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EFFECT OF EPINEPHRINE ON HEPATIC GLYCOGEN PHOSPHORYLASE AND SYNTHETASE ACTIVITIES IN NORMAL AND PERTUSSIS-SENSITIZED RATS

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Abstract—To investigate the nature of the diminished hyperglycemic response to epinephrine in pertussis-sensitized rats, we compared the response to epinephrine of the hepatic enzymes, glycogen phosphorylase and glycogen synthetase, and of peripheral lactate release in normal and sensitized rats. The epinephrine-induced activation of hepatic phosphorylase was markedly diminished in pertussis rats. Pertussis sensitization did not alter either the decrease in hepatic glycogen synthetase activity or the elevation of plasma lactate produced by epinephrine administration. The findings indicate that a diminished activation of hepatic phosphorylase, associated with and possibly dependent upon a relative hyperinsulinism, can account for the attenuated hyperglycemic response to epinephrine in pertussis rats.

THE ADMINISTRATION of *Bordetella pertussis* vaccine to mice and rats results in a marked attenuation of epinephrine-induced hyperglycemia.[1–3] The exact mechanism involved is unknown, although Szentivanyi *et al.*[1] have proposed that a pertussis-sensitized animal exhibits metabolic alterations similar to that produced by β-adrenergic receptor blockade. More recently it has been reported that pertussis sensitization results in a significant elevation of plasma immuno-assayable insulin levels.[3]

It is now established that epinephrine stimulates the formation of cyclic 3′,5′-AMP and causes the activation of glycogen phosphorylase (α-1,4-glucan: orthophosphate glucosyl transferase, EC 2.4.1.1) in heart,[4, 5] skeletal muscle[5–7] and liver.[6, 8, 9] It has been demonstrated that the increased phosphorylase activity results from the activation of phosphorylase b kinase by the elevated levels of cyclic 3′,5′-AMP.[4, 7] In addition, it is now clear that the level of hepatic glycogen, and indirectly the rate of hepatic glucose output and the level of plasma glucose, are regulated not only by the rate of glycogen degradation (phosphorylase activity), but also by the rate of glycogen synthesis (glycogen synthetase activity).

Glycogen synthetase (UDP-glucose: α-1,4-glucan-α-4-glucosyl transferase, EC 2.4.1.11) exists in at least two forms (synthetase I and synthetase D) that are interconvertible.[10, 11] All evidence indicates that under physiological conditions only the glucose-6-phosphate independent form (synthetase I) is active.[12–15] Synthetase I can be phosphorylated to the inactive form by the action of synthetase I kinase. It is of interest to note, therefore, that under conditions resulting in elevated cyclic 3′,5′-AMP

levels both synthetase I kinase[16] and phosphorylase b kinase[7] would be activated with the subsequent inhibition of glycogenesis and stimulation of glycogenolysis.[5, 17]

This paper reports studies on the possible mechanism of the attenuated hyperglycemic response to epinephrine in pertussis-sensitized rats and demonstrates a diminished activation of hepatic phosphorylase in these animals.

MATERIALS AND METHODS

UDP-glucose and UDP-glucose dehydrogenase were obtained from Sigma Chemical Co. Other substrates, cofactors and enzymes were obtained from either Sigma Chemical Company or Boehringer Mannheim Corp. MJ 1999* was generously supplied by Mead Johnson Laboratories, Evansville, Ind. *Bordetella pertussis* vaccine was obtained from Eli Lilly & Company and contained not less than 96×10^9 organisms/ml.

Charles River CD-strain male rats (200–250 g) were used for all experiments. Rats were pertussis-sensitized 5 days prior to use by a single injection of the vaccine (1 ml per rat, i.p.).[2] Epinephrine bitartrate (1 mg/kg, calculated as the free base) was administered subcutaneously either 30 or 20 min (phosphorylase and synthetase experiments) prior to sacrificing the animals. In some experiments, MJ 1999 was administered (20 mg/kg, s.c.) 30 min before epinephrine. All rats were anesthetized with pentobarbital sodium (45 mg/kg, i.p.) 40 min before sacrificing. This was necessary in order to eliminate the artifactual activation of hepatic phosphorylase that occurs when animals are stressed at sacrificing.[6]

Blood was withdrawn from the abdominal aorta in a heparinized syringe, chilled on ice, and plasma removed within 15 min for insulin, glucose and lactate determinations. For lactate and glucose analysis, the plasma was deproteinized with perchloric acid followed by neutralization with $KHCO_3$. After removal of the insoluble $KClO_4$, the supernatant fluid was assayed. Livers were rapidly removed, quick-frozen in liquid nitrogen, and stored at −80° until they could be prepared for substrate or enzyme analysis.

Insulins were estimated by the double antibody technique of Hales and Randle.[18] Acid extracts of liver were prepared for substrate analysis by a slight modification of the procedure described by Lowry *et al.*[19] The concentrations of substrates were determined fluorometrically (Farrand model A-3 fluorometer) by measuring the appearance of NADH or NADPH upon the addition of the appropriate enzymes. Glucose and glucose-6-phosphate were determined as described by Lowry *et al.*[19] Because of the very low tissue level of glucose-1-phosphate, it was necessary to assay this substance in Florisil-treated liver extracts[20] by a two-step procedure. First, the glucose-6-phosphate in the sample was removed by incubating 300 μl of tissue extract with 600 μl of a reagent mixture containing: 45 μmoles Tris-HCl, pH 7·8; 0·12 μmole NADP; 1·8 μmoles $MgCl_2$; and 0·3 i.u. glucose-6-phosphate dehydrogenase. The NADPH produced was destroyed by the addition of 5 N HCl.[21] After neutralization of the acid with an equivalent amount of NaOH, the glucose-1-phosphate was measured by the addition of 0·12 i.u. glucose-6-phosphate dehydrogenase and 0·24 i.u. phosphoglucomutase. Lactate was estimated in 1 ml of a reagent mixture containing: 200 μmoles carbonate buffer, pH 9·7; 50 μmoles hydrazine-HCl, pH 9·7; 0·3 μmole NAD; 0·1 mg bovine serum albumin; and 10 i.u. lactic dehydrogenase. UDP-glucose was determined using UDP-glucose dehydrogenase.

* 4-(2-Isopropylamino-1-hydroxyethyl)methane sulfonanilide HCl.

Glycogen phosphorylase activity was determined by a modification of the method of Hers.[22] A 15-μl aliquot of a 1:10 tissue homogenate was incubated with 200 μl of a reagent mixture containing: 6 μmoles glucose-1-phosphate; 10 μmoles glycogen; 10 μmoles NaF; 0·2 μmole 5'-AMP; final pH of 6·1. Samples were incubated for 10 min at 37°. The amount of P_i liberated was determined by the method of Fiske and SubbaRow.[23] Glycogen synthetase activity was estimated as previously described.[13]

RESULTS

Changes in plasma insulin, glucose and lactate

To investigate the proposed similarity of pertussis sensitization and β-receptor blockade, we compared the effects of pertussis sensitization and a β-adrenergic blocking agent (MJ 1999)[24] on plasma glucose and lactate levels in the presence and absence of epinephrine.

In agreement with previous findings,[3] pertussis-sensitized rats exhibited a marked attenuation of epinephrine-induced hyperglycemia accompanied by a significant elevation of plasma insulin levels (Table 1). The level of insulin in normal rats was decreased by epinephrine. Pertussis treatment, however, did not alter the hyperlacticacidemic effect of epinephrine seen in normal animals. In contrast, pretreatment

TABLE 1. EFFECT OF EPINEPHRINE (EPI) ON PLASMA INSULIN, GLUCOSE AND LACTATE IN NORMAL AND PERTUSSIS-SENSITIZED RATS*

Treatment†	Plasma insulin (μunits/ml)	Plasma glucose (mg/100 ml)	Plasma lactate (μmoles/100 ml)
Normal	252 ± 37	144 ± 4	352 ± 20
Pertussis	780 ± 121‡	134 ± 4	484 ± 29‡
Normal + EPI	128 ± 17‡	376 ± 5‡	753 ± 44‡
Pertussis + EPI	697 ± 74‖	177 ± 7§‖	908 ± 103§

* Nonfasting rats were injected with EPI (1 mg/kg, s.c.) 30 min before bleeding. Values are expressed as mean ± S.E. of the mean.
† Six rats per group.
‡ $P < 0·01$ compared with normal.
§ $P < 0·01$ compared with pertussis.
‖ $P < 0·01$ compared with normal + EPI.

TABLE 2. EFFECT OF EPINEPHRINE (EPI) AND MJ 1999 ON PLASMA GLUCOSE AND LACTATE IN NORMAL RATS*

Treatment	No. of rats	Plasma glucose (mg/100 ml)	Plasma lactate (μmoles/100 ml)
Normal	5	140 ± 4	174 ± 20
Normal + EPI	4	315 ± 9†	426 ± 23†
Normal + MJ 1999	5	151 ± 6	175 ± 9
Normal + MJ 1999 + EPI	5	362 ± 18†	221 ± 25‡

* Nonfasting rats were injected with MJ 1999 (20 mg/kg, s.c.) 30 min before EPI (1 mg/kg, s.c.). Rats were bled 30 min after EPI. Values are expressed as mean ± S.E. of the mean.
† $P < 0·01$ compared with normal.
‡ $P < 0·01$ compared with normal + EPI.

of normal rats with MJ 1999 (30 min before epinephrine) significantly diminished the epinephrine-induced elevation of plasma lactate levels (Table 2). It is difficult to explain the variation in the level of plasma lactate in normal rats (Table 1 vs. Table 2) seen from experiment to experiment. However, we have consistently found only minimal variability within an experiment and the response to epinephrine has been a 2- to 3-fold increase, regardless of the basal lactate level (2·1-fold in Table 1 vs. 2·4-fold in Table 2). The hyperglycemic response to epinephrine was not altered by MJ 1999 (315 mg/100 ml vs. 362 mg/100 ml).

Activation of hepatic glycogen phosphorylase

The pertussis-sensitized animal is known to have normal levels of liver glycogen,[2] although the ability to rapidly mobilize the glycogen after epinephrine has not been investigated. Figure 1 illustrates the effect of epinephrine in normal and sensitized rats

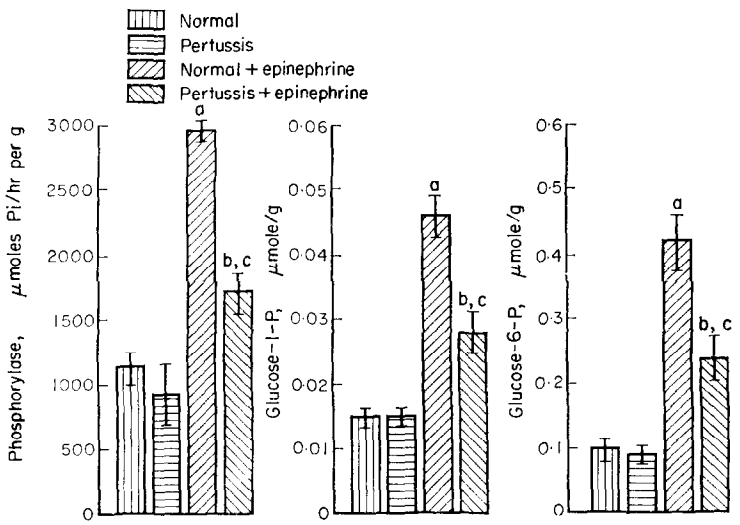

FIG. 1. Effect of epinephrine on liver glycogen phosphorylase, glucose-1-phosphate and glucose-6-phosphate in normal and pertussis-sensitized rats. Nonfasting rats were injected with epinephrine (1 mg/kg, s.c.) 20 min before sacrificing. Values represent the mean ± S.E. of the mean (four to six rats per group). Enzyme activity is expressed as micromoles of product formed per hour per gram wet weight of liver.

a = $P < 0.01$ compared with normal; b = $P < 0.01$ compared with pertussis; c = $P < 0.01$ compared with normal + epinephrine.

on liver phosphorylase activity and levels of the immediate products of glycogenolysis, glucose-1-phosphate and glucose-6-phosphate. Within 20 min after the administration of epinephrine to normal rats, there was about a 3-fold increase in the levels of active phosphorylase, glucose-1-phosphate and glucose-6-phosphate. Prior sensitization with pertussis diminished by 50–70 per cent the epinephrine effect on all three measured parameters. The basal (no epinephrine) levels of phosphorylase, glucose-1-phosphate and glucose-6-phosphate in normal and sensitized rats were not significantly different.

Effect of epinephrine on hepatic glycogen synthetase

The effect of epinephrine on the biosynthetic pathway of hepatic glycogen synthesis in normal and sensitized rats is shown in Fig. 2. The activity of glycogen synthetase and the level of its substrate, UDP-glucose, were determined. Synthetase activity was measured both in the presence (total enzyme activity) and absence (synthetase I activity) of 10 mM glucose-6-phosphate.[11–13] The administration of epinephrine to both normal and pertussis rats resulted in almost complete disappearance of synthetase

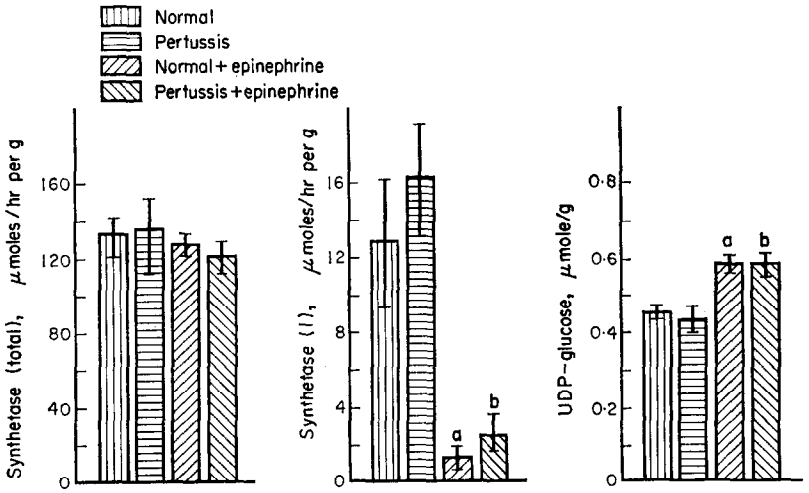

FIG. 2. Effect of epinephrine on liver glycogen synthetase and UDP-glucose in normal and pertussis-sensitized rats. Nonfasting rats were injected with epinephrine (1 mg/kg, s.c.) 20 min before sacrificing. Values represent the mean ± S.E. of the mean (four to six rats per group). Enzyme activity is expressed as micromoles of product formed per hour per gram wet weight of liver. a = $P < 0{\cdot}01$ compared with normal; b = $P < 0{\cdot}01$ compared with pertussis.

I activity with no change in total synthetase. Synthetase I decreased from 10–12 per cent of total synthetase to 1–2 per cent within 20 min after epinephrine administration. Associated with the loss of synthetase I activity was a significant increase in UDP-glucose levels. The effect of epinephrine on glycogen synthetase and UDP-glucose in sensitized rats was not significantly different from that in normal animals.

DISCUSSION

In order to elucidate the possible mechanism of the altered metabolic pattern in pertussis-sensitized rats, we investigated the effects of epinephrine on peripheral glycogenolysis and the hepatic glycogen phosphorylase and synthetase pathways in normal and sensitized rats.

It is well established[6, 24, 25] that skeletal and cardiac muscle glycogenolysis is mediated through an activation of β-adrenergic receptors and results in elevated plasma lactate levels. All these parameters are sensitive to inhibition by the β-blocking agent, MJ 1999. Therefore, our data demonstrating no attenuation in pertussis rats of epinephrine-induced hyperlacticacidemia are not consistent with the proposed[1] similarity of β-receptor blockade and pertussis sensitization. In fact, there was a small

but significant increase in basal lactate levels in pertussis rats. Studies involving the measurement of muscle phosphorylase activity will be needed to further clarify this point.

The fact that epinephrine and glucagon promote an activation of glycogen phosphorylase and inactivation of glycogen synthetase in tissues where the hormones elevate cyclic 3′,5′-AMP levels has led to the proposed reciprocal control of the two systems by the cyclic nucleotide.[5, 17] The effects of epinephrine and glucagon appear to be opposed, at least in liver, by the action of insulin.[9, 17] The reported decrease in hepatic glucose output and stabilization of liver glycogen after insulin[26, 27] may be related to a decrease in tissue cyclic 3′,5′-AMP levels, resulting in decreased phosphorylase and increased synthetase activity and subsequent net glycogen formation. The diminished hyperglycemic response to epinephrine in pertussis-sensitized rats may be explained, at least in part, by the presence of marked hyperinsulinism and a significant inhibition of phosphorylase activation. The differences are further accentuated by the reduction of basal insulin levels in normal rats given epinephrine. It is of interest to speculate that the diminished phosphorylase activation after epinephrine administration to pertussis rats may be secondary to the failure of epinephrine to lower insulin levels in this group as opposed to its action in normal controls. The finding that basal levels of phosphorylase were not significantly different in normal and sensitized rats is not unexpected, since Jefferson *et al.*[9] reported that insulin only slightly decreases basal cyclic 3′,5′-AMP levels (perfused rat liver) while markedly reducing the elevated cyclic nucleotide levels induced by epinephrine and glucagon.

It is difficult to explain why pertussis sensitization (and hyperinsulinism) did not prevent the epinephrine-induced inactivation of glycogen synthetase (loss of synthetase I activity), since this enzyme is usually responsive to insulin.[11, 13, 17] Certainly one possibility is a different degree of sensitivity of synthetase I kinase and phosphorylase b kinase to activation by endogenous levels of 3′,5′-AMP. This provocative aspect, as well as the effect of epinephrine on cyclic 3′,5′-AMP levels in sensitized rats, requires further study.

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