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Effect of Endotoxin on the Activation of Phosphoenolpyruvate Carboxykinase by Tryptophan

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The effect of endotoxin on the activation of phosphoenolpyruvate carboxykinase by tryptophan was studied.

Bacterial endotoxins have been known for many years to lower the carbohydrate reserves in the mammalian host (6). Closer investigation of this phenomenon was warranted by the discovery that cortisone, a gluconeogenic agent (5), protected against endotoxin lethality (M. Brooke, E. H. Kass, O. Hechter, Fed. Proc. p. 560, 1959). Endotoxin-induced carbohydrate depletion appears to be the result of impaired gluconeogenesis (2) rather than increased utilization of sugars (9). The effect of endotoxin on the enzymes involved in the gluconeogenic pathway is currently under investigation. Phosphoenolpyruvate carboxykinase (GTP:oxalacetate carboxy-lyase, transphosphorylating, EC 4.1.1.32), referred to here as PEPCK, is a gluconeogenic enzyme which surmounts one of the main energy barriers in converting non-carbohydrate precursors into carbohydrate. PEPCK is activated by tryptophan in rats (3), and its induction by glucocorticoid is inhibited, apparently by endotoxin in mice (1). Since the increase in PEPCK activity elicited by tryptophan is believed to be mostly the result of enzyme activation (3), the effect of endotoxin on the activation of PEPCK by tryptophan was studied in mouse liver.

PEPCK was assayed by the method of Phillips and Berry (8). Endotoxin (LPS) was extracted from *Salmonella typhimurium*, strain SR-11, by the method of Westphal as described by Nowotny (7). The preparation employed had a median lethal dose (LD_{50}) of 230 μg for ICR female mice weighing 20 $\pm$ 2 g, which were used for all experiments. Tryptophan (Nutritional Biochemicals, Cleveland), endotoxin, and actinomycin D were injected intraperitoneally in the doses specified. To avoid variations due to the circadian rhythm, all mice were sacrificed at 12:00 noon.

Overnight fasting doubles the activity of PEPCK in normal mice. This is seen in Table 1. Administration of 20 mg of tryptophan resulted, after an interval of 2 hr, in a threefold increase in PEPCK activity in both fed and fasted animals (line 2, Table 1). To characterize the tryptophan effect on PEPCK, mice were pretreated with 25 μg of actinomycin D (the LD_{50}) to inhibit messenger ribonucleic acid (mRNA) synthesis. This prevented the induction of PEPCK in response to fasting (line 3, Table 1) and suggests that the increase depends on the synthesis of additional enzyme. Actinomycin D did not depress PEPCK activity below normal levels in fed mice. This is consistent with the results of Phillips and Berry (8) and of Lardy et al. (4), who proposed the existence of preformed mRNA with very long half-life (15 hr) to account for continued synthesis of the enzyme in presence of the inhibitor. Tryptophan, when administered to actinomycin D-treated mice, elicited a twofold increase in PEPCK activity in fed mice and a three- to fourfold increase in fasted animals, as shown in line 4 of Table 1. This confirms the report of Foster et al. (3) that tryptophan causes an increase in PEPCK activity mostly by activating existing enzyme molecules. An LD_{50} of endotoxin given intraperitoneally under conditions similar to those employed with actinomycin D significantly decreased enzyme activity in fasted animals. PEPCK activity in fed, poisoned mice did not differ from that in fasted, poisoned mice (line 5, Table 1). This may be explained by the fact that endotoxin administration impairs the animal's ability to eat, so that the "fed" animals, although having food available, are in reality fasting, hence the same value in both groups. An LD_{50} of endotoxin inhibited PEPCK activation by tryptophan, as judged by the percentage of change, especially in fasted mice (line 6, Table 1). The threefold increase in PEPCK activity resulting from administration of the amino acid to normal or actinomycin D-treated mice (lines 2 and 4)

TABLE 1. *Effect of tryptophan, endotoxin, and actinomycin D on liver PEPCK activity in fed and fasted mice*[a]

Treatment[b]	PEPCK activity[c] (μmoles of PEP per g of liver per 6 min)	
	Fed	Fasted[d]
Control	145 ± 7.1	298 ± 3.5
2 Hr after 20 mg of tryptophan	402 ± 4.9	971 ± 23.4
20 Hr after 25 μg of actinomycin D	141 ± 8.0	114 ± 7.8
20 Hr after 25 μg of actinomycin D + 2 hr after 20 mg of tryptophan	260 ± 12.5	412 ± 10.7
20 Hr after LD_{50} endotoxin	161 ± 20.6	168 ± 12
20 Hr after LD_{50} endotoxin + 2 hr after 20 mg of tryptophan	387 ± 12	300 ± 19

[a] Abbreviations: PEPCK, phosphoenolpyruvate carboxykinase; LD_{50}, median lethal dose.

[b] All mice were sacrificed at 12:00 noon.

[c] Mean ± standard error of the mean from 10 mice.

[d] Starting at 5:00 PM the previous day.

did not occur in either fed or fasted endotoxin-poisoned mice injected with tryptophan. The absolute value for the fed, poisoned group receiving typtophan (line 6, Table 1) is not significantly different from tryptophan-treated mice (line 2, Table 1). Nevertheless, it must be kept in mind that the criteria for inhibition is the decrease in magnitude of activation, rather than comparison of absolute values.

These results suggest that endotoxin inhibits PEPCK induction by fasting and that it reduces its activation by tryptophan. A mediator might be involved, since endotoxin has no effect on PEPCK when added directly to a liver homogenate (*unpublished observations*). The increase in PEPCK activity observed in fasted animals is probably the result of protein synthesis, a process inhibited by both endotoxin and actinomycin D. Furthermore, endotoxin seems to interfere with tryptophan-induced activation of PEPCK, a process depending on a pool of enzyme that can be activated. A twofold effect of endotoxin may, therefore, be postulated. It might selectively decrease synthesis of the enzyme—selectively because endotoxin is known to induce an overall increase in protein synthesis (10)—and it might also inactivate existing enzyme molecules through the action of a mediator.

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LITERATURE CITED

1. Berry, L. J., D. S. Smythe, and L. S. Colwell. 1968. Inhibition of hepatic enzyme induction as a sensitive assay for endotoxin. J. Bacteriol. 96:1191–1199.
2. Berry, L. J., D. S. Smythe, and L. G. Young. 1959. Effects of bacterial endotoxin on metabolism I. Carbohydrate depletion and the protective role of cortisone. J. Exp. Med. 110: 389–405.
3. Foster, D. O., P. D. Ray, and H. Lardy. 1966. A paradoxical effect of L-tryptophan on the phosphoenolpyruvate carboxykinase of rat liver. Biochemistry 5:563–569.
4. Lardy, H. A., D. O. Foster, E. Shrago, and P. D. Ray. 1964. Metabolic and hormonal regulation of phosphopyruvate synthesis. Advan. Enzyme Regul. 2:39–47.
5. Long, C. N. H., B. Katzin, and E. C. Fry. 1940. The adrenal cortex and carbohydrate metabolism. Endocrinology 26: 309–344.
6. Menten, M. L., and H. M. Manning. 1924. Blood sugar studies on rabbits infected with organisms of the enteritidio-paratyphoid B group. J. Med. Res. 44:675–693.
7. Nowotny, A. 1969. Basic exercises in immunochemistry, p. 29. Springer-Verlag, New York.
8. Phillips, L. J., and L. J. Berry. 1970. Circadian rhythm of mouse liver phosphoenolpyruvate carboxykinase. Amer. J. Physiol. 218:1140–1144.
9. Shands, J. W., Jr., V. Miller, H. Martin, and V. Senterfitt. 1969. Hypoglycemic activity of endotoxin. II. Mechanism of the phenomenon in BCG-infected mice. J. Bacteriol. 98: 494–501.
10. Shtasel, T. F., and L. J. Berry. 1969. Effect of endotoxin and cortisone on synthesis of ribonucleic acid and proteins in livers of mice. J. Bacteriol. 97:1018–1025.