Effect of Endotoxin on Phosphoenolpyruvate Carboxykinase Activity in Mouse Liver (36753)

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Berry, Smythe and Young (1) showed that injection of an LD_{50} of endotoxin into mice resulted in a dramatic loss of liver glycogen and body carbohydrate, and that cortisone prevents this loss of carbohydrate. Only recently have attempts been made to explain this carbohydrate loss at an enzymatic level. We (2) reported an increase in the activity of pyruvate kinase and suggest that carbohydrate loss may be associated with increased enzyme activity. Others (3) suggested that endotoxin-poisoned mice are unable to convert body protein into carbohydrate. Enzyme measurements of liver phosphoenol pyruvate carboxykinase (PEPCK) supported this suggestion by showing that endotoxin prevented the induction of this enzyme by cortisone (4).

This report describes the changes in liver PEPCK activity at several intervals after treatment of mice with endotoxin and cortisone, singly and in combination. To determine if protection from the lethal effects of endotoxin is associated with maintenance of normal enzyme levels, the liver PEPCK activity of endotoxin-tolerant mice was measured.

Material and Methods. Animals. Female mice (Swiss-Webster, Laboratory Supply, Indianapolis) weighing 20 ± 2 g were used. The protocol for handling the mice after delivery has been previously described (5). Oxytetracycline (Charles Pfizer and Company, New York) was added to their drinking water for the first 2 days after delivery. Two control groups of animals were used throughout the study: uninjected but fed mice, and uninjected fasted mice. Injected mice were fasted from 5:00 PM the evening before the assay until the time of assay 16 hr later.

Endotoxin. Heat-killed cells of *Salmonella typhimurium* SR-11, suspended in nonpyrogenic saline were used as a source of crude endotoxin in all experiments except as the challenge dose in the tolerance studies. The LD_{50} was approximately 10^{10} cells. One LD_{50} was contained in 0.5 ml of the suspension.

Tolerance production. Mice were made tolerant to the crude endotoxin by a schedule of daily intraperitoneal injections (6). The animals received 0.1, 0.1, 0.2, 0.2, 0.4, and 0.4 LD_{50} doses on successive days; each dose was administered in 0.5 ml of nonpyrogenic saline. The animals were used experimentally 48 hr after the last injection. In the tolerance studies, endotoxin from *Serratia marcescens* was used; it was donated by Dr. L. J. Berry, Department of Microbiology, University of Texas, Austin, TX. Challenged animals received an LD_{50} of this endotoxin (from *S. marcescens*) in 0.5 ml pyrogen-free saline.

Injections. Sterile nonpyrogenic saline was used as diluent for all injectionable substances. Cortisone acetate (Upjohn Co., Kalamazoo, MI) was injected subcutaneously into the interscapular region in 0.5 ml amounts containing 5 mg of the hormone.

Enzyme assay. Mice were sacrificed by cervical dislocation and their livers quickly removed, and approximately 1 g of tissue was homogenized in 7 ml of cold 0.25 *M* sucrose with a Teflon pestle homogenizer. The homogenate was centrifuged at 100,000*g* for 30 min at 4°. The cytosol was removed with a syringe, placed at 4° and assayed for phosphoenolpyruvate carboxykinase activity (7, 8). Changes in absorbance at 340 nm were measured with a Gilford spectrophotometer. The protein concentration of the cytosol was determined by the Folin–Ciocalteau method

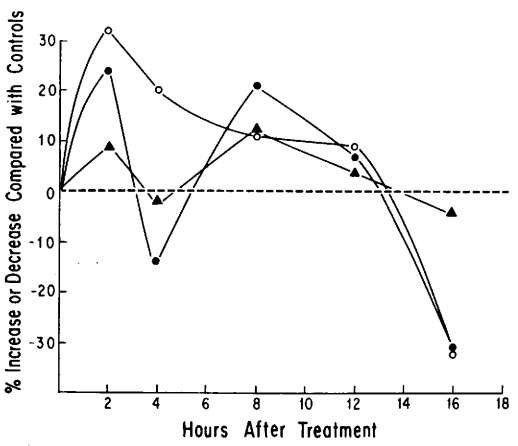

FIG. 1. Changes in liver phosphoenolpyruvate carboxykinase activity after treatment. (- - -) Fasted controls; (○) endotoxin; (●) cortisone acetate and endotoxin; (▲) cortisone acetate.

[cited in (9)]. Enzyme activity was expressed as the milligrams of phosphoenolpyruvate (PEP) per milligram of liver protein per hour at room temperature.

Statistical method. The significance of the differences in the mean responses between experimental treatments was determined by the *t* test.

Results. Phosphoenolpyruvate carboxykinase (PEPCK) activity of mouse liver. Mice were injected with endotoxin alone, cortisone alone, or with cortisone and endotoxin. At intervals after inoculation, the phosphoenolpyruvate carboxykinase activity of the liver was determined and compared with the activity of this enzyme in livers of fasted mice (Fig. 1, Table I). Two significant and different responses were observed in endotoxin-treated mice; (i) induction of the enzyme occurred by 4 hr after injection and (ii) suppression of activity 16 hr after injection (Fig. 1, Table I). Only a slight increase in enzyme activity was observed after injection of cortisone, and cortisone did not prevent the decrease in activity observed 16 hr after injection.

Since mice injected with endotoxin do not eat (10) the effect of starvation on the activity of PEPCK was measured. Starvation for 16 hr resulted in almost a 2-fold increase in activity of the enzyme.

Tolerance and phosphoenolpyruvate carboxykinase activity. Mice made tolerant by daily injections of heat-killed *S. typhimurium* were challenged with *S. marcescens* endotoxin. To determine if tolerance developed, groups of tolerant mice as well as saline injected controls were observed for 3 days after challenge. Tolerant mice survived the injection whereas only 6 to 12 saline-injected mice survived. In parallel groups, assay of the mouse liver enzyme activity 4 hr after injection showed that tolerance prevented induction of the enzyme (Table II).

The induction of tolerance results in increased enzyme activity. The prior increase in enzyme activity may not permit a further increase after challenge with endotoxin.

The decrease in enzyme activity observed 16 hr after injection of endotoxin into tolerant mice was not as significant as was observed in the nontolerant group. Thus induction of tolerance and the subsequent protection against loss of glycogen can be associated with maintenance of the enzyme activity at essentially the same levels as measured in fasted mice.

Discussion. Our results show that injection of endotoxin into mice cause two distinct effects on the liver glyconeogenic enzyme phosphoenolpyruvate carboxykinase (PEP CK). One of these effects, induction of enzyme activity, is observed during the first several hours after treatment. The second effect, a suppression of activity is observed 16 hr after endotoxin injection, the time at which the lethal effect of endotoxin is most pronounced.

The inhibiton of glyconeogenic enzyme (PEPCK) may in part account for the dramatic loss of body carbohydrate shown by Berry, Smythe, and Young (1). Work in our laboratory suggests that the increased activity of pyruvate kinase (PK), a glycolytic enzyme, may prevent glycogen synthesis by recycling glyconeogenic intermediates or by increasing glycolysis (2). Weber *et al.* (11) have shown that the ratio of the activities of the major glyconeogenic enzymes, PEPCK and pyruvate carboxylase (PC) to PK is 0.04. Thus it is important that PK activity be controlled and that PEPCK be induced for a net synthesis of glucose to take place.

TABLE I. Effect of Various Treatments on Mouse Liver Phosphoenolpyruvate Carboxykinase Activity.

	Phosphoenolpyruvate carboxykinase activity time assayed after treatment					
	4 hr			16 hr		
	PEP (mg/mg liver/hr)	Change (%)	Significance	PEP (mg/mg liver/hr)	Change (%)	Significance
1. Fasted	7.00 ± 0.67[a] (8)[b]	—		7.44 ± 0.50 (13)	—	1 vs 2 NS[c]
2. Cortisone acetate	6.84 ± 0.26 (9)	−2	1 vs 4 NS	7.12 ± 0.41 (16)	−4	
3. Endotoxin	8.36 ± 0.24 (8)	+19	1 vs 3 $p < 1.0 > .05$	5.08 ± 0.30 (13)	−32	1 vs 3 $p < .001$
4. Cortisone acetate and endotoxin	6.00 ± 0.60 (9)	−14	1 vs 4 NS[c] 3 vs 4 $p < .02 > .01$	5.16 ± 0.28 (17)	−30	1 vs 4 $p < .001$

[a] Standard error of the mean.
[b] Number of mice assayed.
[c] Not significant.

TABLE II. Liver Phosphoenolpyruvate Carboxykinase Activity in Control and Tolerant Mice Challenged with Endotoxin.

	Phosphoenolpyruvate carboxykinase activity Time assayed after treatment					
	4 hr			16 hr		
	PEP (mg/mg liver/hr)	Change (%)	Significance	PEP (mg/mg liver/hr)	Change (%)	Significance
1. Nontolerant saline	6.80 ± 0.31[a] (6)[b]			7.28 ± 0.39 (12)		
2. Nontolerant endotoxin	7.96 ± 0.34 (6)	+17	1 vs 2 $p < .025$	5.28 ± 0.45 (12)	−27	1 vs 2 $p < .001$
3. Tolerant saline	7.52 ± 0.34 (6)			7.44 ± 0.22 (12)		
4. Tolerant endotoxin	7.24 ± 0.54 (6)	−4	3 vs 4 NS[c]	6.84 ± 0.41 (12)	−9	3 vs 4 NS

[a] Standard error of the mean.
[b] Number of mice assayed.
[c] Not significant.

Experimental data show that PK activity is not maintained at low levels and that PEPCK are not induced but in fact, are reduced.

Although Berry, Smythe and Colwell (4) reported that endotoxin prevented the induction of PEPCK activity by cortisone 4 hr after experimental treatment, our experiments showed an induction of PEPCK by endotoxin during the first several hours after injection of endotoxin. Only a slight increase in enzyme activity, as compared with fasted controls, was observed in animals injected with cortisone. Failure to induce PEPCK in mice by injection of cortisone is at variance with other reported work (12). However, Phillips and Berry (12) found that exogenous cortisone induced PEPCK only when given in the morning. Our failure to prevent the drop in PEPCK activity observed 16 hr after endotoxin injection may be related to the injection of cortisone during the late afternoon. The induction of the enzyme activity by fasting is in agreement with others (13).

The activity of tryptophan oxygenase is maintained at normal levels in mice made tolerant to endotoxin and challenged with a lethal dose of endotoxin (6). In our experiments, induction of tolerance prevented the increase in PEPCK activity observed 4 hr after endotoxin injection. Also, the decrease in activity obtained 16 hr after endotoxin injection was not observed in the tolerant animals. Proof for the induction of the tolerant state was established by the absence of death in parallel experimental groups.

The mechanisms responsible for the changes in enzyme activity in mice given endotoxin are unknown. Studies to determine these are currently in progress.

Summary. Injection of mice with a lethal dose of endotoxin caused an initial increase in liver phosphoenolpyruvate carboxykinase (PEPCK) activity. This increase was not observed in animals tolerant to endotoxin. A significant decrease in enzyme activity was observed 16 hr after injection, and cortisone did not prevent the fall in activity observed. The decrease in enzyme activity obtained in mice 16 hr after injection with endotoxin was

not observed in tolerant mice injected with endotoxin.

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