INFECTION AND IMMUNITY, Nov. 1972, p. 883-885
Vol. 6, No. 5

Mouse Liver Fructose-1,6-Diphosphatase and Glucose-6-Phosphatase Activities After Endotoxin Poisoning

R. E. McCALLUM[1] AND L. JOE BERRY
Department of Microbiology, University of Texas at Austin, Austin, Texas 78712

Received for publication 5 July 1972

Hepatic fructose-1,6-diphosphatase and glucose-6-phosphatase were significantly decreased in fasted mice 17 hr after the intraperitioneal injection of a median lethal dose of *Salmonella typhimurium* endotoxin. Liver glycogen levels were essentially depleted at 17 hr.

For nearly 50 years bacterial endotoxins have been known to deplete an animal of its carbohydrate reserves (9, 19). Such a depletion could result from several causes; the most likely, however, seems to be an impaired ability to carry out gluconeogenesis, glycogen synthesis, or both. Berry et al. (3) demonstrated that injection of a lethal dose of killed *Salmonella typhimurium* results in the reduction of liver glycogen and almost complete loss in total body carbohydrate. Cortisone not only protected mice against the lethality of endotoxin but also prevented the total loss of glycogen. Shands et al. (14, 15), employing mice hyperreactive to endotoxin, clearly demonstrated impaired gluconeogenesis as responsible for the decreased conversion of pyruvate-2-^{14}C into blood glucose. Moon (10) has shown that a severe hypoglycemia accompanies sensitivity of endotoxin-poisoned mice to exogenous tryptophan administration. Inhibition of phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis, during endotoxin poisoning has recently been shown by Berry et al. (2). Studies by LaNoue et al. (7) and Williamson et al. (18) using rat liver slices and perfused rat livers have shown the overall rate of gluconeogenesis to be impaired by *Pseudomonas aeruginosa* infection as well as *Escherichia coli* endotoxin. The activity of glucose-6-phosphatase in liver was significantly lower in infected and poisoned animals than in controls.

The present study was undertaken to characterize further the liver carbohydrate depletion in endotoxin-treated animals and to determine the possible contribution of altered activity of the gluconeogenic phosphatases to these changes.

[1] Present address: Department of Microbiology and Immunology, University of Oklahoma Health Science Center, Oklahoma City, Okla. 73190.

Female ICR mice (Texas Inbred Mice Co., Houston, Tex.) weighing 18 to 20 g were fasted at the beginning of each experiment (1700 hr) and were kept fasting until sacrifice. The endotoxin employed in these studies was extracted and purified from *S. typhimurium*, strain SR-11, as described by Nowotny (12). The median lethal dose (LD_{50}) of the purified lipopolysaccharide was determined by the method of Reed and Muench (13), using a 48-hr end point, and was found to be approximately 235 μg/mouse. Groups of 10 mice each were injected intraperitoneally with an LD_{50} of endotoxin suspended in sterile nonpyrogenic saline (Baxter Laboratories, Morton Grove, Ill.). Control mice received sterile saline alone. In certain experiments, actinomycin D (20 μg/mouse; Schwarz/Mann, Orangeburg, N.Y.) was injected intraperitoneally at the beginning of the experiment (1700 hr). All mice were sacrificed by decapitation 17 hr after treatment (1000 hr), livers were quickly removed, blotted dry, and weighed, and glycogen content and enzyme activity were determined. Liver glycogen was assayed by the method of Kemp and Kits van Heyningen (6). Hepatic fructose-1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) activity was measured by the method of Taketa and Pogell (16) in 30,000 $\times$ g supernatant fluids of 25% liver homogenates prepared in 0.14 M KCl (5). Glucose-6-phosphatase (glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) activity was measured by the method of Harper (4) in 2.5% liver homogenates prepared in 0.1 M cacodylate buffer, *p*H 6.5. All results were analyzed by the rank order test (17) and the Student t test (8) for significant differences ($P \leq 0.05$).

Hepatic fructose-1,6-diphosphatase and glucose-6-phosphatase activities and liver glycogen

levels were compared in fed control mice, mice fasted for 17 hr, and fasted mice treated with either an LD_{50} of endotoxin or 20 μg of actinomycin D (Table 1). The time 17 hr after treatment was chosen because it is only a short time before the first deaths from endotoxin, and any metabolic effect related to death would be expected to be maximal at that time. As expected, overnight fasting produced a partial reduction in liver glycogen from 5.35 mg/100 mg to 1.07 mg/100 mg. This decrease seemed to parallel increases in both fructose-1,6-diphosphatase and glucose-6-phosphatase activity after the 16-hr fasting period. Actinomycin D treatment suggests that the increases observed in fasting mice involve synthesis of new enzyme protein. Seventeen hours after treatment with an LD_{50} of endotoxin, liver glycogen levels in fasted mice were depleted to 0.15 mg/100 mg. The activities of both of the gluconeogenic phosphatases in fasted mice treated with endotoxin were reduced below the values seen in fasted controls. In fact, the enzyme activities measured in endotoxin-poisoned animals were comparable to those seen in mice treated with actinomycin D. These results possibly suggest similarities in the action of endotoxin and actinomycin D. The lack of effect of actinomycin D on liver glycogen levels at 17 hr, however, remains unexplained. This may be due to several possibilities: e.g., recovery from the effects of the poison may have occurred by 17 hr; carbohydrate homeostasis may not depend directly on enzyme induction; and the hypoglycemia and glycogen depletion by endotoxin may not involve direct effects on fructose-1,6-diphosphatase and glucose-6-phosphatase. For these reasons further study is in progress.

TABLE 1. *Effect of endotoxin treatment (1 LD_{50}) on mouse liver fructose-1,6-diphosphatase, glucose-6-phosphatase, and glycogen*[a]

Exptl treatment	FDPase activity[b]	G6Pase activity[c]	Liver glycogen (%)
Fed control	5.49±0.12[d] (20)[e]	38.8±1.4 (20)	5.35±0.51 (20)
Fasting alone (17 hr)	6.46±0.32 (20)	50.9±3.1 (19)	1.07±0.23 (20)
1 LD_{50} of *S. typhimurium* lipopolysaccharide (ip, 17-hr fasting)	4.97±0.21[f] (18)	40.0±1.8[f] (19)	0.15±0.03[f] (19)
20 μg of ac inomycin D (ip, 17-hr fasting)	4.13±0.37[f] (18)	33.8±3.5[f] (20)	1.10±0.10 (20)

[a] Abbreviations: FDPase, fructose-1,6-diphosphatase; G6Pase. glucose-6-phosphatase; ip, intrapritoneally.

[b] Micromoles of fructose-6-phosphate formed per hour per milligram of protein.

[c] Micromoles of inorganic phosphate liberated per minute per milligram (dry weight) of liver.

[d] Mean ± standard error.

[e] Number of animals.

[f] Significantly different from fasted control values ($P \leq 0.05$).

The data clearly indicate that endotoxin inhibits the induction of fructose-1,6-diphosphatase and glucose-6-phosphatase due to fasting. The relationship between altered activity of the gluconeogenic phosphatases and rapid liver carbohydrate depletion remains unclear. In other experiments performed in this laboratory, it has been found that liver glycogen is rapidly reduced (as early as 1 hr after treatment with an LD_{50} of endotoxin) in poisoned mice, whereas the gluconeogenic phosphatases do not appear to be affected until 12 to 17 hr after treatment. No significant differences were noted at 12 hr in glucose-6-phosphatase activity between fasted controls and endotoxin-treated mice, although slight decreases were noted in fructose-1,6-diphosphatase activity in poisoned animals (82% of the control values). Greater changes were seen in the gluconeogenic phosphatases at 22 hr after treatment than at 17 hr (data not presented). Fructose-1,6-diphosphasase and glucose-6-phosphatase were reduced to approximately 61 and 63% of the control values, respectively. The altered activity of the phosphatases is therefore not likely to be the major cause of liver carbohydrate loss during endotoxin poisoning. The fact that fructose-1,6-diphosphatase and glucose-6-phosphatase have relatively long half-lives compared with other gluconeogenic enzymes (11) may relate to their delayed response to endotoxin. This particular aspect is currently under investigation.

Closely related to these studies are those of LaNoue et al. (7). Three rat liver enzymes were measured at 5 hr after the intravenous injection of twice the LD_{50} of *E. coli* lipopolysaccharide. Neither phosphoenolpyruvate carboxykinase nor fructose-1,6-diphosphatase changed, but glucose-6-phosphatase was significantly diminished (from 28.7 to 22.5 units). The ability of liver slices prepared from animals similarly injected to convert pyruvate-2-^{14}C into glucose in a Krebs-Ringer bicarbonate medium was also depressed. It is important to emphasize the difference in design between these experiments and the ones described above.

In conclusion, the effect of endotoxin in lowering the gluconeogenic phosphatases, fructose-1,6-diphosphatase and glucose-6-phosphatase, appears after the time when rapid depletion in liver carbohydrate is seen in mice treated with an LD_{50} of endotoxin. Although impaired gluconeogenesis has been shown to play a central role in carbohydrate loss during endotoxin poisoning

(3, 7, 15), altered glycogen synthesis may in addition lead to sugar depletion. This possibility should be considered in studying possible mechanisms.

This investigation was supported by a Public Health Service grant from the National Institute of Allergy and Infectious Diseases (AI 10087).

LITERATURE CITED

1. Berry, L. J., and D. S. Smythe. 1960. Some metabolic aspects of host-parasite interactions in the mouse typhoid model. Ann. N.Y. Acad. Sci. 88:1278–1286.
2. 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.
3. 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.
4. Harper, A. E. 1965. Glucose-6-phosphatase, p. 788–782. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. Academic Press Inc., New York.
5. Hurvitz, A. I., and R. A. Freedland. 1968. Influence of dietary protein on hydrocortisone-mediated adaptive enzymatic changes in rat liver. Arch. Biochem. Biophys. 127:548–555.
6. Kemp, A., and A. J. M. Kits van Heyningen. 1954. A colorimetric micromethod for the determination of glycogen in tissues. Biochem. J. 56:646–648.
7. LaNoue, K. F., A. D. Mason, Jr., and J. P. Daniels. 1968. The impairment of gluconeogenesis by gram negative infection. Metab. (Clin. Exp.) 17:606–611.
8. Lewis, A. E. 1966. Biostatistics. Reinhold Publishing Corp., New York.
9. Menten, M. L., and H. M. Manning. 1924. Blood sugar studies on rabbits infected with organisms of the enteritidis-paratyphoid group. J. Med. Res. 44:675–676.
10. Moon, R. J. 1972. Carbohydrate metabolism and survival of endotoxin-poisoned mice given tryptophan. Infect. Immunity 5:288–294.
11. Nordlie, R. C., and H. A. Lardy. 1963. Mammalian liver phosphoenolpyruvate carboxykinase activities. J. Biol. Chem. 238:2259–2263.
12. Nowotny, A. 1969. Basic exercises in immunochemistry. Springer-Verlag, New York.
13. Reed, L. J., and H. A. Muench. 1938. A simple method for estimating fifty percent end points. Amer. J. Hyg. 27:493–499.
14. Shands, J. W., Jr., V. Miller, and H. Martin. 1968. The hypoglycemic activity of endotoxin. I. Occurrence in animals hyper-reactive to endotoxin. Proc. Soc. Exp. Biol. Med. 130:413–417.
15. Shands, J. W., Jr. V. Miller, H. Martin, and V. Senterfitt. 1969. Hypoglycemic activity of endotoxin. II. Mechanism of phenomenon in BCG-infected mice. J. Bacteriol. 98:494–501.
16. Taketa, K., and B. M. Pogell. 1965. Allosteric inhibition of rat liver fructose-1,6-diphosphatase by adenosine-5′-monophosphate. J. Biol. Chem. 240:651–662.
17. Wilcoxon, F., and R. A. Wilcox. 1949. Some rapid approximate statistical procedures. American Cyanimid Co., New York.
18. Williamson, J. R., C. Refino, and K. LaNoue. 1970. Effects of *E. coli* lipopolysaccharide B treatment of rats on gluconeogenesis, p. 145–151. *In* R. Porter and J. Knight (ed.), Energy metabolism in trauma. J. & A. Churchill, London.
19. Zeckwer, I. S., and H. Goodell. 1925. Blood sugar studies. I. Rapid alterations in the blood sugar level of rabbits as a result of intravenous injections of killed bacteria of various types. J. Exp. Med. 42:43–56.