

The effect of lipopolysaccharide, interleukin-1 and tumour necrosis factor on the hepatic accumulation of 5-hydroxytryptamine and platelets in the mouse

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1 Injection of lipopolysaccharide (LPS; 0.5–500 $\mu\text{g kg}^{-1}$) into mice induced a dose-dependent, slowly developing increase in hepatic content of 5-hydroxytryptamine (5-HT). This sustained increase could not be attributed to an LPS-induced alteration of the pharmacokinetic handling of 5-HT by stimulation of its uptake or inhibition of its degradation.

2 Regional differences were apparent in the tissue content of histamine and 5-HT between mast cell-deficient (W/W^v) and normal (+/+) mice. LPS administration (0.5 mg kg⁻¹) gave comparable increases in the hepatic level of 5-HT in mast cell-deficient and normal mice.

3 Reserpine pretreatment (1 mg kg⁻¹) selectively reduced 5-HT levels in the blood, spleen, liver, brain and lung of normal mice. Prior treatment with this agent also abolished the LPS (0.5 mg kg⁻¹)-induced hepatic accumulation of 5-HT.

4 Accumulation of 5-HT in the liver by LPS (0.1 mg kg⁻¹) was temporally associated with both a fall in the levels of circulating platelets, and a reduction in the concentration of 5-HT in the blood. The LPS dose-dependent (0.5–500 $\mu\text{g kg}^{-1}$) increase in hepatic 5-HT content was associated with a similar dose-dependent reduction in the circulating levels of 5-HT.

5 Interleukin-1, α and β (10 $\mu\text{g kg}^{-1}$) and tumour necrosis factor α (TNF α) (1 mg kg⁻¹) significantly enhanced the accumulation of 5-HT within the liver. Administration of TNF α (10 $\mu\text{g kg}^{-1}$) potentiated the increase in hepatic 5-HT content seen with IL-1 β (10 $\mu\text{g kg}^{-1}$).

6 Electron microscopy revealed numerous platelets in the sinusoidal and perisinusoidal Disse spaces within the liver, in animals pretreated with LPS (0.1 mg kg⁻¹). The platelets retained their intact structure and showed no evidence of degranulation.

7 These data suggest that the LPS and cytokine-induced mobilization of 5-HT in the liver is associated with the hepatic translocation of platelets. This migration appears to be independent of platelet aggregation.

Keywords: Lipopolysaccharide; platelet; interleukin-1; tumour necrosis factor; 5-hydroxytryptamine; liver; endotoxin

Introduction

In the pathogenesis of septic shock, vasoactive amines such as histamine and 5-hydroxytryptamine (5-HT) have been implicated as endogenous mediators contributing to liver dysfunction (Halpern *et al.*, 1963; Iff & Vas, 1966). Previous data have demonstrated that injection of lipopolysaccharides (LPS) into mice induces a rise in both histamine and 5-HT levels within the liver (Endo, 1982, 1983a). The LPS-induced increase in hepatic histamine levels has been attributed to an induction of histidine decarboxylase, an enzyme involved in its synthesis. This induction has also been reported in other tissues such as the lung, spleen and bone marrow (Endo, 1982; 1983b; 1989; Endo *et al.*, 1986). However, the mechanisms responsible for the accumulation of 5-HT within the liver remain to be established. It is known that the rise in 5-HT observed after LPS injection occurs after a time lag of 1 h, reaches a maximum at 4.5–6 h and returns to baseline within 2 days (Endo 1983a). This rise is extremely sensitive to LPS, significant accumulation occurring at concentrations as low as 0.1 $\mu\text{g kg}^{-1}$ (Endo, 1984). Unlike histamine, the increase in 5-HT appears to be relatively specific for the liver and does not occur in other tissues such as the lung, spleen, intestine or kidney (Endo, 1983a). In addition, the accumulation of 5-HT is not attributable to an enhancement of its synthesis (Endo, 1987), which suggests this amine is transported into the liver from the surrounding tissues.

The aim of the present study was to investigate the mechanisms and identify the possible sources responsible for the

hepatic accumulation of 5-HT. Mast cells in rodents contain and release 5-HT in addition to histamine (Moran *et al.*, 1962; Prouvost-Danon *et al.*, 1966). Thus, we used mice known to be deficient in mast cells (Kitamura *et al.*, 1978; Suda *et al.*, 1985) to ascertain the role played by these cells in the mobilization of 5-HT into the liver. Recently, it has been suggested that cytokines released by macrophages may play a role in the LPS-induced rise in hepatic 5-HT (Endo *et al.*, 1985; Endo, 1991) and we have also therefore investigated the effects of recombinant interleukin-1 (IL-1, α and β) and tumour necrosis factor α (TNF α).

Methods

Animals

Male, 6 week old WBB6F1 (B-W/ + x C57BL/6-W^v/ +) - W/W^v (mast cell-deficient), WBB6F1-+/+ (normal litter mates of W/W^v) and ddY mice were obtained from Shizuoka Agricultural Association, Japan.

Determination of 5 hydroxytryptamine and histamine

To avoid the rapid degradation of 5-HT in the blood, pre-weighed tubes containing 3 ml of 0.4 M HClO₄, 2 mM EDTA and 0.1% cysteine-HCl were used to collect 5 drops of blood, which was then rapidly mixed and weighed. The tubes were then quickly transferred into a dry ice/ethanol bath for freezing, where they were kept for up to 2 days. For the estimation of tissue content of 5-HT and histamine, the respective organs

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were rapidly removed, weighed and frozen in dry ice and ethanol. 5-HT was measured according to a previously described method (Tadano *et al.*, 1980) modified to allow the simultaneous measurement of many samples. Briefly, 5-HT within the tissues and blood was extracted in 0.4 M HClO₄ containing 2 mM EDTA and 0.1% cysteine-HCl in an Ultra Turrax homogenizer. The extract was neutralized to approximately pH 4.5 with 2 M KOH before 1 ml was applied to a phosphylated-cellulose packed column and equilibrated with 0.01 M phosphate buffer (pH 6.2). The column was washed with 4 ml of the phosphate buffer and 0.8 ml of 0.1 M HCl containing 0.1% cysteine-HCl, before the 5-HT was eluted by another 1.5 ml of 0.1 M HCl containing 0.1% cysteine-HCl. The 5-HT content of the eluate was determined fluorometrically.

Histamine content was determined as described previously (Endo, 1983c).

Estimation of platelet count

Approximately 5 drops of blood from stunned, decapitated mice were collected into a preweighed test tube containing 0.5 ml of 4 mM EDTA in 0.01 M phosphate buffered saline. The tube was weighed and the number of platelets was automatically counted in a Celltac MEK-4150 cell counter.

Effect of lipopolysaccharide on 5-hydroxytryptamine synthesis and degradation

To examine whether LPS administration alters the pharmacokinetic handling of 5-HT, normal mice were injected with either saline or LPS (0.5 mg kg⁻¹) i.v. and 4 h later various doses of 5-HT (5–20 mg kg⁻¹) were given i.p. After 2 h, the mice were killed and their livers analysed for differences in 5-HT content. The effect of pargyline, an irreversible inhibitor of monoamine oxidase, was examined on the LPS-induced rise in hepatic content of 5-HT. Saline or pargyline (100 mg kg⁻¹) were given by intraperitoneal injection, followed 1 h later by various doses of LPS (0.1–10 µg kg⁻¹) i.p. The mice were killed 4 h later and their livers analysed for 5-HT content.

Examination of possible sources of 5-hydroxytryptamine

The 5-HT content of a variety of tissues was examined in mast cell-deficient (W/W⁻) and normal (+/+) mice to assess the role of the mast cell as a possible source of 5-HT by examining its tissue distribution. To ascertain whether the LPS-induced rise in 5-HT was attributable to an influx of mast cells, LPS was administered (0.5 mg kg⁻¹, i.v.) to mast cell-deficient and normal litter mate controls. The mice were killed 4.5 h later and their livers analysed for 5-HT content.

Effect of reserpine

To assess the relative levels of 5-HT in organs which could be mobilized by LPS, a variety of tissues were examined in ddY mice, 24 h after saline or reserpine (1 mg kg⁻¹) pretreatment. To examine whether tissues depleted of their 5-HT content contributed to the LPS-induced liver accumulation, mice pretreated with either saline or reserpine (1 mg kg⁻¹) were injected 24 h later with either saline or LPS (0.5 mg kg⁻¹, i.v.). After 4.5 h the mice were killed and their livers analysed for 5-HT content.

Relationship between hepatic and circulating levels of 5-hydroxytryptamine

Various doses of LPS (0.5–500 µg kg⁻¹) were injected i.v. into normal mice, to examine the dose-dependent relationship between circulating and hepatic levels of 5-HT. The mice were killed 4.5 h later and the respective tissues analysed for 5-HT content. Assessment of the temporal relationship between hepatic and circulating levels of 5-HT was performed as

follows. At various time intervals after the injection of 0.1 mg kg⁻¹ LPS, ddY mice were killed and their blood and livers analysed for 5-HT content. To calculate the total amount of 5-HT in the blood, its volume was calculated according to the method of Wish *et al.* (1950).

Lipopolysaccharide-induced thrombocytopenia

Since platelets are recognized as blood elements containing significant amounts of 5-HT, the temporal relationship between the levels of circulating platelets and hepatic 5-HT content was examined in normal mice. The mice were killed at various times after LPS administration (0.1 mg kg⁻¹) when platelet counts and hepatic 5-HT content were measured.

Effect of cytokine administration

IL-1 α , IL-1 β and TNF α at doses of 10 µg kg⁻¹ or 1 mg kg⁻¹, alone and in combination, were injected intraperitoneally into ddY mice. After 4 h their liver content of 5-HT was measured and cellular structure was examined by electron microscopy.

Electron microscopy

For visualization of the hepatic cellular events, 4.5 h after saline or LPS (0.1 mg kg⁻¹) injection i.v., the livers from stunned, decapitated mice were rapidly removed and chopped into small pieces. The specimens were fixed with a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, post-fixed with 1% aqueous OsO₄, and stained *en bloc* with 1% aqueous uranyl acetate for 1 h at 4°C. The samples were dehydrated with a graded series of ethanols, passed through propylene oxide and embedded in EPON 812. Ultra-thin sections mounted on copper grids were stained with uranyl acetate and lead citrate and examined with a Hitachi H-700 transmission electron microscope. For the visualization of possible fibrin disposition, the staining procedure according to Carstairs (1965) was followed.

Materials

Lipopolysaccharide (*E. coli*; 055B5) was purchased from Difco Lab., Detroit, U.S.A.; reserpine (Apoplone) from Daiichi Seiyaku Co. Ltd., Tokyo, Japan and pargyline hydrochloride from Sigma Chemical Co., St. Louis, U.S.A. Recombinant human interleukin-1 α (IL-1 α) and recombinant human tumour necrosis factor α (TNF α) were provided by Dainippon Pharmaceutical Co., Osaka, Japan, prepared according to Furutani *et al.*, (1985) and Yamada *et al.* (1985). Recombinant human IL-1 β was donated by the Ohtsuka Pharmaceutical Co., Tokushima, Japan and prepared according to Kikumoto *et al.* (1987). On a SDS-polyacrylamide gel, electrophoresis of each of the cytokines gave a single band. The contaminants of LPS in the preparations of IL-1 α , IL-1 β and TNF α were less than 0.02, 0.1 and 0.04 ng per mg protein respectively (*Limulus* test). The remainder of the laboratory reagents were obtained from Wako Pure Chemical Industries Ltd., Tokyo, Japan. The drugs and cytokines were dissolved in sterile saline and injected in a volume of 0.1 ml per 10 g body weight.

Statistical analysis

Experimental values shown in the text, figures and tables are given as mean \pm standard deviation. The statistical significance of these differences were analysed by Student's unpaired *t* test and *P* values less than 0.05 were considered to be significant.

Results

Lipopolysaccharide-induced alteration of 5-hydroxytryptamine uptake/degradation

There was no significant difference between saline or LPS (0.5 mg kg⁻¹) pretreated mice in the amount of 5-HT that was

increased in the liver by the prior administration of 5-HT (data not shown). In addition, there was no effect of pargyline (100 mg kg^{-1}) on the LPS-induced accumulation of 5-HT in the liver.

Examination of possible sources of 5-hydroxytryptamine

The histamine and 5-HT content of various tissues in mast cell-deficient mice and their normal litter mates is shown in Table 1. In mast cell-deficient mice, histamine levels were significantly lower in the spleen, lung, thymus and skin; in contrast, only the cutaneous 5-HT content was found to be significantly lower in these animals ($P < 0.01$).

Lipopolysaccharide-induced accumulation of 5-hydroxytryptamine in the liver of mast cell-deficient mice

Accumulation of hepatic 5-HT following injection of LPS into normal and mast cell-deficient mice is shown in Figure 1. LPS (0.5 mg kg^{-1}) induced a significant accumulation of 5-HT in the liver of normal and mast cell deficient mice ($P < 0.01$).

Effect of reserpine pretreatment

Reserpine pretreatment (1 mg kg^{-1} , i.p.) administered 24 h before tissue 5-HT analysis, revealed that various organs, notably the brain, liver, spleen, lung, thymus and blood, but not intestine, had been significantly depleted of their 5-HT content (Table 2). Administration of LPS (0.5 mg kg^{-1}), 24 h

Table 1 Histamine and 5-hydroxytryptamine (5-HT) levels in normal (+/+) and mast cell deficient (W/W^v) mice

	Histamine (nmol g^{-1})		5-HT (nmol g^{-1})	
	+/+	W/W ^v	+/+	W/W ^v
Brain	0.62 ± 0.02	0.56 ± 0.02	3.7 ± 0.3	3.8 ± 0.3
Liver	0.79 ± 0.04	0.69 ± 0.03	2.3 ± 0.1	2.6 ± 0.2
Spleen	6.0 ± 0.6	$0.9 \pm 0.1^*$	88 ± 11	86 ± 20
Lung	2.3 ± 0.2	$0.3 \pm 0.1^*$	5.7 ± 1.8	5.5 ± 1.9
Thymus	10.0 ± 2.0	$0.8 \pm 0.3^*$	1.3 ± 0.1	1.4 ± 0.2
Skin	80.0 ± 7.0	$2.0 \pm 0.4^*$	0.9 ± 0.2	$<0.3^*$
Intestine	5.0 ± 0.8	4.3 ± 0.5	46 ± 5	41 ± 4
Blood	0.29 ± 0.05	0.21 ± 0.04	19 ± 2	22 ± 2

* $P < 0.01$. Values represent the mean and standard deviation of results from 4 mice.

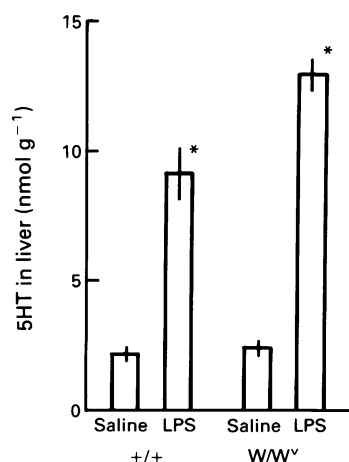


Figure 1 Lipopolysaccharide (LPS, 0.5 mg kg^{-1})-induced 5-hydroxytryptamine (5-HT) accumulation within the liver of normal (+/+) and mast cell deficient (W/W^v) mice. Each column represents the mean and vertical lines indicate the standard deviation of results from 4 animals. * $P < 0.01$.

Table 2 5-Hydroxytryptamine (5-HT) levels in saline and reserpine-pretreated ddY mice

	5-HT (nmol g^{-1})	
	Saline	Reserpine-treated
Brain	3.4 ± 0.1	$2.0 \pm 0.4^*$
Liver	1.9 ± 0.1	$0.2 \pm 0.1^*$
Spleen	50 ± 6	$1.3 \pm 0.2^*$
Lung	6.5 ± 0.8	$0.3 \pm 0.1^*$
Thymus	1.0 ± 0.1	$0.3 \pm 0.1^*$
Muscle	0.5 ± 0.1	0.4 ± 0.1
Skin	0.8 ± 0.2	0.5 ± 0.1
Ear	1.4 ± 0.3	1.0 ± 0.2
Intestine	50 ± 6	52 ± 16
Blood	14 ± 2	$0.3 \pm 0.1^*$

* $P < 0.01$. Values represent the mean and standard deviation of results from 4 mice.

after reserpine pretreatment failed to induce the rise in hepatic 5-HT seen in saline pretreated mice (Figure 2). Prior administration of reserpine depleted circulating levels of 5-HT, which were unaffected by the subsequent addition of LPS (Figure 2).

Dose and time-dependent lipopolysaccharide-induced mobilization of 5-hydroxytryptamine

LPS ($0.5\text{--}500 \mu\text{g kg}^{-1}$) injection induced a dose-dependent rise in hepatic 5-HT content within the liver. This mobilization was maximal between $5\text{--}50 \mu\text{g kg}^{-1}$ and was reflected in a dose-dependent reduction in circulating levels of 5-HT (Figure 3). Measurement of the kinetics underlying the translocation of 5-HT revealed a significant rise in the hepatic content 2 h after the LPS (0.1 mg kg^{-1}) was administered (Figure 4). Examination of the kinetics of the circulating levels of 5-HT following LPS (0.1 mg kg^{-1}) injection showed a significant depletion within 1 h (Figure 4), circulating and hepatic levels

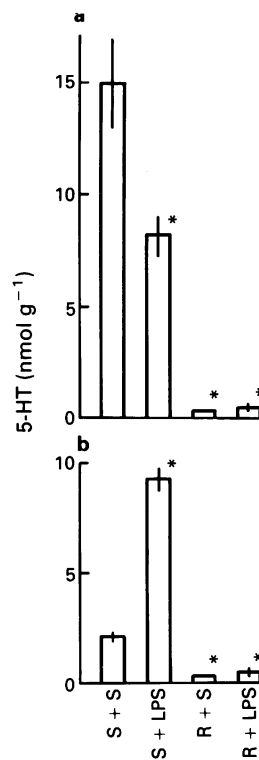


Figure 2 The effect of lipopolysaccharide (LPS, 0.5 mg kg^{-1}) on 5-hydroxytryptamine (5-HT) levels in the blood (a) and liver (b) of saline (S) or reserpine (R) pretreated ddY mice. Each column represents the mean and vertical lines indicate the standard deviation of results from 4 animals. * $P < 0.01$.

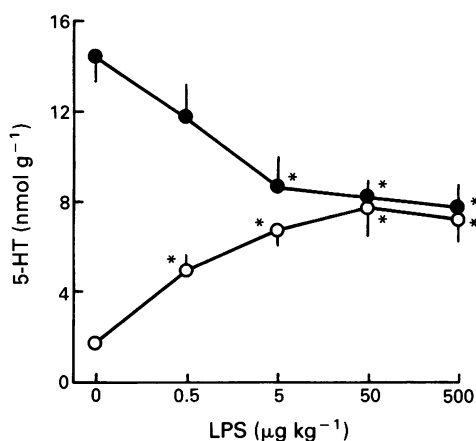


Figure 3 Dose dependent relationship of lipopolysaccharide (LPS, 0.5–500 $\mu\text{g kg}^{-1}$) administration on the level of 5-hydroxytryptamine (5-HT) in the blood (●) and liver (○) in ddY mice. Each point represents the mean and vertical lines indicate standard deviation of results from 4 animals. * $P < 0.01$.

plateaued within 4.5 h. An analysis of the amount of 5-HT lost from the blood 4.5 h after LPS administration indicated that approximately 70% accumulated within the liver (amount lost from the blood, 13.1 nmol; amount accumulated within the liver, 9.4 nmol; $n = 8$).

Lipopolysaccharide-induced thrombocytopenia

The temporal relationship between circulating levels of platelets and hepatic accumulation of 5-HT stimulated by LPS (0.1 mg kg^{-1}) is shown in Figure 5. A close reciprocal relationship was evident, significant liver accumulation of 5-HT and platelet depletion occurring within 3 and 4 h of LPS injection, respectively. Maximal hepatic accumulation of 5-HT and thrombocytopenia were evident at 5.5 h.

Effects of interleukin-1 α , -1 β and tumour necrosis factor α

As demonstrated in Figure 6, IL-1 α , IL-1 β (10 $\mu\text{g kg}^{-1}$) and TNF α (1 mg kg^{-1}) all induced a rise in the hepatic accumulation of 5-HT. These effects showed a similar time course to that observed on LPS administration (data not shown). The

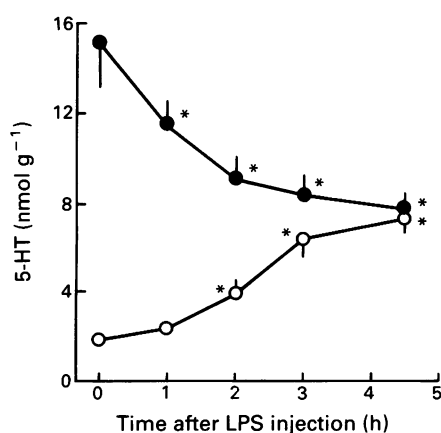


Figure 4 Temporal relationship between the 5-hydroxytryptamine (5-HT) content in the blood (●) and liver (○) after lipopolysaccharide (LPS, 0.1 mg kg^{-1}) administration in ddY mice. Each point represents the mean and vertical lines indicate the standard deviation of results from 4 animals. * $P < 0.01$.

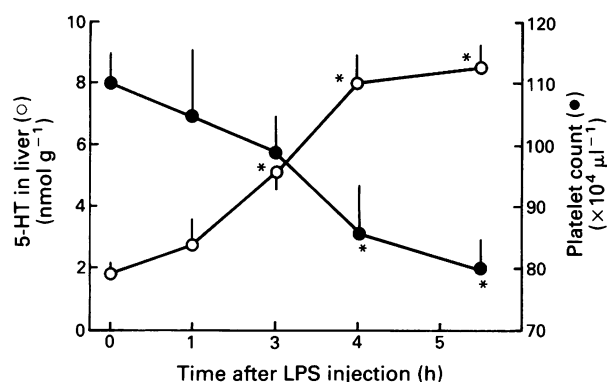


Figure 5 Temporal relationship between the increase in 5-hydroxytryptamine (5-HT) content in the liver (○) and the decrease in platelet count in the blood (●) following lipopolysaccharide (LPS, 0.1 mg kg^{-1}) administration. Each point represents the mean and vertical lines indicate the standard deviation of results from 4 normal mice. * $P < 0.01$.

concurrent administration of IL-1 α and IL-1 β produced an accumulation of 5-HT within the liver which was equal to the administration of either cytokine alone. However, TNF α potentiated the effects of both IL-1 α and IL-1 β . The combination of TNF α (10 $\mu\text{g kg}^{-1}$) and IL-1 (α or β ; 10 $\mu\text{g kg}^{-1}$) resulted in an accumulation of 5-HT that corresponded to the level induced by 1 mg kg^{-1} TNF α , or to the maximal level obtained on LPS injection.

Electron microscopy

A notable feature of the livers taken from LPS-treated mice was the presence of numerous platelets, located in the sinusoidal and perisinusoidal spaces between hepatocytes and endothelial cells. An estimation of the platelet numbers in 5 objective fields gave values of 0.8 ± 0.7 for normal liver and 5.8 ± 2.1 for the livers of LPS-pretreated mice. The platelets seen within the liver still retained the characteristic dense granules and microtubules indicating that these cells had not undergone degranulation.

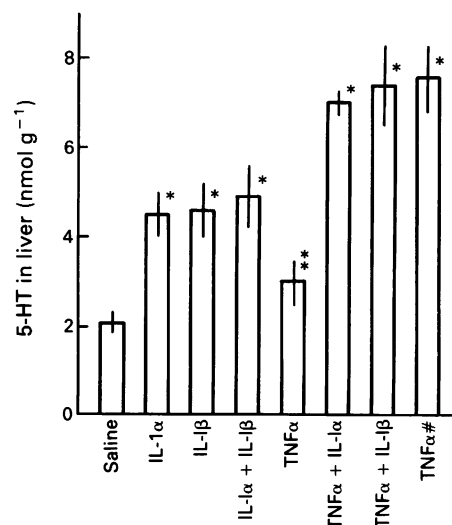


Figure 6 The effect of various cytokines, alone and in combination, on hepatic 5-hydroxytryptamine (5-HT) accumulation in ddY mice. Interleukin (IL) 1 α and 1 β were administered at 10 $\mu\text{g kg}^{-1}$, i.p., tumour necrosis factor α (TNF α) was administered at 10 $\mu\text{g kg}^{-1}$ and 1 mg kg^{-1} , i.p. (#). Each column represents the mean and vertical lines indicate the standard deviation of results from 4 animals. * $P < 0.01$; ** $P < 0.01$.

Additionally, there was no apparent deposition of fibrin in the perisinusoidal spaces as detected by Carstairs' staining method. Figure 7a displays the typical lack of interaction between a platelet and a Kupffer's cell (KC) in a normal liver. In contrast, the majority of the platelets in the sinusoidal spaces of the liver in LPS-treated mice (Figure 7b-d) were surrounded by well developed cell processes from Kupffer's cells and there were electron dense sites between platelets and Kupffer's cells. All of the KC in the specimens from LPS-treated mice were Mac-1 positive, indicating that they had been previously activated.

In the liver of LPS-treated mice, many more polymorphonuclear neutrophils were observed in the sinusoidal rather

than the perisinusoidal spaces. However, there was no obvious attachment of the platelets to the neutrophils. A similar profile of cell accumulation was observed with the combination of IL-1 α and TNF α (both 10 $\mu\text{g kg}^{-1}$).

Discussion

These studies, whilst confirming previous results showing LPS-induced accumulation of 5-HT in the liver (Endo, 1982; 1983a), have attempted to investigate the underlying mechanisms. Previous data have demonstrated that the increase in 5-HT within the liver cannot be attributed to the stimulation

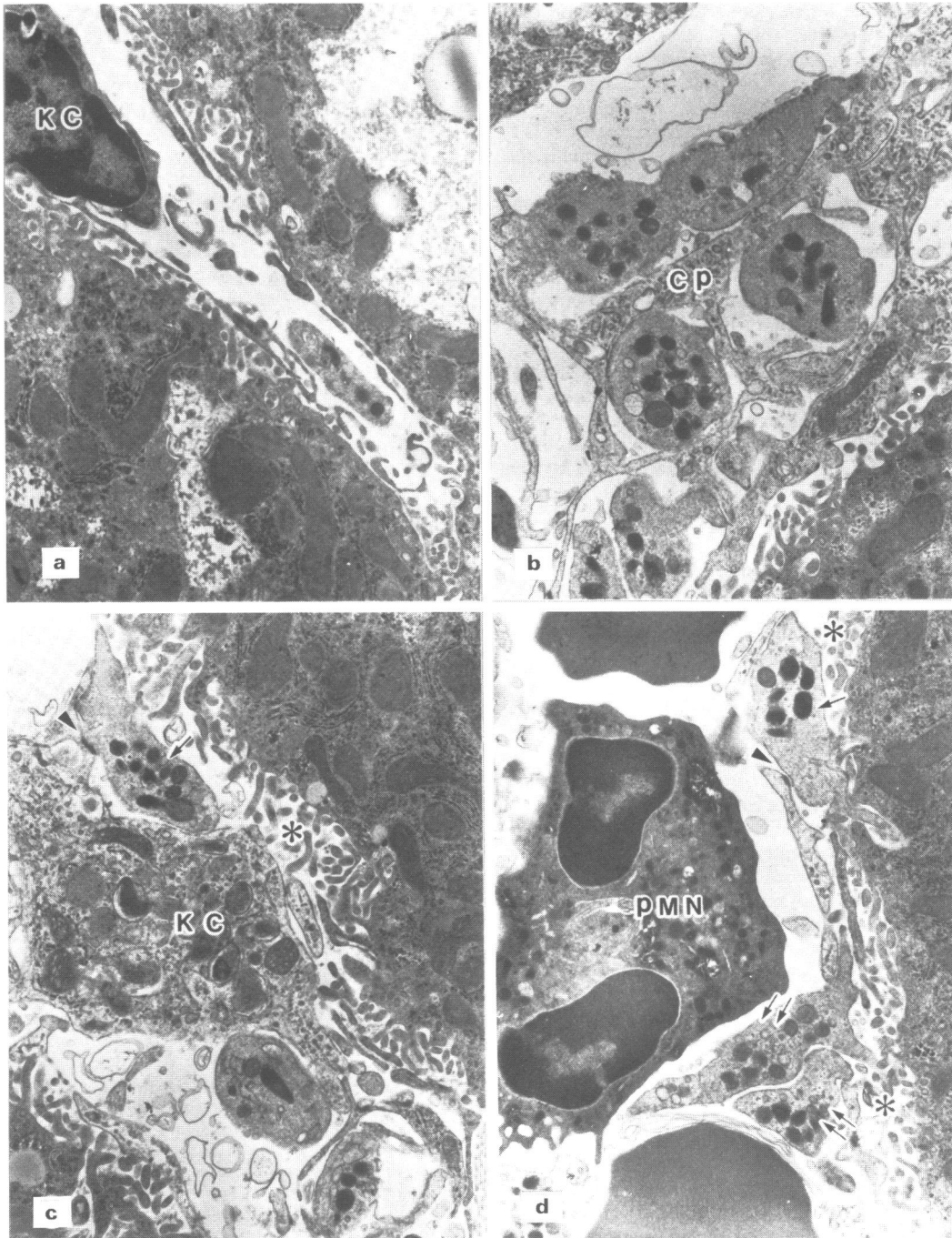


Figure 7 Electron micrographs of hepatic sinusoidal spaces in normal (a) and lipopolysaccharide (LPS)-treated (b-d) ddY mice. (a) A platelet and a Kupffer's cell (KC) are seen in the sinusoidal space. (b) Platelets in the sinusoidal space are surrounded by the cell processes (CP) of a Kupffer's cell. (c) A platelet (arrow) is localized in a space of Disse (*). There is an electron dense site (arrow head) suggesting intercellular contact. (d) Two platelets (arrows) are penetrating into a space of Disse. A platelet (an arrow) is seen in a space of Disse, and there is a presumed cell contact site (arrow head) between the Kupffer's cell and the platelet, and an extension of the platelet pseudopod into the hepatocyte. A polymorphonuclear neutrophil (PMN) is also present in the sinusoidal space.

of its synthesis (Endo, 1987). Furthermore, no evidence was obtained in this study to suggest that LPS enhanced stimulation of 5-HT uptake into the liver or inhibited its degradation. As such, the hepatic increase observed is postulated to be the result of mobilization from 5-HT containing tissues.

The histamine content in various tissues was shown to be lower in mast cell-deficient (W/W^u) than normal litter mate control (+/+) mice (Yamatodani *et al.*, 1982; present study). However, there is little difference in these two groups of mice with respect to tissue 5-HT content except for the skin. This may suggest that only cutaneous mast cells contain significant amounts of 5-HT. Since mast cell deficient and normal mice have comparable increases in liver 5-HT content following LPS administration, it is concluded that mast cells *per se* do not contribute to the 5-HT accumulation observed.

Despite containing the highest concentration of 5-HT per gram of tissue and its almost total depletion by reserpine-pretreatment, the spleen is not thought to be responsible for the mobilization of 5-HT into the liver, because in some experiments the 5-HT level in the spleen was enhanced by LPS (Endo, 1983a). The intestine is a rich source of 5-HT, however, it is not depleted by reserpine-pretreatment. If the intestine is the source of 5-HT for the liver, possibly via the connecting portal system, then reserpine pretreatment might be expected to have no effect on the LPS-induced stimulation of hepatic 5-HT accumulation. Thus, the intestine is probably not responsible for the increase in 5-HT observed.

From the inverse relationship between hepatic and circulating 5-HT levels, it is suggested that 5-HT is mobilized, following LPS administration, from the blood into the liver. Furthermore, reserpine pretreatment caused an almost total depletion of 5-HT from the blood and prevented the LPS-induced rise in 5-HT within the liver. From the data it can be calculated that 70% of the 5-HT lost from the blood accumulates within the liver. This could be explained simply by the engorgement or congestion of the liver with blood; however, no increase in total liver mass was observed on LPS administration (data not shown). From the time-dependent studies examining the levels of circulating platelets and 5-HT accumulation within the liver, the reciprocal relationship was highly suggestive of hepatic platelet accumulation. Furthermore, the total amount of 5-HT accumulated within the liver expressed as a percentage of the total blood content (32%) is almost identical to the amount of platelets lost from the circulation (30%). Whether these platelets represent a separate subpopulation of cells able to accumulate within the liver in response to LPS or cytokines, without degranulating, remains to be established. Although there is little information concerning the effect of LPS on platelets, it is known that 5-HT is released within 1 min of LPS injection from rabbit platelets (Davies *et al.*, 1963). This acute release of 5-HT may be responsible for the loss of circulating 5-HT from the blood within 1 h in the absence of significant hepatic platelet accumulation. This would also help explain why only 70% of the 5-HT lost from the circulation accumulates in the liver.

Examination of liver sections confirmed the presence of platelets within the sinusoidal and perisinusoidal spaces. Many platelets in sinusoidal spaces were surrounded by cell processes from Kupffer's cells, and there were electron dense sites between platelets and Kupffer's cells, which indicate that cellular interactions may play an important role in the migration of platelets from the sinusoidal into the Disse spaces.

In addition to LPS, various types of inflammatory agents also promote the accumulation of 5-HT within the liver of the mouse (Endo, 1983a; 1984). LPS is known to stimulate macrophages to produce cytokines such as IL-1 α , IL-1 β and TNF α (Oppenheim *et al.*, 1986; Old, 1987). In the present

study, it was shown that each of these cytokines was able to induce accumulation of 5-HT within the liver. Endo (1991) showed that the effects of IL-1 (α and β) were significant at doses as little as 0.1 $\mu\text{g kg}^{-1}$ with maximum accumulation occurring at 10 $\mu\text{g kg}^{-1}$. Since no additional increase in 5-HT levels within the liver was observed with a combination of these two cytokines, it may be concluded that the respective pathways are closely interlinked if not identical. Moreover, there was an apparent synergism between IL-1 (α and β) and TNF α , corresponding to the maximum level induced by LPS. These results suggest that the increase in platelet and 5-HT content in the liver by LPS may be mediated via a combination of these cytokines.

Injection of LPS into experimental animals with immune adherence positive platelets has previously been shown to induce a biphasic response in the numbers of circulating platelets (Ulevitch *et al.*, 1975; Morrison & Ulevitch, 1978). The acute thrombocytopenia, occurring within several minutes is known to be accompanied by the release of 5-HT and histamine (Davies *et al.*, 1966) and platelet aggregation (Davies, 1966). However, since abrogation of this response does not prevent disseminated intravascular coagulation (DIC), it is the slowly developing phase which is believed to be important in the DIC induced by LPS or Gram-negative sepsis (Ulevitch *et al.*, 1975; 1978; Mathison & Ulevitch, 1981). This delayed thrombocytopenia is independent of complement (Ulevitch *et al.*, 1975; 1978; Morrison & Ulevitch, 1978) and occurs even in primates which lack immune adherence sites on their platelets (Ulevitch *et al.*, 1978). However, the role of platelets in this phenomenon has not been fully investigated. This study has proposed that the slowly developing thrombocytopenia following LPS administration in the mouse is associated with the accumulation of platelets into the sinusoidal and Disse spaces of the liver.

Due to its central role in thermal regulation, gluconeogenesis and in the production of clotting factors, the liver is intrinsically involved in the symptomology of Gram negative sepsis, i.e. fever, hypoglycaemia and stimulation of the coagulation system resulting in thrombocytopenia or DIC, hypotension or shock and death. Furthermore it has been previously shown that D-galactosamine, an agent which attacks the liver in a similar manner to human viral hepatitis, in combination with a small amount of LPS induces a lethal, fulminant hepatitis (Galanos *et al.*, 1979; Tiegs *et al.*, 1989). It has already been established that the toxic principle of Gram negative bacteria is LPS or endotoxin and that the actions of LPS may be mediated via IL-1 or TNF α (Oppenheim *et al.*, 1986; Old, 1987; Lehmann *et al.*, 1987; Wallach *et al.*, 1988, Mathison *et al.*, 1988; Endo, 1989; 1991). It is also known that platelets contain many factors such as hepatocyte growth factor, platelet-derived growth factor, and transforming growth factor β (Nakamura *et al.*, 1986) which may be important in the physiological defence system against bacterial invasion. Although the mechanisms for platelet accumulation in the liver remain to be clarified, the present findings suggest a new role for these cellular elements in the pathophysiological response to LPS.

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