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# *In Vivo* vs *In Vitro* Effects of Endotoxin on Glycogenolysis, Gluconeogenesis, and Glucose Utilization[1] (39776)

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*Introduction.* Gram-negative bacterial lipopolysaccharides, i.e., endotoxins, administered *in vivo* produce marked alterations in glucose homeostasis, viz, depressed hepatic gluconeogenesis, enhanced peripheral glucose catabolism, and profound, progressive hypoglycemia (1, 2). Despite intensive investigation the precise mechanism of endotoxin's metabolic actions is obscure. As an initial approach, the issue of a direct effect of endotoxin as opposed to an indirect or mediated effect requires clarification.

In an attempt to develop appropriate *in vitro* metabolic systems for analyzing endotoxin's mechanisms of action, the present study evaluated the ability of endotoxin *in vivo* and *in vitro* to influence glycogenolysis and gluconeogenesis in isolated hepatocytes, glucose uptake in hemidiaphragms, and glucose oxidation in epididymal fat pads.

*Materials and methods.* Male rats of the Holtzman strain (Holtzman Company, Madison, Wis.) were used. For hepatocyte isolation (3) the donor rats were 300 ± 10 g. For optimal hemidiaphragm and epididymal fat pad preparations the donor rats were 100 ± 10 g. The endotoxin used was the Boivin lipopolysaccharide of *Salmonella enteritidis* iv (Difco Laboratories, Detroit, Mich.). Insulin and glucagon were obtained from the Sigma Chemical Company, St. Louis, Mo.

Hepatocytes were isolated and gluconeogenesis was evaluated as described previously (3-5). Glycogenolysis was quantitated as net glucose production using hepatocytes from fed rats incubated in glucose-free Krebs-Ringer bicarbonate solution. Hemidiaphragm glucose uptake was quantitated using decrements in incubation media glucose content as determined on the Yellow Spring Model 23A glucose analyzer (Yellow Springs, Ohio). Glucose oxidation of epididymal fat pads was evaluated using the metabolic collection flask of Saba and DiLuzio (6) and conversion of D-[U-$^{14}$C]glucose to $^{14}CO_2$. The various incubation parameters are specified in the data tables. Data were analyzed for statistical significance using Student's *t* test for unpaired comparisons.

*Results. Effect of endotoxin on glycogenolysis.* Hepatocytes were isolated from fed donor rats treated with either saline (control group) or endotoxin (Table I). Glycogenolysis was increased 1.6 times in the postendotoxin hepatocytes. In contrast, endotoxin added *in vitro* to control hepatocytes failed to alter the rate of hepatocyte glycogenolysis (Table I). However, glucagon augmented glycogenolysis *in vitro* which affirmed the ability of the isolated hepatocytes to respond to a glycogenolytic agent.

*Effect of endotoxin on gluconeogenesis.* Hepatocytes isolated from overnight-fasted, control donor rats manifested gluconeogenesis from lactate and also responded to insulin via a depression in the rate of gluconeogenesis (Table II). Hepatocytes isolated from rats treated with endotoxin *in vivo* had a depressed rate of gluconeogenesis. In contrast, endotoxin added *in vitro* to isolated control hepatocytes had no effect on gluconeogenesis.

*Effect of endotoxin on glucose uptake and oxidation.* Hemidiaphragms from rats treated with endotoxin *in vivo* had increased glucose uptake (Table III). In contrast, endotoxin added *in vitro* had no effect on diaphragm glucose uptake. Epididymal fat pads from control donor rats had a brisk rate of glucose oxidation which was augmented 3.6-fold by insulin addition (Table IV). Fat pads from rats treated with endotoxin prior to sacrifice had enhanced glucose oxidation. In contrast, endotoxin *in vitro* had no effect on glucose oxidation in epididymal fat pads.

*Discussion.* A number of physiologic ef-

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TABLE I. EFFECT OF ENDOTOXIN ON GLYCOGENOLYSIS IN ISOLATED HEPATOCYTES.[a]

| Donor group | Number of preparations | Glycogenolysis (μmole of glucose-mg of protein/min) |
|---|---|---|
| Control | 7 | 3.5 ± 0.24 |
| Control plus 100 μM glucagon | 5 | 6.0 ± 0.52* |
| Postendotoxin (1 mg, 1 hr prior) | 5 | 5.6 ± 0.62* |
| Endotoxin *in vitro* (μg/ml) | | |
| 5 | 7 | 3.3 ± 0.55** |
| 20 | 7 | 3.5 ± 0.28** |
| 40 | 6 | 3.7 ± 0.27** |
| 100 | 6 | 3.3 ± 0.56** |

[a] Hepatocytes (3 ml) were incubated at $4 \times 10^6$/ml in glucose-free Krebs-Ringer bicarbonate buffer for 60 min at 37° in a Dubnoff shaking incubator at 100 oscillations/min. The samples were deproteinized by adding 1.0 ml of 1.8% $Ba(OH)_2$ and 1.0 ml of 2% $ZnSO_4$. Glucose was determined using the glucose oxidase method (Worthington Biochemical Corp., Freehold, N. J.).

* $P < 0.01$ as compared to control.

** No statistically significant differences as compared to control group.

TABLE II. EFFECT OF ENDOTOXIN ON GLUCONEOGENESIS IN ISOLATED HEPATOCYTES.[a]

| Donor group | Number of preparations | Gluconeogenesis (μmole of glucose/g of protein/min) |
|---|---|---|
| Control | 5 | 1.8 ± 0.23 |
| Control plus insulin (100 μU/ml) | 6 | 0.91 ± 0.19* |
| Postendotoxin (3 mg, 3 hr prior) | 5 | 0.80 ± 0.13* |
| Endotoxin *in vitro* (μg/ml) | | |
| 0 | 4 | 1.6 ± 0.22** |
| 50 | 4 | 1.9 ± 0.25** |
| 100 | 4 | 1.5 ± 0.20** |
| 200 | 4 | 1.7 ± 0.18** |
| 400 | 4 | 2.0 ± 0.17** |

[a] Hepatocytes (3 ml) were incubated at $4 \times 10^6$/ml in glucose-free Krebs-Ringer bicarbonate solution with 10 m*M* L-lactate for 60 min at 37° in a Dubnoff shaking incubator at 100 oscillations/min. Glucose was determined as specified under Table I.

* $P < 0.001$ as compared to control group.

** No statistically significant differences as compared to control group.

fects of endotoxin have been ascribed to the production of endogenous mediators, e.g., pyrogen responses, serum trace metal alterations, and glucocorticoid antagonism (7-10). However, in other test systems a direct *in vitro* effect of endotoxin has been reported, e.g., lipolysis in isolated adipocytes, liver homogenate glycogen degradation, and cyclic AMP alterations (11-14).

In the present studies no *in vitro* effects of endotoxin were demonstrable on isolated hepatocyte glycogenolysis and gluconeogenesis, hemidiaphragm glucose uptake, or epididymal fat pad glucose oxidation. In contrast, administration of endotoxin *in vivo* and removal of the appropriate tissue for *in vitro* measurements revealed increased hepatocyte glycogenolysis, decreased hepatocyte gluconeogenesis, enhanced hemidiaphragm glucose uptake, and increased fat pad glucose oxidation. These data would

TABLE III. EFFECT OF ENDOTOXIN ON GLUCOSE UPTAKE IN HEMIDIAPHRAGMS.[a]

| Donor group | Number of preparations | Glucose uptake (μg of glucose/mg dry wt/180 min) |
|---|---|---|
| Control | 32 | 42.6 ± 1.21 |
| Postendotoxin (1 mg, 3 hr prior) | 28 | 66.4 ± 1.08* |
| Endotoxin *in vitro* (μg/ml) | | |
| 0 | 23 | 36.8 ± 0.85** |
| 10 | 23 | 38.4 ± 1.33** |
| 100 | 21 | 38.7 ± 2.08** |
| 1000 | 21 | 38.9 ± 2.27** |

[a] Hemidiaphragms were incubated in 2 ml of Gey's solution containing 2 mg/ml of D-glucose. After incubation for 180 min glucose uptake was quantitated by measuring the decrements in glucose content of the incubation media using a Yellow Springs Model 23A glucose analyzer.

* $P < 0.001$ as compared to control group.

** No statistically significant differences as compared to control group.

TABLE IV. EFFECT OF ENDOTOXIN ON GLUCOSE OXIDATION IN EPIDIDYMAL FAT PADS.[a]

| Donor group | Number of preparations | Glucose oxidation (dpm of $^{14}CO_2$/g/120 min) |
|---|---|---|
| Control | 23 | 54, 065 ± 4892 |
| Control plus insulin (100 μU/ml) | 9 | 194,710 ± 23,358* |
| Postendotoxin | 9 | 117,390 ± 11,166* |
| Endotoxin *in vitro* (μg/ml) | | |
| 50 | 5 | 47,417 ± 5888** |
| 100 | 5 | 48,277 ± 3382** |
| 200 | 5 | 47,892 ± 3288** |

[a] Epididymal fat pads were incubated in 5 ml of Krebs-Ringer phosphate buffer containing 1 mg/ml of D-glucose and 0.5 μC/ml of D-[$^{14}C$]glucose and evolved $^{14}CO_2$ was collected directly in scintillation vials (6).

* $P < 0.001$ as compared to control group.

** No statistically significant difference as compared to control group.

tend to support an indirect or mediated mechanism of endotoxin's action on carbohydrate homeostasis vis-á-vis a direct effect of the lipopolysaccharide at the cellular level. In accord with our past studies (15, 16) insulin or an insulin-like agent (ILA) is a likely candidate as the mediator. Studies are currently in progress to evaluate the production of ILA mediators *in vitro* by endotoxin.

*Summary*. *Salmonella enteritidis* lipopolysaccharide administered iv to male rats of the Holtzman strain resulted in increased glycogenolysis and decreased gluconeogenesis in isolated hepatocytes, increased glucose uptake in isolated hemidiaphragms, and enhanced glucose oxidation in epididymal fat pads. Endotoxin added *in vitro* to the identical test systems failed to affect the rates of glycogenolysis, gluconeogenesis, glucose uptake, and glucose oxidation. A mediated as opposed to a direct mechanism of endotoxin action is supported.

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1. Hinshaw, L. B., Circ. Shock **3,** 1 (1976).
2. Filkins, J. P., Buchanan, B. J., and Cornell, R. P., Circ. Shock **2,** 129 (1975).
3. Cornell, R. P., and Filkins, J. P., Proc. Soc. Exp. Biol. Med. **145,** 203 (1974).
4. Filkins, J. P., and Cornell, R. P., Amer. J. Physiol. **227,** 778 (1974).
5. Cornell, R. P., and Filkins, J. P., Proc. Soc. Exp. Biol. Med. **147,** 371 (1974).
6. Saba, T. M., and DiLuzio, N. R., J. Lipid Res. **7,** 566 (1966).
7. Berry, L. J., *in* "Microbial Toxins V" (S. Kadis, G. Weinbaum, and S. J. Ajl, eds.), p. 165. Academic Press, New York (1971).
8. Berry, L. J., Toxicon, in press (1977).
9. Beisel, W. R., Ann. Rev. Med. **26,** 9 (1975).
10. Moore, R. N., Goodrum, K. J., and Berry, L. J., J. Reticuloendothel. Soc. **19,** 187 (1976).
11. Spitzer, J. A., Proc. Soc. Exp. Biol. Med. **145,** 186 (1974).
12. Zwadyk, P., Jr., and Snyder, I. S., Proc. Soc. Exp. Biol. Med. **142,** 299 (1973).
13. Bitensky, M. W., Gorman, R. E., and Thomas, L., Proc. Soc. Exp. Biol. Med. **138,** 773 (1971).
14. Gimpel, L., Hodgins, D. S., and Jacobson, E. D., Circ. Shock **1,** 31 (1974).
15. Buchanan, B. J., and Filkins, J. P., Circ. Shock **3,** 223 (1976).
16. Buchanan, B. J., and Filkins, J. P., Circ. Shock **3,** 267 (1976).

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