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Hepatic Excretory Function in the Endotoxin-Tolerant Rat (40595)

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Intrahepatic cholestasis is a well-recognized complication of gram-negative bacterial infection, especially in newborns (1–4). Although the underlying pathogenesis of this syndrome is unknown, its occurrence with or without bacteremia (1–4) suggests that some bacterial factor may be involved. This factor may well be the lipopolysaccharide (LPS, endotoxin) of the bacterial cell wall (5).

We and others have found that LPS from both *Escherichia coli* and *Salmonella enteritidis* exert dose-dependent cholestatic effects on the perfused liver (6–8). The *E. coli* LPS exerted its major effect on the bile acid-independent fraction (BAIF) of bile (9) and preferentially inhibited the sodium, potassium-adenosinetriphosphatase (Na^+,K^+-ATPase) of rat liver plasma membranes enriched with bile canaliculi (10). This inhibition might account for the decreased bile flow as Na^+,K^+-ATPase activity may be a determining factor in the regulation of BAIF (11).

The acute effects of endotoxin may not accurately mimic the effects of endotoxin liberated by infecting organisms. Since the levels of LPS in the blood are increased during infection (12, 13), the prolonged exposure to LPS could lead to endotoxin tolerance (13) and the state of tolerance might modulate some of the toxic effects of LPS (14). Accordingly, we have tested the effects of endotoxin tolerance (15) on bile flow and sulfobromophthalein (BSP) excretion in the perfused rat liver and on canalicular Na^+,K^+-ATPase activity.

Materials and methods. Male CD rats (200 to 300 g; Charles River Laboratories) were rendered tolerant to endotoxin by daily injection of *E. coli* endotoxin (055:B5; Difco Laboratories) (Day 1, 0.01 mg; Day 2, 0.025 mg; Day 3, 0.1 mg; Day 4, 0.2 mg; Day 5, 0.6 mg; Days 6 and 7, no treatment; Days 8 and 9, 1.0 mg each day (15)) dissolved in sterile nonpyrogenic saline. The control animals received saline only. By 24 hr after the last injection, tolerance to the lethal effects of LPS had developed as demonstrated by the survival of all four LPS-pretreated rats given an acute lethal dose (5 mg/rat, i.p.), which killed all four of the control animals. After completion of the tolerance-inducing regimen, the animals were permitted a 24- to 48-hr rest period prior to use.

Preparation of the rat liver for perfusion was accomplished as described previously (6, 7). The perfusion medium was a red blood cell-free Krebs–Henseleit buffer (pH 7.45) augmented with 120 mg glucose, 2 g bovine serum albumin, and 500 U heparin per 100 ml of perfusate. The total perfusate volume was 200 ml. Sodium taurocholate (0.5 μmole/min) was infused into the recirculating perfusate during the entire experimental period to replace the bile acids normally present in the enterohepatic circulation.

After a 30-min equilibration period, the *E. coli* endotoxin was introduced into the perfusate. Fifteen minutes later, 20 mg of BSP was added and the experiment continued for an additional 45 min. The bile and hepatic perfusate flow rates were monitored as outlined earlier (6). The excretion of BSP was measured by obtaining samples of bile (20 μl) and perfusate (50 μl), adding 0.2 *N* NaOH and determining the absorbance at 575 nm using a Gilford 300 N spectrophotometer.

In separate experiments, rat liver plasma membranes enriched with bile canaliculi were prepared using a slight modification (10, 16) of the method of Song and co-workers (17); their purity and enrichment were ascertained as described in an earlier report (10).

The total ATPase was measured in a 5-ml reaction mixture containing 20 m*M* imidazole

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buffer (pH 7.8), 0.2 mg protein, 150 m*M* NaCl, 5 m*M* KCl, 2.5 m*M* $MgCl_2$, and 2.5 m*M* Na_2ATP. In the Mg^{2+}-ATPase assay, the KCl was omitted and the difference between the total and Mg^{2+}-ATPase was taken to be the Na^+,K^+-ATPase activity (16). The specific activity of the ATPases is expressed as μmoles inorganic phosphorus (P_i) released/mg protein/hr. All results are expressed as means ± SEM. The *t* test was used to compare means and the 5% level was assumed to denote significance (18).

Results. The endotoxin-pretreatment regimen led to a significant reduction in bile flow and BSP excretion (Table I). In control experiments, the bile flow and BSP excretory rates were 1.56 ± 0.06 μl/min/g liver and 14.6 ± 0.6 μg/min/g liver, respectively. However, during perfusion of livers from tolerant animals, these rates were lower (1.22 ± 0.13 and 10.6 ± 0.6, respectively; both $P < 0.05$). Addition of the *E. coli* LPS to the perfusate at a concentration of 20 μg/ml failed to produce a further reduction in bile flow or BSP clearance, although, at the same concentration, the LPS significantly decreased bile flow (1.17 ± 0.07 μl/min/g liver; $P < 0.05$) and BSP excretion (11.9 ± 0.3 μg/min/g liver; $P < 0.05$) in livers isolated from control animals.

The Na^+,K^+-ATPase activity in membranes isolated from tolerant rats was significantly lower (15.9 ± 0.8 μmoles P_i/mg protein/hr; $P < 0.01$) than that of control membranes (23.4 ± 1.1). While the addition of LPS produced significant dose-dependent reductions in the Na^+,K^+-ATPase activity of membranes prepared from controls, it caused no further modification in the activity of this enzyme in preparations obtained from endotoxin-tolerant rats (Fig. 1). That these inhibitory effects were specific for the Na^+,K^+-ATPase is demonstrated by the fact that there were no significant differences in the basal activities of Mg^{2+}-ATPase from control (50.7 ± 2.4 μmoles P_i/mg protein/hr or tolerant animals (53.5 ± 3.5).

Discussion. The results demonstrate the endotoxin-tolerant state leads to a decrease in bile flow and hepatic organic anion excretion in the perfused rat liver and to a reduction in the basal activity of Na^+,K^+-ATPase in canalicular-enriched liver plasma membranes. In addition, these models are refractory to further inhibition by an acute dose of LPS. Although the role of Na^+,K^+-ATPase in the regulation of bile secretion is controversial (11, 19–21), the present data, demonstrating parallel changes in bile flow and Na^+K^+-ATPase activity, are consistent with the concept that this enzyme is involved in bile formation.

These data appear to have a bearing on the mechanisms of tolerance and its expression. With respect to the cholestatic effects of endotoxin, tolerance does not appear to be a state of "enzymatic adaptation" (22), an inhibition of the release of metabolic mediators (23, 24), or the result of a hyperactive hepatic reticuloendothelial system (25), all hypotheses of-

TABLE I. EFFECTS OF ACUTE ENDOTOXIN TREATMENT ON BILE FLOW AND SULFOBROMOPHTHALEIN (BSP) EXCRETION NORMAL AND ENDOTOXIN-TOLERANT RATS

Endotoxin concentration (μg/ml)	Bile flow (μl/min/g liver)	BSP excretion (μg/min/g liver)
	Nontreated rats	
0 (5)[a]	1.56 ± 0.06	14.6 ± 0.6
20 (5)	1.17 ± 0.07*	11.9 ± 0.3*
	Endotoxin-tolerant rats	
0 (5)	1.22 ± 0.13*	10.6 ± 0.7*
20 (5)	1.34 ± 0.05*,**	11.5 ± 0.4*,**

[a] Numbers in parentheses are numbers of animals in each group.

* $P < 0.05$ versus nontreated controls.

** Not significantly different from endotoxin-tolerant rats.

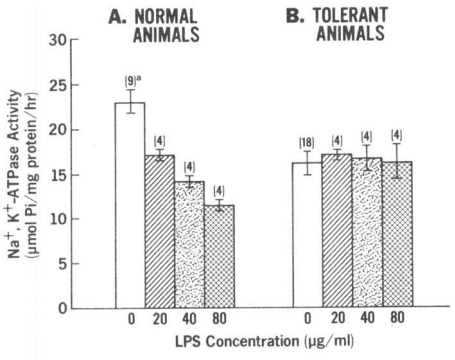

FIG. 1. Effects of various concentrations of *E. coli* endotoxin on the Na^+,K^+-ATPase activity of bile canalicular-enriched rat liver plasma membranes isolated from control and endotoxin-tolerant animals. [a] Numbers in parentheses are numbers of experiments in each group.

fered to explain other aspects of "tolerance." Indeed, "tolerance" to the acute cholestatic effects of LPS may simply reflect the cholestatic effects of chronic LPS administration, rather than protection from the cholestasis of acute doses.

The cholestatic effects of LPS may be a consequence of its high affinity for biological membranes (26) and its effects on their enzymes. Endotoxins have been reported to decrease ATPase activity in leukocytes (27) and rat liver plasma membranes enriched with bile canaliculi (10). Furthermore, there are receptors for endotoxin on membranes (28, 29) and the binding of endotoxin to membranes induces changes in their configuration (30, 31). Changes in membrane fluidity or lipid composition have been reported to alter Na^+,K^+-ATPase activity (32, 33). Since LPS has been reported to localize in hepatic parenchymal cells (34), its binding to the hepatic plasma membrane may induce changes in membrane configuration which, in turn, could account for the observed decreases in Na^+,K^+-ATPase activity. During the development of tolerance, the continuous exposure of the membrane to the LPS might induce membrane changes which result in the reduction of Na^+,K^+-ATPase activity and bile flow similar to that seen during acute endotoxin intoxication (6, 10). However, due to the chronic exposure of the membrane to the LPS, a subsequent acute dose might not be able to cause any further conformational changes. These suppositions could account for the apparent lack of response of endotoxin-tolerant animals when challenged by an acute lethal dose of endotoxin.

Summary. The isolated perfused liver of endotoxin-tolerant rats exhibited lower bile flow and sulfobromophthalein (BSP) excretion rates than did similar preparations from control animals. In addition, the Na^+,K^+-ATPase activity of bile canalicular-enriched liver plasma membranes isolated from tolerant animals was less than that observed in canalicular membranes isolated from normal rats. Although, acute endotoxin treatment inhibited bile flow and BSP excretion in the isolated perfused rat liver and Na^+,K^+-ATPase in canalicular membranes from controls, this treatment caused no further reductions in these parameters in preparations obtained from endotoxin-tolerant rats. Thus, the tolerant rat appeared to be refractory to the acute effects of endotoxin. Although it is possible that an interaction of endotoxin with the liver plasma membrane could account for these results, the underlying mechanism(s) for the observed refractoriness is unknown.

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