-hatched bars represent diabetics and the open bars, normals. For adjacent bars in B, the left bar represents kinase assayed in the absence of added cAMP, while the bar on the right represents kinase assayed in the presence of added cAMP. For adjacent bars in C, the left bar represents phosphorylase assayed in the absence of AMP, while the right bar represents phosphorylase assayed in the presence of AMP. For adjacent bars in D, the left bar represents synthase assayed in the absence of added Glc-6-P, while the right bar represents synthase assayed in the presence of added Glc-6-P. The per cent numbers above the left bars in B, C, and D represent the per cent of the enzymes in the active form. Plus (+) or minus (−) on the abscissa represents plus or minus perfusion with epinephrine.

**Fig. 6.** α Effector activation of phosphorylase in diabetic hearts. Hearts from normal and diabetic rats were perfused for 10 min without added hormone and then for 90 s without and with 0.28 μM phenylephrine. Heart perfusions and protocols were described under "Experimental Procedures" and in the text. Each mean represents 10 hearts. The cross-hatched bars represent diabetics and the open bars, normal. Adjacent bars are as described for Fig. 5. Plus (+) and minus (−) on the abscissa represent perfusion with or without phenylephrine.

**Fig. 7.** Glucagon effects in diabetic hearts. Hearts from normal and diabetic rats were perfused for 10 min without added hormone and then for 90 s without and with 0.1 μM glucagon. Heart perfusions and protocols were described under "Experimental Procedures" and in the text. Each mean represents 10 hearts. The cross-hatched bars represent diabetics and the open bars, normals. Adjacent bars are as described for Fig. 5. Plus (+) or minus (−) on the abscissa represents perfusion with or without glucagon.
**Table I**

Effects of epinephrine and diabetes on cardiac phosphorylase kinase and phosphorylase phosphatase.

<table>
<thead>
<tr>
<th>Type</th>
<th>Epinephrine</th>
<th>Phosphorylase kinase ratio</th>
<th>Phosphorylase phosphatase μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0.114 ± 0.006</td>
<td>6.56 ± 0.49</td>
</tr>
<tr>
<td>Normal</td>
<td>+</td>
<td>0.142 ± 0.007*</td>
<td>5.80 ± 0.51*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0</td>
<td>0.126 ± 0.007*</td>
<td>6.23 ± 0.48*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>+</td>
<td>0.184 ± 0.010*</td>
<td>5.36 ± 0.57*</td>
</tr>
</tbody>
</table>

* p < 0.005 versus normal without epinephrine.

* Not significantly different than normal or diabetic without or with hormone.

* Not significantly different than normal without epinephrine.

Epinephrine Hypersensitivity and Diabetes

Hearts were perfused for 10 min without added hormone and then for 60 s without or with 0.28 μM epinephrine as described under "Experimental Procedures" and in the text. Each mean represents from 12 to 24 hearts.

Epinephrine was used to counter the β effect of epinephrine, the hypersensitivity of the diabetic hearts to phosphorylase activation remained apparent, while the effect of epinephrine on phosphorylase in normal hearts was completely abolished.

This suggested that the diabetic hypersensitivity might be related to an α effect which was not exhibited in the normal heart. Using 0.28 μM phenylephrine, a concentration of the α agonist which produced no apparent change in cAMP or protein kinase in these studies, it was demonstrated that diabetic hearts responded with increases in phosphorylase α and decreases in synthase I, whereas there was no effect in hearts from normal rats. The discrepancy between our data and that of Kecely et al. (23) where they showed phosphorylase activation by phenylephrine in the normal hearts might possibly be explained by the difference in protocols. Their perfusions consisted of a 15-min equilibration followed by a 3-min hormone exposure. Perhaps this low concentration of phenylephrine requires more time than 90 s to show the phosphorylase effect in normal hearts. The present data strongly imply that an α effect on phosphorylase activation is unmasked by the diabetic state. However, the same hypersensitivity was observed when hearts were perfused with glucagon. Therefore, it is reasonable to assume that a step beyond effector-receptor interaction common to all three hormones is involved in the hypersensitivity.

Since CAMP and protein kinase responses to these effectors in diabetic hearts were either the same as or lower than the responses in normal hearts, the affected step most probably involved the phosphorylase kinase reaction or the phosphorylase phosphatase reaction. Analyses of hearts perfused without and with epinephrine demonstrated that phosphorylase phosphatase activity, unlike glycogen synthase phosphatase (16, 24), was not diminished by diabetes. Furthermore, epinephrine was without apparent effect on phosphorylase phosphatase in both normal and diabetic hearts. However, the results did show that phosphorylase kinase in the diabetic hearts was activated twice as much as in the normal hearts in response to the same epinephrine concentration. Since protein kinase in diabetic hearts was shown to be normally sensitive or hypoactive to epinephrine and glucagon and completely unresponsive to phenylephrine, it follows that the hypersensitivity of phosphorylase activation resides in a hypersensitivity of phosphorylase kinase activation by some factor beyond protein kinase.

Catecholamines are known to alter calcium flux (increased inward current) in cardiac muscle (1) presumably by acting through cAMP, and phosphorylase kinase has long been known to be activated by calcium (25). A recent report by Owen and Carrier (26) has demonstrated a diabetes-related increase in norepinephrine-induced calcium-dependent activation of contractile responses in rat aortas. Therefore, since diabetes may either calcium metabolism in rat aortas, it is credible to extrapolate that to rat heart. Several studies have shown that α effectors can increase the force of cardiac contraction (27-31), an effect which can be blocked by α but not β antagonists (27). Therefore, it would seem reasonable to predict that α agonists might be acting to increase the force of contraction through a calcium-mediated effect. Following these lines of reasoning, one could hypothesize that diabetes somehow alters cardiac calcium metabolism or calcium-sensitive systems. The hypersensitivity of phosphorylase activation in the diabetic could then possibly be explained by a cAMP-mediated alteration in calcium flux by glucagon and the β component of epinephrine and an α effector-mediated alteration of calcium flux by phenylephrine and the α component of epinephrine. However, the increased calcium flux in the diabetic could not explain the diabetes-related increase in the pH 6.8/8.2 activity ratio for phosphorylase kinase and phosphorylase phosphatase. Analyses of hearts perfused with normal or diabetic hearts or without or with epinephrine demonstrated that phosphorylase phosphatase activity, unlike glycogen synthase phosphatase (16, 24), was not diminished by diabetes. Furthermore, epinephrine was without apparent effect on phosphorylase phosphatase in both normal and diabetic hearts. However, the results did show that phosphorylase kinase in the diabetic hearts was activated twice as much as in the normal hearts in response to the same epinephrine concentration. Since protein kinase in diabetic hearts was shown to be normally sensitive or hypoactive to epinephrine and glucagon and completely unresponsive to phenylephrine, it follows that the hypersensitivity of phosphorylase activation resides in a hypersensitivity of phosphorylase kinase activation by some factor beyond protein kinase.

**DISCUSSION**

The present study demonstrates that epinephrine, phenylephrine, and glucagon cause activation of glycogen phosphorylase to a greater degree in hearts from alloxan diabetic rats than in hearts from normal rats. When the β blocker, propranolol, was used to counter the β effect of epinephrine, the hypersensitivity of the diabetic hearts to phosphorylase activation remained apparent, while the effect of epinephrine on phosphorylase in normal hearts was completely abolished. This suggested that the diabetic hypersensitivity might be related to an α effect which was not exhibited in the normal heart. Using 0.28 μM phenylephrine, a concentration of the α agonist which produced no apparent change in cAMP or protein kinase in these studies, it was demonstrated that diabetic hearts responded with increases in phosphorylase α and decreases in synthase I, whereas there was no effect in
related to variations in the degree of diabetes induced by alloxan from one series of animals to the next. Two reports (32, 33) have demonstrated that cAMP responses to isoproterenol and epinephrine in isolated perfused rat hearts are depressed by diabetes. Therefore, the decreases in responsiveness of cAMP could be accounted for by a decrease in \( \beta \) receptor populations.

While the present study did not address the question of whether the hypersensitivity problem could be restored to normal by treatment of diabetic animals with insulin or inclusion of insulin in the perfusate, a recent study by Ingebretsen et al. (34) has shown that in vitro administration of insulin diminished the response. Therefore, it appears that insulin deficiency is responsible for the alteration.

An additional possibility, at least in regards to the effects of \( \alpha \) effectors and \( \beta \) blockers, is that diabetes produces an increase in the number or affinity of \( \alpha \) adrenergic receptors in the heart. Only further research will determine whether this mechanism is involved in the \( \alpha \)-related hypersensitivity of phosphorlyase activation in the diabetic heart.

The relationship that this phenomenon has to the increased risk of cardiovascular disease associated with diabetes mellitus remains to be elucidated. A recent report by Cryzik et al. (35) confirms the fact that diabetic dead twice as often from myocardial infarctions than matched nondiabetic controls. A diabetes-related alteration in cardiac responsiveness to catecholamines and/or calcium fluxes could be an important factor.

REFERENCES