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ACTIVATION OF MUSCLE PHOSPHORYLASE BY SUBCUTANEOUSLY INJECTED EPINEPHRINE AS RELATED TO OTHER METABOLIC CHANGES *IN VIVO*

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Abstract—Gastrocnemius muscle was quickly excised from the rat 30 min after subcutaneous injection of epinephrine and the tissue was extracted with 60% glycerol and analyzed for phosphorylase a content and the ratio of active to inactive form of phosphorylase b kinase. The subcutaneous injection of epinephrine into fasted rats was without effect on both enzyme activities, whereas epinephrine administration into glucose-fed rats or streptozotocin-diabetic rats caused a significant formation of the active forms of both phosphorylase and phosphorylase b kinase. Feeding of glucose and induction of diabetes produced lower control ratios of the active to the inactive form for both enzymes with an inverse correlation with blood levels of glucose and lactate. The lower the control activity, the more activation elicited by epinephrine. When both enzymes were assayed in the protein–glycogen complex, activation by epinephrine was detected even in muscle of the normal rat receiving neither glucose nor streptozotocin. These findings *in vivo* are interpreted as support for the concept that breakdown of muscle glycogen is induced by subcutaneously injected epinephrine via activation of phosphorylase b kinase and phosphorylase, although there is no conclusive evidence that the enzymes are really activated in normal fasted rats.

THE MECHANISM by which epinephrine and other beta-adrenergic agonists stimulate muscle glycogenolysis is explained in terms of stimulation of a series of reactions initiated by activation of adenyl cyclase.[1–5] Cyclic AMP consequently formed stimulates a cyclic AMP-dependent protein kinase, and this enzyme catalyzes the phosphorylation and activation of phosphorylase b kinase. Activated phosphorylase b kinase then catalyzes the formation of phosphorylase a from phosphorylase b and glycogen breakdown occurs. Although support for this cascade of enzymic reactions has been obtained *in vivo*,[3–7] only rapid responses (up to 60 sec) to the catecholamine infused directly into the circulation were measured in all studies in which glycogen breakdown was not correlated with increased enzyme activity. Breakdown of muscle glycogen appears to be a slower reaction and is more readily observable at 30 min in response to less acute administration (i.e. subcutaneous injection) of epinephrine. If the cascade reactions described above are necessarily responsible for the epinephrine-induced glycogenolysis in skeletal muscle, activation of phosphorylase b kinase and phosphorylase should be detected whenever the tissue level of glycogen is lowered by catecholamines.

Recent studies from this laboratory have shown that most of the metabolic actions of epinephrine *in vivo*, such as hyperglycemia, hyperlactacidemia, increased hepatic glyconeogenesis and muscle glycogenolysis and glycolysis, are observed 30 min after subcutaneous injection of epinephrine into the fasted rat at a dose of 10–20 μg/100 g

of body weight (in preparation). It was also found that muscle glycogen was the major source of blood lactate, increasing in response to epinephrine under this condition. It is conceivable, therefore, that glycogen breakdown in muscle plays a significant role in epinephrine-induced hyperglycemia as well, since blood lactate is the important precursor of blood glucose in the fasted rat. The purpose of the present study was to obtain evidence of whether phosphorylase b kinase as well as phosphorylase is activated when blood glucose and lactate are elevated and muscle glycogen is lowered by subcutaneous injection of epinephrine.

MATERIALS AND METHODS

Animal experiments. Male Wistar rats weighing 150–180 g were fasted for 20 hr prior to experiments. Adrenodemedullated rats were used 1 week after operation and diabetic rats were prepared by intravenous injection of 6·5 mg streptozotocin/100 g of body weight 4 days before the experiments.

When necessary, 50% glucose (1 ml/100 g of body weight) was force-fed through a stomach tube and anti-insulin serum (1 ml/100 g) was intravenously injected 15 and 10 min respectively prior to the subcutaneous injection of epinephrine tartrate (20 μg/100 g). Twenty min later, the skin of the rat (anesthetized with nembutal, 5 mg/100 g intraperitoneally) was cut around the ankle and pulled back so as to expose the gastrocnemius. Exactly 30 min after epinephrine administration, the gastrocnemius was dissected with scissors, quickly frozen with aluminium tongs chilled in liquid N_2 and plunged into liquid N_2 to be stored until extraction. At the same time, the rat was bled by cutting the carotid artery and the blood was collected in a centrifuge tube. Serum was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and analyzed for glucose[8] and lactate.[9] Glycogen in the frozen tissue was determined according to the method of Seifter *et al.*[10]

Preparation of tissue extract. Frozen tissues were weighed and ground to a fine powder in liquid N_2 in a mortar with the aid of a glass rod. The powdered tissue was thoroughly homogenized in 10 vol. of 60% glycerol containing 50 mM NaF and 5 mM EDTA at $-25°$.[11] The homogenate was then centrifuged at 8500 *g* for 10 min at $-5°$ and the supernatant, diluted 5-fold in 0·1 M NaF, was treated with charcoal to remove adenine nucleotides. The charcoal-treated extract was immediately assayed for phosphorylase and phosphorylase b kinase as described below.

Phosphorylase and phosphorylase b kinase assays. The extract (0·1 ml) was assayed for phosphorylase in the direction of glycogen synthesis in both the presence and absence of 5′-AMP (1 mM) using 0·1 ml of assay mixture (30 mM NaF, 2·5 mM EDTA and 0·4% glycogen in 30 mM citrate buffer at pH 6·1).[12] The reaction was started by the addition of 0·1 ml of 10 mM glucose 1-phosphate and stopped by 0·025 ml of 60% $HClO_4$ after 10 min at 30°. Inorganic phosphate liberated during the incubation was determined according to Fiske and Subbarow.[13] Activation of phosphorylase is expressed as the ratio of phosphorylase a (assayed in the absence of 5′-AMP) to total activity (in the presence of 5′-AMP). Phosphorylase b kinase was assayed by the method of Krebs *et al.*[14] with phosphorylase b purified from rabbit muscle[15] as substrate. The extract (0·1 ml) was mixed with 0·1 ml phosphorylase b in 15 mM neutral cysteine and 0·1 ml of 0·125 M Tris (pH 8·6; final pH 8·2) or 0·125 M β-glycerophosphate (pH 6·8). The mixture was placed in a water bath at 30° and the reaction was started by addition of 0·1 ml of 60 mM $MgCl_2$, 18 mM ATP

at pH 7·0. At 5 min, a 0·1-ml aliquot was removed, transferred to 1·9 ml of a mixture of 40 mM β-glycerophosphate and 30 mM cysteine (pH 6·8) and assayed for phosphorylase a as described above. All assays with appropriate controls were performed in duplicate at pH 6·8 and at pH 8·2. The degree of activation is expressed as the ratio of activity at pH 6·8 to that at pH 8·2.

Preparation of the protein–glycogen complex. In some experiments, phosphorylase and phosphorylase b kinase activity associated with muscle glycogen particles were assayed using the protein–glycogen complex prepared by the method of Meyer *et al.*[16] The powdered rat muscle, prepared as described above, was homogenized in 2 vol. of 4 mM EDTA and 2 vol. of 0·1 M NaF, and the homogenate was centrifuged at 0° for 40 min at 40,000 *g*. The supernatant solution, after being decanted through glass wool to remove lipid material, was further centrifuged at 0° for 90 min at 80,000 *g* and the sedimented pellet was taken up in an appropriate amount of H_2O to be analyzed for phosphorylase and phosphorylase b kinase.

Materials. Antiserum to insulin was prepared in guinea-pigs after three subcutaneous injections of insulin (1 mg/animal at each time) in complete Freund's adjuvant. The injections were given at 2-week intervals and the animals were bled from the carotid artery 10 days after the last immunization. Streprozotocin was a kind gift from the Upjohn Co. through the courtesy of Dr. W. E. Dulin. Nucleotides and glucose 1-phosphate were obtained from the Sigma Chemical Co.

RESULTS

Activation of phosphorylase and phosphorylase b kinase by epinephrine in glucose-fed rats. Subcutaneous injection of epinephrine into fasted normal rats resulted in no changes of phosphorylase and phosphorylase b kinase at 30 min, despite a significant increase in blood glucose and lactate as well as a decline of muscle glycogen (Expt. 1, Table 1). In order to detect activation of the enzymes, similar experiments were repeated in which we tried to potentiate the action of exogenous epinephrine by

TABLE 1. EFFECT OF EPINEPHRINE ON SERUM GLUCOSE, SERUM LACTATE, MUSCLE GLYCOGEN, PHOSPHORYLASE AND PHOSPHORYLASE b KINASE IN FASTED OR GLUCOSE-FED RATS*

	Expt. 1 Normal fasted rat		Expt. 2 Adrenodemedullated glucose-fed rat	
	Control(5)	Epinephrine(5)	Control(6)	Epinephrine(6)
Serum glucose (mg/100 ml)	128 ± 2·1	207 ± 1·6 <0·01†	235 ± 28·1	407 ± 27·6 <0·01
Serum lactate (mg/100 ml)	19·1 ± 1·6	35·9 ± 6·7 <0·05	37·3 ± 4·7	46·1 ± 5·7 <0·05
Muscle glycogen (mg/g tissue)	5·7 ± 0·70	3·9 ± 0·60 <0·01	4·6 ± 0·36	3·4 ± 0·48 <0·01
Phosphorylase activity (−AMP/+AMP)	0·28 ± 0·02	0·29 ± 0·07 NS	0·22 ± 0·055	0·46 ± 0·067 <0·01
Phosphorylase b/kinase (pH 6·8/pH 8·2)	0·20 ± 0·021	0·20 ± 0·032 NS	0·11 ± 0·009	0·23 ± 0·027 <0·01

* The data, mean ± standard error of the mean, were obtained on blood specimens and gastrocnemius taken at 30 min after subcutaneous injection of epinephrine, 20 μg/100 g of body weight, or saline in the same volume. In Expt. 2, 50% glucose, 1 ml/100 g, was force-fed to fasted adrenodemedullated rats 15 min prior to injection of epinephrine. Figures in parentheses are the number of observations.

† Significance level for epinephrine effect; NS, not significant.

TABLE 2. EFFECT OF EPINEPHRINE ON SERUM GLUCOSE, SERUM LACTATE, MUSCLE GLYCOGEN, PHOSPHORYLASE AND PHOSPHORYLASE b KINASE IN RATS INJECTED WITH ANTI-INSULIN SERUM*

	Control(6)	Epinephrine(6)
Serum glucose (mg/100 ml)	219 ± 25·5	392 ± 21·6 <0·01†
Serum lactate (mg/100 ml)	58·9 ± 9·6	46·6 ± 5·0 NS
Muscle glycogen (mg/g tissue)	4·9 ± 0·36	3·7 ± 0·26 <0·01
Phosphorylase activity (−AMP/+AMP)	0·33 ± 0·039	0·46 ± 0·071 <0·01
Phosphorylase b kinase (pH 6·8/pH 8·2)	0·081 ± 0·013	0·214 ± 0·021 <0·01

* The data were obtained on blood specimens and gastrocnemius taken at 30 min after subcutaneous injection of epinephrine, 20 μg/100 g of body weight, or saline in the same volume. The rats were force-fed 50% glucose, 1 ml/100 g, and anti-insulin serum, 1 ml/100 g, was injected 15 and 30 min respectively prior to the injection of epinephrine. Figures in parentheses are the number of observations.

† Significance level for epinephrine effect.

changing the dose and route of epinephrine injection or by decreasing the endogenous catecholamine by means of adrenodemedullation. In no case were the epinephrine-induced changes in blood glucose, blood lactate and muscle glycogen levels accompanied by an increase of phosphorylase or phosphorylase b kinase.

When the fasted adrenodemedullated rats had been force-fed with 50% glucose, however, conversion of phosphorylase b to phosphorylase a as well as conversion of nonactivated phosphorylase b kinase to the activated form was observed together with other metabolic changes at 30 min after epinephrine injection (Expt. 2, Table 1). Similar activation of both enzymes was obtained with the fasted non-operated rats

TABLE 3. EFFECT OF EPINEPHRINE ON SERUM GLUCOSE, LACTATE, MUSCLE GLYCOGEN, PHOSPHORYLASE AND PHOSPHORYLASE b KINASE IN STREPTOZOTOCIN-INDUCED RATS*

	Expt. 1 With glucose feeding		Expt. 2 Without glucose feeding	
	Control(4)	Epinephrine(6)	Control(4)	Epinephrine(4)
Serum glucose (mg/100 ml)	612 ± 34·9	736 ± 28·1 NS†	247 ± 18·1	570 ± 21·1 <0·01
Serum lactate (mg/100 ml)	41·1 ± 4·1	53·7 ± 3·3 <0·05	33·0 ± 3·0	43·9 ± 5·8 <0·05
Muscle glycogen (mg/g tissue)	6·1 ± 0·36	2·5 ± 0·32 <0·01	8·0 ± 0·38	5·1 ± 0·25 <0·01
Phosphorylase activity (−AMP/+AMP)	0·19 ± 0·014	0·46 ± 0·036 <0·01	0·18 ± 0·026	0·52 ± 0·025 <0·01
Phosphorylase b kinase (pH 6·8/pH 8·2)	0·071 ± 0·01	0·23 ± 0·049 <0·01	0·155 ± 0·007	0·231 ± 0·008 <0·01

* The data were obtained on blood specimens and gastrocnemius taken at 30 min after subcutaneous injection of epinephrine, 20 μg/100 g of body weight, or saline in the same volume into streptozotocin-induced diabetic rats. Where indicated, 50% glucose, 1 ml/100 g, was force-fed to the fasted rats 15 min prior to injection of epinephrine. Figures in parentheses are the number of observations.

† Significance level for epinephrine effect.

injected intravenously with 50% glucose 5 min prior to epinephrine injection (data not shown).

Activation of phosphorylase and phosphorylase b kinase in insulin-deficient rats. The above findings indicated that administration of glucose into rats provides a favorable condition for epinephrine activation of phosphorylase and phosphorylase b kinase. Since glucose administration is one of the most potent stimuli to secretion of insulin from pancreatic β-cells, it might be likely that the insulin secreted in response to glucose is involved in the epinephrine-induced activation of the muscle enzymes. The effect of epinephrine was therefore studied in rats in which circulating insulin was neutralized with anti-insulin serum. Blood levels of glucose and lactate rose strikingly after injection of anti-insulin serum to glucose-fed rats (Table 2). After epinephrine, blood glucose was further elevated but lactate did not show any statistical change. It is also seen that both phosphorylase and phosphorylase b kinase were activated by epinephrine with a simultaneous decrease of muscle glycogen after epinephrine under this condition.

Experiment 1 in Table 3 shows the response of the glucose-fed streptozotocin-diabetic rats to epinephrine. Blood levels of glucose and lactate in diabetic rats were extraordinarily high after glucose feeding and the further increases after additional treatment with epinephrine were barely significant. In the same rat, the diminution of muscle glycogen induced by epinephrine was very striking and was associated with strong activation of phosphorylase and phosphorylase b kinase. It can be concluded from Tables 2 and 3, therefore, that epinephrine-induced activation of phosphorylase and phosphorylase b kinase observed after glucose feeding is not mediated through the action of endogenous insulin. Instead, it is very likely that the increased level of blood sugar plays a role in potentiating the action of epinephrine, resulting in conversion of the inactive form to the active form of the muscle enzymes. This view is supported by the fact that the b to a conversion of muscle phosphorylase took place in diabetic rats having high levels of blood glucose even if no glucose had been administered (Expt. 2, Table 3).

Relation of blood level of glucose or lactate to the activities of phosphorylase b kinase and phosphorylase. Surveying the results in Tables 1–3 reveals that the ratio of the active to the inactive form of phosphorylase b kinase in the absence of epinephrine was reduced by glucose feeding or induction of diabetes and that the glucose- or diabetes-induced reduction was exaggerated by their superposition (0·20 for normal, Expt. 1, Table 1; 0·11 for glucose-fed, normal, Expt. 2, Table 1; 0·16 for diabetes, Expt. 2, Table 3; 0·08 for glucose-fed, anti-insulin serum injected, Table 2). A similar trend was noted in the case of muscle phosphorylase of control rats receiving no epinephrine.

All of the pH 6·8/8·2 ratios of phosphorylase b kinase as well as of the relative amounts of phosphorylase a of control rats presented in Tables 1–3 are plotted in Fig. 1 as a function of blood glucose or lactate level. One set of data obtained for the normal rat without any further treatment which is not recorded in the tables is added to Fig. 1. Although Fig. 1 shows some scatter, there is a tendency toward an inverse relationship between the activities of both enzymes on the one hand and blood glucose and lactate levels on the other. The degree of activation by epinephrine of both enzymes is also plotted as a function of the basal enzymic activities obtained without epinephrine in Fig. 2. It is seen that epinephrine activates phosphorylase b

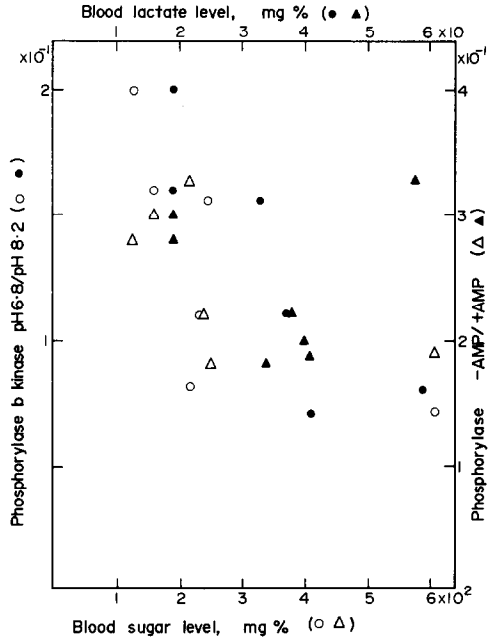

FIG. 1. Relation of phosphorylase b kinase activity ratio and phosphorylase activity ratio in control muscle to blood levels of glucose and lactate. The control values in Tables 1–3 are used to plot these relationships. Open circles (phosphorylase b kinase) and triangles (phosphorylase) are plotted as a function of blood glucose, while the solid circles and triangles are plotted as a function of blood lactate.

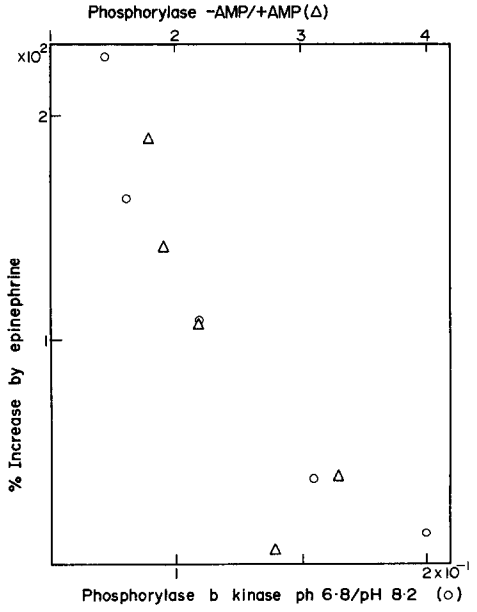

FIG. 2. Activation by epinephrine of phosphorylase b kinase and phosphorylase as a function of control values. The percent increase by epinephrine of phosphorylase b kinase activity ratio (open circle) and phosphorylase activity ratio (open triangle) is calculated from the data in Tables 1–3 and plotted as a function of control values.

kinase and phosphorylase only when their activities before epinephrine injection have been lowered. Based on the relationships illustrated in Figs. 1 and 2, we were led to the conclusion that the increase in blood glucose and/or lactate leads to suppression of the activities of phosphorylase b kinase and phosphorylase in the muscle, which enhances the sensitivity of the enzymes to epinephrine to such a degree as to be detectable under the present condition.

Activation of phosphorylase and phosphorylase b kinase in the protein–glycogen complex. Fischer *et al.*[16–19] have shown that the enzymes involved in the phosphorolysis of glycogen are associated with a protein–glycogen complex that can be isolated from rabbit muscle as a specific structural entity of the cell. They suggested that this integrated enzyme system would retain some of the enzymic properties in intact muscle. It is probable, therefore, that the activity of phosphorylase and phosphorylase b kinase estimated in the protein–glycogen complex reflects their activity in the intact muscle cell more closely than in other more disrupted systems *in vitro*. In accord with their findings,[17] phosphorylase was essentially in its inactive b-form (only 2–3 per cent was in the a-form) in the protein–glycogen complex of rat muscle prepared by a technique exactly the same as their procedure proposed for rabbit muscle. Since their original homogenization medium does not contain NaF, an inhibitor of phosphorylase phosphatase, the absence of phosphorylase a would be likely to have resulted from its conversion to the b-form during homogenization and centrifugation. In order to retain the state of the enzymes in the cell, the protein–glycogen complex was prepared in a medium which contains 10 mM NaF in addition to the ionic components used by Meyer *et al.*[16] The enzyme activities in the glycogen particles thus obtained are shown in Table 4. It is seen that about 50 per cent of the phosphorylase was present in the active a-form before epinephrine injection and that epinephrine was very effective in further increasing phosphorylase a, even in the rat receiving no glucose. Likewise, phosphorylase b kinase in the same particle was activated by epinephrine.

TABLE 4. EFFECT OF EPINEPHRINE ON PHOSPHORYLASE AND PHOSPHORYLASE b KINASE OF THE PROTEIN–GLYCOGEN COMPLEX PREPARED FROM SKELETAL MUSCLE OF NORMAL FASTED RATS*

	Control	Epinephrine
Phosphorylase activity(9) (−AMP/+AMP)	0·56 ± 0·131	0·99 ± 0·075 0·01*
Phosphorylase b kinase(4) (pH 6·8/pH 8·2)	0·14 ± 0·033	0·39 ± 0·047 0·01

* The data were obtained on the protein–glycogen complex prepared from gastrocnemius taken from normal fasted rats 30 min after subcutaneous injection of epinephrine, 20 μg/100 g of body weight, or saline in the same volume. Figures in parentheses are the number of observations.

† Significance level for epinephrine effect.

Although convincing evidence is not available at present that the phosphorylase activity measured in the isolated protein–glycogen complex really reflects the situation in the intact cell of rat muscle, the present result is a strong indication that

the breakdown of glycogen induced *in vivo* by subcutaneous injection of epinephrine is associated with the activation of phosphorylase b kinase and phosphorylase in accord with the cascade reactions proposed, based mainly on observations *in vitro*.

DISCUSSION

All of the studies which have so far revealed the activation of phosphorylase and phosphorylase b kinase *in vivo* by epinephrine dealt with the rapid response of the muscle suddenly exposed to a high concentration of the catecholamine injected directly into the blood stream. In contrast, subcutaneously injected epinephrine causes no detectable changes in phosphorylase b kinase and phosphorylase activities despite a significant breakdown of muscle glycogen as exemplified in Expt. 1 in Table 1. It is unlikely, however, that the breakdown of glycogen occurring in muscle exposed to gradually increasing epinephrine is dependent on a mechanism other than that involving activation of phosphorylase b kinase and phosphorylase. When the fasted rat was fed glucose, both enzymes were activated after subcutaneous injection of epinephrine. Figs. 1 and 2 suggest that glucose feeding renders phosphorylase b kinase and phosphorylase more sensitive to epinephrine as a result of reduced control activity. It is likely, therefore, that the degree of activation of both enzymes achieved *in vivo* by the subcutaneous injection of epinephrine into the fasted rat was so small, as compared with what has been obtained *in vitro*, by intravenous injection or by subcutaneous injection into the glucose-fed or diabetic rat, as to be obscured by experimental errors associated with studies *in vivo*.

A possible role of glucose in the regulation of muscle phosphorylase activity was suggested by Holmes and Mansour[20,21] based on their studies with the use of rat diaphragm *in vitro*. They reported that glucose added to the incubation medium caused a marked decrease in phosphorylase a levels by stimulating the conversion of phosphorylase a to phosphorylase b catalyzed by phosphorylase phosphatase. The inhibitory effect of glucose *in vitro* on liver phosphorylase was also observed during perfusion of rat liver.[22] The inverse correlation between phosphorylase a and blood glucose levels illustrated in Fig. 1, though not highly significant in a statistical sense, is in accord with these observations *in vitro*. In our studies *in vivo* however, it remains to be settled that glucose is the only factor in blood in determining the level of phosphorylase a, because glucose feeding and induction of diabetes, which are effective in reducing the phosphorylase a level, bring forth a rise not only of blood glucose but also of blood lactate. The conversion of phosphorylase a to phosphorylase b induced by glucose feeding or by induction of diabetes appears to result from inactivation of phosphorylase b kinase rather than from activation of phosphorylase phosphatase, since these treatments caused a simultaneous reduction of the ratio of the active to the inactive form of phosphorylase b kinase. Thus, a somewhat different mechanism from that proposed by Holmes and Mansour[20,21] would be involved in the regulation of phosphorylase by blood glucose (or lactate) *in vivo*. In any event, the lower the activity of phosphorylase and phosphorylase b kinase was maintained, the more were both enzymes activated by epinephrine, resulting in a statistically significant activation even after a mild administration such as subcutaneous injection.

The possibility that phosphorylase and phosphorylase b kinase are activated, even after subcutaneous injection of epinephrine into fasted rats receiving neither glucose

nor streptozotocin, was further tested by assaying both enzymes in the protein–glycogen complex which apparently is better than other enzyme preparations in retaining the state of the enzymes in the intact muscle cell. However, the values obtained here for the phosphorylase a content of the protein–glycogen complex are questionable as an exact measure of the enzymic activities in intact muscle cells for the following reasons. About half of the phosphorylase was in the active a-form in the protein–glycogen complex. This value is much higher than that obtained in the extract with 60% glycerol at −25°. Since the phosphorylase a content was only 2–3 per cent upon preparation of the complex in medium without NaF the interconversion between the active and inactive forms of phosphorylase and phosphorylase b kinase is likely to take place during homogenization at 0° before the inhibitors, EDTA and NaF, penetrate the muscle powder, as shown by Danforth *et al.*[11] Therefore, an explanation for the extraordinarily high values of phosphorylase a is that the phosphorylase a content as well as the ratio of the active to inactive form of phosphorylase b kinase in our preparation of the protein–glycogen complex involves artifacts produced during manipulation *in vitro*. It should be emphasized that, despite the apparent artifact involved, the per cent of a-form of the total phosphorylase as well as the pH 6·8/8·2 ratio of phosphorylase b kinase increased to a statistically significant degree after epinephrine administration. Our procedure for isolation of the protein–glycogen complex, unlike the original one by Meyer *et al.*[16] involves freezing of muscle in liquid N_2 before homogenization. It is probable that the integrity of the subcellular particulates is damaged to a certain extent by freezing. Probably for this reason, the total (a + b) activity of phosphorylase in the final pellet was found to be lower than that in the 60% glycerol extract. This finding suggests that the enzyme had been liberated in part from the glycogen particles during centrifugation. An alternative explanation then might be that not the total but only fractions of phosphorylase and phosphorylase b kinase are associated with our preparation of the protein–glycogen complex, with different affinities between the active and inactive states of the enzymes, resulting in more association of the active than of the inactive form.

Based on these arguments, the present results strongly suggest that epinephrine injected subcutaneously into the fasted rat caused the breakdown of muscle glycogen via activation of phosphorylase and phosphorylase b kinase, although the degree of activation recorded in Table 4 apparently fails to represent the real situation in intact cells quantitatively. It is also likely that starvation is a "stress" situation and that the catecholamine-sensitive enzymes such as phosphorylase b kinase were activated by high titers of circulating catecholamines. As a result, the effects of exogenous catecholamines in the fasted state would be much smaller than their effects in rats having higher blood glucose levels. Probably, the endogenous catecholamines increasing in starvation stress originated not only from the adrenal medulla but also from the adrenergic nerve endings, because the enzymes in muscle of fasted adrenodemedullated rats also showed no measurable response to exogenous catecholamine. Further work is necessary to determine finally the exact mechanism by which epinephrine activates muscle phosphorylase and phosphorylase b kinase *in vivo*.

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