Hepatic Vascular Response to Endotoxin* (34077)

JAMES P. FILKINS

Department of Physiology and Biophysics, University of Tennessee Medical Units, Memphis, Tennessee 38103

The vascular effects of lipopolysaccharides derived from gram-negative bacteria, *i.e.*, endotoxins, have been intensively investigated due primarily to their implication in the pathogenesis of septic and other forms of shock (1). In addition, since the intestinal tract harbors a vast reservoir of gram-negative flora, it is probable that endogenous endotoxin occasionally, if not regularly, permeates the intestinal barriers (2). Due to its strategic location between the portal and caval circulation, the liver presumably receives the highest concentration of gut toxins. Since the liver has the dual ability to sequester and detoxify endotoxin, it plays a major role in safeguarding the heart and systemic circulation (3). If, however, endotoxemia is prolonged and severe, the liver vasculature suffers marked damage which may so impair liver function as to contribute to the progression of irreversible shock (4).

In order to delineate the vascular effects of endotoxin on the liver *per se*, the present study evaluated the alterations in perfusate flow accompanying endotoxin administration in the isolated rat liver preparation. Evidence is presented that (1) transient hepatic vascular constriction and diminished perfusion accompany endotoxemia, and (2) the vascular reaction depends on the interaction of endotoxin, buffy coat cells, and plasma.

Methods. Male rats of the Holtzman strain (300 ± 20 g) maintained on Purina Chow and water *ad libitum* were used both as liver and blood donors. Heparinized blood (25–30 units/ml) was obtained by cardiac puncture under light ether anesthesia. The perfusate media employed were (1) fresh rat blood diluted with balanced salt solution (Table I) to a final hematocrit of 30–35%; (2) fresh heparin-plasma diluted 1:1 with balanced salt solution; (3) twice-washed rat erythrocytes suspended in balanced salt solution to a hematocrit of 30–35%; and (4) balanced salt solution. In all perfusions the volume of perfusion media was 75 ml.

The general procedure of liver perfusion was modified from Miller *et al.* and Brauer *et al.* as described previously (5). In brief, polyethylene cannulae were placed in the portal vein and thoracic inferior vena cava and the livers were rapidly extirpated and placed in a constant environment chamber which provided a temperature of 38 ± 0.5° and oxygenated perfusate media. Hydrostatic portal perfusion pressure was maintained constant in each preparation but was varied initially between 10 and 16 cm H_2O in order to yield flow rates in all preparations of 20–25 ml/min. After portal perfusion and hepatic transit, the caval efflux passed through a graduated 10-ml flow tube into a collection flask. A revolving disc pump elevated the perfusate from the collection flask to flow

TABLE I. Composition of Balanced Salt Perfusate Media.

Substance	Concentration
NaCl	137.0 m*M*
KCl	2.68 m*M*
$CaCl_2$	1.80 m*M*
$MgCl_2 \cdot 6H_2O$	0.49 m*M*
$NaHCO_3$	11.9 m*M*
$NaH_2PO_4 \cdot 1H_2O$	0.67 m*M*
D-Glucose	5.45 m*M*
Penicillin	20 U/ml
Streptomycin	25 μg/ml
pH	7.4

* Supported by grants from the Tennessee Heart Association and the United States Public Health Service, National Heart Institute (HE-11499).

down over a multilobed gas exchange tube which terminated in the perfusion reservoir. Flow measurements were performed by clamping the outflow from the graduated flow tube and timing the interval to fill to 10 ml.

Endotoxin was obtained from Difco Laboratories, Detroit, Michigan, as the Boivin preparation from *Salmonella enteritidis*. Endotoxin was prepared fresh daily in saline and all additions were made into the perfusate collection flask and rapidly mixed via a magnetic stirrer. In all experiments 15 min of flow equilibration preceded the introduction of endotoxin and the ensuing measurements of flow.

Results. Effects of varying doses of endotoxin on flow in livers perfused with various media. In corroboration of Nolan and O'Connell (6) endotoxin at doses of 1, 2, 3, and 12 mg induced progressive, marked decreases in flow in livers perfused with homologous blood (Fig. 1). The vasoconstriction was transient—lasting 4–6 min—and exhibited tachyphylaxis, *i.e.*, a second 3.0-mg endotoxin dose failed to induce a marked diminution of blood flow even when introduced as long as 2 hr after an initial flow-depressing dose of 3.0 mg endotoxin. Since no visible swelling of the liver occurred during the flow decreases, the resistance to perfusion was probably presinusoidal. In contrast to the results of Nolan and O'Connell (6) endotoxin failed to elicit vasoconstriction in livers perfused with either balanced salt solution alone, rat erythrocytes in salt solution, or heparin-plasma (Fig. 1). This difference in results may reflect the care taken in this study to completely flush all blood out of the liver prior to insertion into artificial perfusion media. In addition, Nolan and O'Connell (6) introduced endotoxin directly into the portal vein which yields much higher perfusion concentrations; and it is known that the responses of blood vessels of the splanchnic bed vary greatly as the endotoxin dose and route of administration are altered (7).

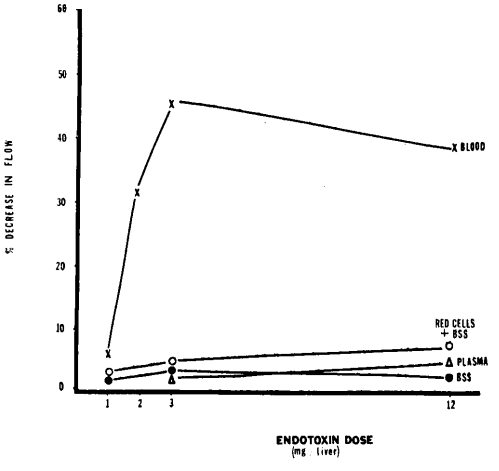

FIG. 1. Effect of endotoxin on perfusate flow in isolated rat livers. Each point represents 4–6 separate perfusions.

Blood constituents involved in endotoxin-induced hepatic vasoconstriction. In order to partially elucidate the blood ingredients responsible for hepatic vasoconstriction, livers were first perfused with balanced salt solution and then various combinations of endotoxin and blood ingredients were added to the perfusate. As indicated in Table II, 3 mg of endotoxin when mixed and incubated for 30 min with 10 ml of fresh, heparinized rat blood produced a 46% decrease in flow upon addition to the perfusate. In contrast, neither blood alone nor endotoxin in saline caused appreciable changes in flow. Similarly, no flow decrements were noted when endotoxin was introduced in singlular combination with heparin–plasma, buffy coat cells, or red cells; however, the multiple combination of buffy coat cells, heparin–plasma, and endotoxin yielded a 39% decrease in flow. The vasoconstrictive effect was also not produced by heparin–plasma obtained from rats injected 120 min previously with 3 mg of endotoxin nor by heparin–plasma harvested from 20 ml of blood incubated with 3 mg of endotoxin. These data indicate that the continuing presence of plasma, buffy coat cells, and endotoxin is necessary to evoke a decrease in flow.

Discussion. In attempts to dissect out the mechanisms mediating specific organ and vascular alterations induced by endotoxemia, previous *in vitro* studies have been performed in perfused lungs (8), intestinal mucosa (9), ear vessels (10), mesentery (10), and isolated veins (11). In agreement with the results of this study on the perfused liver, the

TABLE II. Determinants of Hepatic Vascular Response to Endotoxin.[a]

Perfusate additions	Number of perfusion experiments	Maximum flow decrease in ml (mean ± SEM)	% Decrease in flow
None	8	2.0 ± .25	7.0
Endotoxin in saline (3 mg)	6	1.0 ± .10	3.0
Blood (10 ml)	4	1.5 ± .15	5.0
Blood (10 ml) + endotoxin (3 mg)	4	13.0 ± .15	46.0
Heparin–plasma (10 ml)	4	1.0 ± .05	3.0
Heparin–plasma + endotoxin	4	1.5 ± .10	5.0
Buffy coat (from 10 cc of blood)	4	1.5 ± .15	5.0
Buffy coat + endotoxin	4	1.5 ± .30	5.0
Red cells (from 10 cc of blood)	4	1.5 ± .15	5.0
Red cells + endotoxin	4	1.5 ± .15	5.0
Buffy coat + heparin–plasma	4	2.0 ± .25	7.0
Buffy coat + heparin–plasma + endotoxin	4	11.0 ± .90	39.0
Heparin–plasma (10 ml) from endotoxin-treated rat	4	1.5 ± .10	5.0
Heparin–plasma (10 ml) from blood incubated with endotoxin	4	1.5 ± .10	5.0

[a] Protocol: Livers were perfused with balanced salt solution for 15 min of control flow at 20–25 ml/min; various additions to perfusate were then injected into the collection flask and rapidly mixed with magnetic stirrer; flow measurements were made for the ensuing 15 min.

above-cited investigations concur that (1) endotoxin does not act singularly, nor perhaps directly, on the blood vessels, but rather (2) endotoxin interacts with blood constituents in order to trigger certain of its vascular effects. The ingredients needed to evoke a vascular response appear to include endotoxin, plasma, and buffy coat cells.

It is probable that plasma is vital for a supply of protein factors such as complement, opsonin, or elements of the coagulation system (12). The buffy coat cells include both platelets and leukocytes and their relative contribution to the hepatic vascular action of endotoxin merits further study. Since bradykinin is a hepatic vasoconstrictor (13), perhaps the recent demonstrations of kinin generation by leukocytes (14), and the contribution of kinins to endotoxin shock (15) are pertinent to this study. In addition, endotoxin acting in conjunction with a plasma protein may damage platelets (16) and release serotonin, which is a potent hepatic vasoconstrictor (17).

Summary. Endotoxin induced transient, tachyphylactic decreases in flow in rat livers perfused with whole blood. In contrast, no flow decrements accompanied endotoxin administration to livers perfused with rat erythrocytes in balanced salt solution, heparin–plasma, or balanced salt solution. By varying the combination of endotoxin and the major blood constituents, the hepatic vasoconstrictor response was found dependent on plasma, buffy coat cells, and endotoxin.

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Received April 1, 1969. P.S.E.B.M., 1969, Vol. 131.