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ENDOTOXIN INHIBITS GLUCURONIDATION IN THE LIVER

AN EFFECT MEDIATED BY INTERCELLULAR COMMUNICATION

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Abstract—Endotoxin [lipopolysaccharide (LPS) 50 μg/mL] added to the perfusion medium increased glucose production and inhibited the glucuronidation of *p*-nitrophenol in perfused mouse liver both in recirculating and non-recirculating systems, while sulfation of *p*-nitrophenol was unchanged. The effects of endotoxin could be prevented by the addition of cyclooxygenase inhibitors, while PGD_2 and PGE_2 also caused a decrease in *p*-nitrophenol glucuronidation in perfused liver. In isolated hepatocytes endotoxin failed to affect *p*-nitrophenol conjugation, while PGD_2 and PGE_2 decreased the rate of it. Our results suggest that endotoxin inhibits glucuronidation through an intercellular communication presumably mediated by eicosanoids.

Key words: glucuronidation; endotoxin; eicosanoids; intercellular communication; mouse liver

Infections and inflammatory states induce alterations of hepatic drug metabolism. These effects may be manifested at the level of gene expression mediated by various cytokines and other factors [1, 2]. However, these states also cause immediate changes in liver metabolism. Endotoxin, the LPS§ component of the cell wall of gram negative organisms is an agent widely used to mimic infections and inflammation-related metabolic alterations in experimental systems [3].

Recently it has been shown that endotoxin stimulates glycogenolysis in perfused liver by means of intercellular communication mediated by prostaglandins between the Kupffer and/or endothelial cells and the parenchymal liver cells [3]. Similar effects on glycogenolysis have been observed with platelet-activating factor [4], phorbol myristate acetate [5], Zymosan [6], melittin [7], colloidal carbon [8] and thrombin [9].

Liver plays a crucial role in glycogen storage and biotransformation. These two liver functions are in close relationship through the cofactor supply for biotransformation [10]. The rate of glucuronidation in phase II of biotransformation is determined by the extent of glycogen pools [11], since UDP-glucuronic acid supply is derived predominantly from glycogenolysis [12, 13].

Glycogenolysis and the glucuronidation of planar phenols catalyzed by UDPGTs [14–16] seems to be regulated in the opposite direction: the well-known cAMP and Ca^{2+} mediated stimulation of glycogen breakdown is associated with a cAMP [17, 18] and a putative Ca^{2+} [19, 20] dependent inhibition of glucuronidation. Hepatic nerve stimulation has been reported to inhibit *p*-nitrophenol extraction in perfused rat liver [19]. The effect of nerve stimulation is mediated by prostanoids [21]. PGD_2—the major eicosanoid produced by Kupffer cells [22]—and PGE_2 increase glycogenolysis in hepatocytes. They stimulate the activity of glycogen phosphorylase [23]. This effect is supposed to be mediated by calcium-dependent phosphorylation [24].

Our study was undertaken to investigate whether a prostaglandin-mediated stimulating effect on glycogenolysis by endotoxin in the liver may result in a depression of *p*-nitrophenol glucuronidation at the same time.

MATERIALS AND METHODS

Materials. Collagenase type IV, β-glucuronidase type IX, arylsulfatase type VIII and LPS (*E. coli* serotype 0111:B4) were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.), PGE_2 and PGD_2 were from Upjohn (Kalamazoo, MI, U.S.A.).

Isolated liver perfusion. Male CFLP mice (25–30 g body weight) were used throughout the experiments. Livers were cannulated through the portal vein and perfused for 30 min with non-recirculating Krebs–Henseleit bicarbonate buffer (pH 7.4) containing 8.5 mM glucose and 5 mM pyruvate, continually saturated with O_2:CO_2 (95:5, v/v), at 37°. After 30 min the perfusion buffer was supplemented with 0.1 mM *p*-nitrophenol and the perfusion was continued. LPS (50 μg/mL) was given as a 3 min pulse at the time indicated. Effluent was collected at 1 min intervals. In a series of experiments after 30 min livers were perfused for a further 15 min in a recirculating system (20 mL perfusion volume). In

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§ Abbreviations: LPS, lipopolysaccharide; UDPGT, UDP-glucuronosyltransferase.

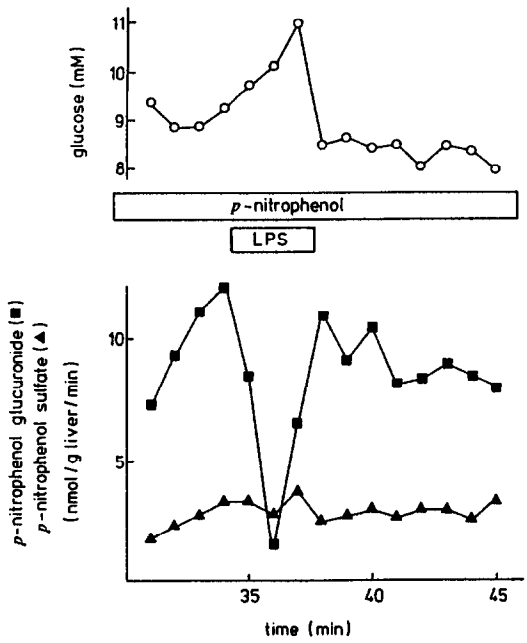

Fig. 1. Influence of endotoxin on the glucose and *p*-nitrophenol glucuronide output of perfused mouse liver. After a 30 min perfusion the liver was perfused further in a non-recirculating system in the presence of 0.1 mM *p*-nitrophenol. LPS (50 μg/mL) was added as a 3-min pulse. Data are from one representative experiment of four separate ones.

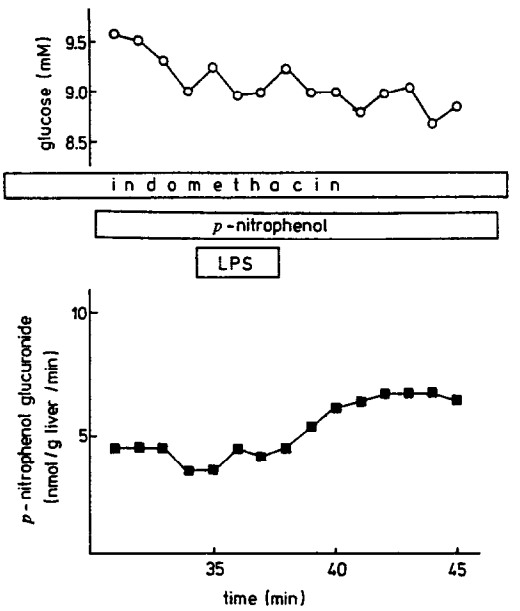

Fig. 2. Effect of endotoxin on the glucose and *p*-nitrophenol glucuronide output of perfused mouse liver in the presence of indomethacin. After 30 min perfusion the liver was perfused further in a non-recirculating system. Indomethacin (10^{-5} M) was present during the whole perfusion period. LPS (50 μg/mL) was added as a 3-min pulse. Data are from one representative experiment of three separate ones.

these cases LPS (50 μg/mL) and *p*-nitrophenol (0.1 mM) were added immediately after 30 min; LPS was present or absent throughout the perfusion. The velocity of the perfusion was constant: 1 mL/min/g liver in both systems.

Isolation and incubation of hepatocytes Isolated hepatocytes were prepared by the collagenase perfusion method as described earlier [25]. Viability of the cells checked by the trypan blue exclusion test was approx. 90%. Hepatocytes (2×10^6 cells/mL) were incubated in the above-mentioned perfusion buffer supplemented with 1% albumin. Glucose and pyruvate were omitted from the medium when glucose production was measured.

Measurement of metabolites. Conjugation of *p*-nitrophenol in isolated hepatocytes was investigated by measuring *p*-nitrophenol disappearance. *p*-Nitrophenol glucuronide and sulfate formation were determined enzymatically as described earlier [12]. Production of glucose was measured by the glucose oxidase–peroxidase method [26].

RESULTS

Mouse livers were perfused with 0.1 mM *p*-nitrophenol and the effect of LPS added to the perfusion medium was studied on *p*-nitrophenol conjugation. In once-through perfusion the addition of LPS resulted in a transient increase in glucose production in agreement with previous observations in perfused rat liver [3]. At the same time it caused an immediate decrease in *p*-nitrophenol glucuronide formation, while *p*-nitrophenol sulfate production was unaffected (Fig. 1).

In a series of experiments conducted after 30 min perfusion was continued with a recirculating perfusion system. In these experiments LPS also caused a decrease in *p*-nitrophenol glucuronidation in the course of a further 15 min perfusion (control: 16.9 ± 3.8, LPS: 8.1 ± 2.2) while *p*-nitrophenol sulfate formation was unchanged (control: 7.7 ± 1.9, LPS: 8.9 ± 1.4, nmol/min/g liver, means ± SD, N = 3). It has been suggested that LPS may reduce the glucuronidation of *p*-nitrophenol by inhibiting aglycon uptake. Our results excluded this possibility: the addition of LPS even increased the disappearance of *p*-nitrophenol from the perfusion buffer (data not shown).

It has been shown that the increasing effect of LPS on glucose production is mediated by PGD_2 and PGE_2, which are secreted mainly by Kupffer cells upon addition of LPS [3]. Therefore, the possible role of eicosanoids was investigated. First, the liver was preperfused with 10 μM indomethacin or meclofenamate and the effect of LPS examined. The addition of inhibitors of the cyclooxygenase pathway in non-recirculating perfusion prevented the enhancing effect of LPS on glucose production. The formation of *p*-nitrophenol glucuronide was

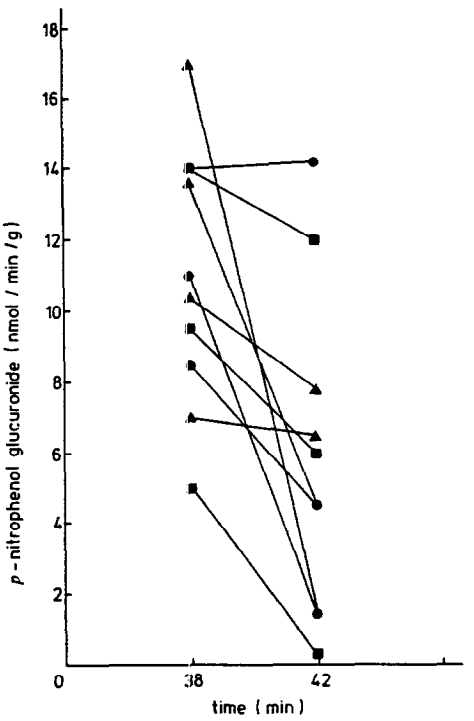

Fig. 3. Effect of LPS (▲), PGE_2 (●) and PGD_2 (■) on the *p*-nitrophenol glucuronide output of perfused liver. After a 30 min perfusion the liver was perfused further in a non-recirculating system in the presence of 0.1 mM *p*-nitrophenol. Prostaglandins (30 μM) and LPS (50 μg/mL) were added as a 3-min pulse at 40 min of perfusion. *p*-Nitrophenol glucuronide formation was measured 2 min before and after these additions. The effects of LPS and PGD_2 were significant at $P < 0.05$ level.

Table 1. Effect of endotoxin, PGD_2 and PGE_2 on *p*-nitrophenol conjugation and glucose production in isolated mouse hepatocytes

		p-Nitrophenol disappearance (nmol/min/g liver)	Glucose production (nmol/min/g liver)
Control		48.1 ± 4.3 (4)	790 ± 102 (4)
PGD_2	3 μM	46.3 ± 2.5 (3)	843 ± 51 (4)
	30 μM	39.3 ± 5.2 (4)	873 ± 61 (4)
	300 μM	32.3 ± 12.7 (3)	1014 ± 60 (4)
PGE_2	3 μM	48.1 ± 8.7 (3)	1011 ± 102 (4)
	30 μM	36.5 ± 10.3 (4)	1082 ± 297 (4)
	300 μM	25.5 ± 10.6 (3)	1028 ± 49 (4)
LPS	100 μg/mL	44.6 ± 5.8 (4)	763 ± 109 (4)

p-Nitrophenol disappearance was measured for 30 min in the presence of 0.1 mM *p*-nitrophenol. Glucose production of the cells was measured in the absence of glucose and gluconeogenic precursors for 30 min. Data are means ± SD (N).

somewhat lower in the presence of indomethacin (probably due to a competition) but the addition of LPS did not result in any change (Fig. 2). Meclofenamate (10 μM) prevented the inhibitory effect of LPS on glucuronidation and did not alter the basal rate of *p*-nitrophenol glucuronidation (data not shown). Second, the effect of prostaglandins was investigated on *p*-nitrophenol conjugation in perfused liver. In accordance with previous data [23] PGD_2 and PGE_2 increased the secretion of glucose (data not shown). At the same time both PGD_2 and PGE_2 decreased *p*-nitrophenol glucuronide formation (Fig. 3); however, their inhibitory effect on glucuronide formation was less explicit than that of LPS and the effect of PGD_2 was barely significant. Third, isolated hepatocytes were incubated in the presence of 100 μM *p*-nitrophenol and the effect of LPS on its conjugation was studied. LPS added at 25–100 μg/mL concentrations did not influence the conjugation of *p*-nitrophenol (Table 1).

As the possible role of prostanoids as mediators of the LPS effect was presumed, the effect of PGD_2 and PGE_2 on conjugation of *p*-nitrophenol was examined. For comparison their effect on glucose production was also measured. Table 1 shows that PGD_2 and PGE_2 decrease the disappearance of *p*-nitrophenol in a dose-dependent manner in isolated hepatocytes.

DISCUSSION

Based on these observations it is suggested that endotoxin inhibits the glucuronidation of *p*-nitrophenol in the liver via an indirect mechanism involving intercellular communication. LPS decreased *p*-nitrophenol conjugation in perfused liver both in the non-recirculating (Fig. 1) and recirculating systems but failed to inhibit it in isolated hepatocytes (Table 1). In isolated mouse hepatocytes at 100 μM aglycone concentration *p*-nitrophenol is conjugated mainly with glucuronate and to a small degree with sulfate [12]. Inhibition of glucuronidation was responsible for the decrease in conjugation—sulfation of *p*-nitrophenol remained unaltered (Fig. 1). These observations are in accordance with previous data: depression of glucuronidation has been shown to underlie the inhibition of conjugation by various agents such as dibutyryl cAMP [17, 18] glucagon, insulin [13], and by calcium mobilization from the endoplasmic reticulum [20]. These agents are also involved in the regulation of the carbohydrate metabolism of hepatocytes. The source of UDP-glucuronate synthesis and glucose secretion is glycogenolysis [12, 13]. Therefore, one is led to suppose that the increase in glucose secretion and the decrease in glucuronidation are related.

Prostanoids are possible mediators of the effect of LPS on drug conjugation. However, while indomethacin and meclofenamate prevented inhibition by LPS, their decreasing effect on *p*-nitrophenol conjugation in perfused liver was less expressed than that of LPS (Fig. 3), and only a moderate decrease in conjugation (and a modest increase in glycogenolysis) was shown in isolated hepatocytes, albeit at a rather high concentration of PGD_2 and PGE_2 (Table 1). Based on these findings

the contribution of other eicosanoids in the intercellular communication mediated by the LPS effect cannot be excluded. The possible role of thromboxane A_2 and other cyclooxygenase products has recently been reported in the mediation of the 2,5-di(tert-butyl)hydroquinone effect on the Ca^{2+} efflux from the parenchymal cells in perfused rat liver [27].

The findings presented here suggest that in the liver pathological stimuli through local hormones from non-parenchymal liver cells may influence the rate of the UDP-glucose consuming glucuronidation and glucose secretion at the same time.

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