Br. J. Pharmacol. (1992), **107**, 888–894

Ornithine and histidine decarboxylase activities in mice sensitized to endotoxin, interleukin-1 or tumour necrosis factor by D-galactosamine

[1]Yasuo Endo, Takashi Kikuchi & *Masanori Nakamura

Department of Pharmacology, *Department of Anatomy, School of Dentistry, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan

1 An injection of D-galactosamine (GalN) into mice together with a lipopolysaccharide (LPS or endotoxin), interleukin-1 (IL-1) or tumour necrosis factor (TNF), sensitized the mice and induced fulminant hepatitis with severe congestion resulting in rapid death. Since LPS and these cytokines induce ornithine decarboxylase (ODC) and histidine decarboxylase (HDC) in the liver and spleen of mice, the effects of GalN on the induction of ODC and HDC in these organs were examined.

2 The induction of ODC by LPS, IL-1 or TNF was suppressed by GalN in the liver, and this suppression preceded the hepatic congestion. There was good agreement between the degree of hepatic congestion and the suppression of ODC induction by various amounts of GalN. The induction of ODC in the spleen was suppressed only at the highest dose of GalN examined.

3 GalN is known to deplete uridine 5'-triphosphate (UTP), resulting in the suppression of RNA and protein synthesis. An injection of uridine, the precursor of UTP, diminished the GalN-induced suppression of ODC induction by LPS and prevented the hepatic congestion and death.

4 LPS-pretreatment before injection of LPS plus GalN prevented the suppression of ODC activity and prevented the hepatic congestion and death.

5 An injection of putrescine, the product of ODC, prolonged survival time and delayed the development of hepatic congestion. However, injection of an ODC inhibitor into the mice given LPS did not produce hepatic congestion.

6 The induction of HDC in the liver by LPS, IL-1 or TNF was not suppressed by GalN and, at high doses, the response to LPS was enhanced. An inhibitor of HDC neither prevented the hepatic congestion nor enhanced the protective effect of putrescine.

7 Although GalN in combination with IL-1α induced a markedly higher HDC activity than was observed when it was combined with TNFα, and suppressed the induction of ODC, the former combination at the doses used did not produce hepatic congestion or death. However, the sensitization to TNFα by GalN was markedly potentiated by IL-1α.

8 These results suggest that suppression of the induction of ODC by GalN may be one cause of the sensitization to LPS, IL-1 or TNF, and that the induction of HDC, i.e. histamine formation, may not be involved in this sensitization.

9 These results are consistent with the hypothesis that both IL-1 and TNF are involved in the sensitization to LPS.

Keywords: Ornithine decarboxylase; polyamines; histamine; histidine decarboxylase; lipopolysaccharide; endotoxin; D-galactosamine; interleukin-l; tumour necrosis factor; hepatitis

Introduction

D-Galactosamine (GalN) is metabolized by enzymes of the galactose pathway which are most abundant in the liver. Since this pathway consumes uridine nucleotides, the administration of GalN rapidly depletes uridine nucleotides, primarily in the liver, and results in a decrease in RNA synthesis (Decker & Keppler, 1974). This agent, within 2 days of its injection into rats, induces hepatic necrosis resembling human viral hepatitis (Decker & Keppler, 1974). In addition, GalN sensitizes various experimental animals to the lethal effect of lipopolysaccharides (LPS) (Galanos *et al.*, 1979). The lethality induced by GalN plus LPS is thought to be due to the hepatic failure that occurs rapidly (within 10 h) after their injection (Galanos *et al.*, 1979; Tiegs *et al.*, 1989).

It has been suggested that the effect of LPS is mediated by the cytokines, interleukin-1 (IL-1) and/or tumour necrosis factor (TNF), because IL-1 and TNF can substitute for LPS, their lethal effects are also augmented by GalN (Lehmann *et al.*, 1987; Wallach *et al.*, 1988; Tiegs *et al.*, 1989) and because their production is stimulated by LPS (Oppenheim *et al.*, 1986; Beutler & Cerami, 1987). However, it is not clear what mechanisms or biochemical events are involved in the sensitization to LPS in the presence of GalN.

Ornithine decarboxylase (ODC) produces putrescine, a precursor of spermidine and spermine, from ornithine. ODC is a rate-limiting enzyme in the synthesis of these polyamines which have been implicated as regualtors of cellular growth and functions, and ODC is induced rapidly by various growth stimuli (Pegg & McCann, 1982). We have shown that ODC is induced in the liver of mice *in vivo* within a few hours after injection of various inflammatory agents (Endo, 1982; 1984; Endo *et al.*, 1985), and that LPS is the most potent inducer among them (Endo, 1984). This action of LPS seems to be mediated by IL-1 and/or TNF, because these cytokines, either by themselves or acting synergistically in combination, induce ODC (Endo *et al.*, 1985; Endo, 1989). IL-1 and TNF have an anti-proliferative action on some tumour cells *in vitro*, and we have shown evidence that suppression of ODC activity in such tumour cells may be involved in the anti-proliferative action of IL-1 or TNF (Endo *et al.*, 1988). Therefore, it is of interest to examine how GalN affects the induction of ODC by LPS, IL-1 or TNF.

[1] Author for correspondence.

Histamine is produced by histidine decarboxylase (HDC). We have shown that HDC is also induced in the liver of mice by LPS, IL-1 and TNF (Endo, 1982; 1983a; 1989; Endo *et al.*, 1986). Since the GalN-induced sensitization to LPS, IL-1 or TNF is usually accompanied by severe congestion, and histamine is a potent stimulator of post capillary venule permeability, the histamine produced by the induction of HDC might be expected to be involved in the development of the hepatic congestion.

In this study, therefore, the effects of GalN on the induction of ODC and HDC in the liver by LPS, IL-1 and TNF were examined. Since LPS, IL-1 and TNF induce ODC and HDC in the spleen, also, the effects of GalN on the spleen were compared to those on the liver.

Methods

Determination of putrescine and histamine and assay of ornithine and histidine decarboxylase activities

The mice were killed by decapitation at indicated time intervals after injections of test samples, the liver and spleen rapidly removed and kept in dry ice until assayed.

Putrescine and histamine levels were determined as described previously (Endo, 1983b). Briefly, they were extracted from the tissues with 0.4 M $HClO_4$, separated on a small phosphorylated cellulose column and the amounts present determined fluorometrically after reaction with fluorescamine or *o*-phthalaldehyde.

HDC and ODC activities were assayed by a previously described method (Endo, 1983b), which was modified both in order to remove any histamine and putrescine in the tissues and to simplify the method. The liver (0.2 g) or a single whole spleen was placed in a teflon tube with phosphorylated cellulose powder (50 mg) and 2 ml of ice-cold 0.02 M sodium phosphate buffer (pH 6.2) containing pyridoxal 5'-phosphate (20 μM) and dithiothreitol (200 μM) and homogenized with an Ultra Turrax homogenizer (Janke & Kunkel Co., Germany). The supernatant of the homogenate obtained after centrifugation (10,000 *g* for 15 min at 4°C) was used as the enzyme solution. The histamine and putrescine present in the tissues were bound to the phosphorylated cellulose and removed almost completely from the enzyme solution.

The procedures for simultaneous assay of HDC and ODC activities were the same as described previously (Endo, 1983b). Briefly, the enzyme solution was incubated at 37°C for 3 h with both histidine and ornithine. After terminating the enzyme reaction by adding $HClO_4$ (final concentration 0.33 M), histamine and putrescine formed in the reaction mixture were recovered separately by chromatography on a small phosphorylated cellulose column and quantitated fluorometrically as described above. The small amounts of histamine or putrescine in the non-incubated reaction mixture (i.e., zero-activity; $HClO_4$ was added without incubation) were subtracted from the amounts of these amines in the incubated samples. Since the enzyme reaction progressed linearly for at least 3 h, the activities of HDC and ODC were expressed as nmol of histamine or putrescine formed during 1 h by the enzyme contained in 1 g of the tissue (nmol h^{-1} g^{-1}).

Estimation of the degree of hepatic congestion

When the livers were removed for the assay of ODC and HDC activities, the degree of hepatic congestion was scored by macroscopic observation, i.e., (1) no congestion; (2) slight (congestion in up to 20% of the liver); (3) medium (congestion in 20 to 50% of the liver); (4) severe (congestion in more than 50% of the liver), and the mean ± s.d. of the score was calculated. The incidence of hepatic congestion is also shown together with the mean scores of hepatic congestion.

Materials and animals

Lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 prepared by Boivin's method was obtained from Difco Laboratories (Detroit, MI, U.S.A.). Recombinant human interleukin-1α (IL-1α) and recombinant human tumour necrosis factor α (TNFα) were provided by Dainippon Pharmaceutical Co., Osaka, Japan. DL-α-Difluoromethylornithine (DFMO) and DL-α-monofluoromethylhistidine (FMH) were gifts from Dr Wilkins of Merrell Dow Research Institute (Strasburg, Cedex, France) and Dr Kollonitsch of Merck Sharp & Dohme Research Laboratories (Rahway, NJ, U.S.A.), respectively. Uridine and putrescine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Female BALB/cA and C57BL/6N mice (6 weeks old) were obtained from the Mouse Center at our University. Reagents were dissolved in sterile saline and injected intraperitoneally into mice (0.05–0.1 ml/10 g body weight).

Statistical differences

Statistical differences between two means of data were evaluated by Student's unpaired *t* test and *P* values less than 0.05 were considered to be significant.

Results

Lethality induced by the combination of a lipopolysaccharide with D-galactosamine

The sensitivity to LPS varies among strains of mice. Therefore, the lethal effect of the combination of LPS with GalN was tested in two strains of mice, C57BL/6N and BALB/cA, raised in our University (Table 1). GalN by itself, even at a dose of 1500 mg kg^{-1}, caused no deaths and no obvious ill effects when mice were observed for up to 3 days. Most mice survived for 20 h after the injection of LPS alone at a dose of 10 mg kg^{-1}. However, the combination of smaller amounts of GalN and LPS markedly enhanced lethality. Sudden death occurred within 5–10 h without symptoms until shortly before death, as noted by Galanos *et al.* (1979), and severe congestion in the liver was observed in all the mice that died.

Kinetics of the induction of histidine and ornithine decarboxylases and the effects of D-galactosamine

In normal mice both ODC and HDC activities are very low in the liver (below 1 and 0.2 $nmol^{-1}$ g^{-1}, respectively), and a

Table 1 Lethality of the combination of lipopolysaccharide (LPS) with D-galactosamine (GalN)

GalN (mg kg^{-1})	*LPS* (μg kg^{-1})	*Lethality* C57BL/6N	BALB/cA
1500	0	0/5	0/5
0	10000	0/5	1/5
0	1000	0/5	0/5
800	100	4/4	4/4
800	10	4/4	4/4
800	1	4/4	0/4
800	0.1	0/4	–
800	10	4/4	4/4
600	10	4/4	3/5
400	10	4/4	–
200	10	0/4	–

LPS was injected into mice immediately after the injection of GalN. The lethality values are the sum total of deaths occurring by 20 h. The dashes indicate that no observations were made with these drug combinations.

sham injection with saline does not enhance these activities. However, an injection of LPS induced marked increases in ODC and HDC activities in the liver of both C57BL/6N and BALB/cA mice (Figure 1). GalN suppressed the induction of ODC in the liver of both strains of mice, but the HDC activity in the liver of C57BL/6N mice was not suppressed at all. In the liver of BALB/cA mice given a larger amount of GalN (800 mg kg^{-1}), the induction of HDC activity was enhanced. Such an enhancement of HDC activity in the liver was also observed in C57BL/6N mice when injected with large amounts of GalN (Figure 2).

The ODC and HDC activities in the spleen of normal mice are below 5 and 1 nmol h^{-1} g^{-1}, respectively. These activities were also markedly enhanced by LPS, but not by sham injection. However, GalN did not cause any significant effect on the induction of ODC or HDC activities by LPS in the spleen of either C57BL/6N or BALB/cA mice in this experiment (data not shown).

The scores (and incidence) of hepatic congestion in C57BL/6N mice in this experiment were 1 ± 0 (0/12) within 3.5 h and 1.8 ± 1.0 (2/4) and 3.5 ± 0.6 (4/4) at 4.5 h and 5.5 h, respectively. The scores (and incidence) or hepatic congestion in BALB/cA mice were 1 ± 0 (0/12) within 6 h and 3.5 ± 0.6 (4/4) at 7.5 h. These results indicate that the suppression of ODC induction occurs before the development of hepatic congestion.

In the following experiments C57BL/6N mice were used, because hepatic congestion occurred in this strain more rapidly than in BALB/cA mice. In most of the following experiments, a combination of 50 μg kg^{-1} LPS and 600 mg kg^{-1} GalN was used. All the mice treated with this combination died within 7 h (Figure 6).

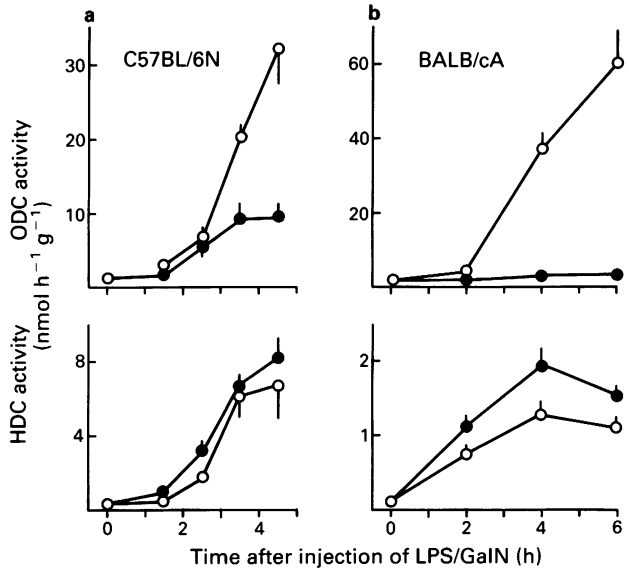

Figure 1 Kinetics of the induction of ornithine decarboxylase (ODC) and histidine decarboxylase (HDC) in the liver by lipopolysaccharide (LPS) and the effect of D-galactosamine (GalN). Mice were killed at the time intervals indicated after the injection of LPS alone (○) or a mixture of LPS and GalN (●). (a) In C57BL/6N mice: the doses of LPS and GalN were 50 μg kg^{-1} and 600 mg kg^{-1}, respectively. (b) In BALB/cA mice: the doses of LPS and GalN were 10 μg kg^{-1} and 800 mg kg^{-1}, respectively. Each value is the mean (± s.d., vertical lines) from 4 mice.

Dose- and time-dependent effects of D-galactosamine

GalN, administered to C57BL/6N mice 1 h before the injection of LPS, suppressed the ODC induction by LPS in the liver in a dose-dependent manner (Figure 2). However, GalN was not effective in suppressing HDC induction in the liver and, at higher doses, enhanced HDC activity, although GalN itself did not induce HDC activity at these doses. GalN opposed ODC and HDC inductions in the spleen only at the highest dose used. The scores (and incidence) of hepatic congestion in this experiment were 1.3 ± 0.5 (1/4) at 400 mg kg^{-1} of GalN and 2.5 ± 1.3 (3/4) at 600 mg kg^{-1}.

As shown in Figure 3, the suppressant effect of GalN on the ODC induction was marked when GalN was administered 1 h before LPS injection or simultaneously with LPS, and the scores (and incidence) of hepatic congestion under these conditions were 2.8 ± 1.0 (4/4) and 1.8 ± 1.0 (2/4), respectively. On the other hand, when GalN was injected 1 or 2 h after LPS injection, its effect was not significant and hepatic congestion did not occur. In this experiment, GalN showed no significant effect on the induction of HDC in the liver nor on the induction of ODC and HDC in the spleen. When GalN (600 mg kg^{-1}) was injected into 5 mice 1 h after LPS injection (50 μg kg^{-1}), all the mice survived for 20 h and no hepatic congestion was observed.

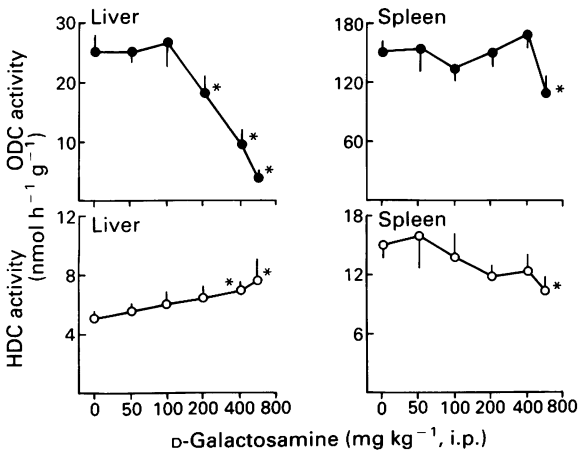

Figure 2 The effects of D-galactosamine (GalN) on the induction of ornithine decarboxylase (ODC) and histidine decarboxylase (HDC) in the liver and spleen. GalN (50–800 mg kg^{-1}) was injected into C57BL/6N mice 1 h before injection of lipopolysaccharide (LPS) (50 μg kg^{-1}). The mice were killed 4.5 h after LPS injection. Each value is the mean (± s.d., vertical lines) from 4 mice.
*$P<0.05$ vs control (LPS alone).

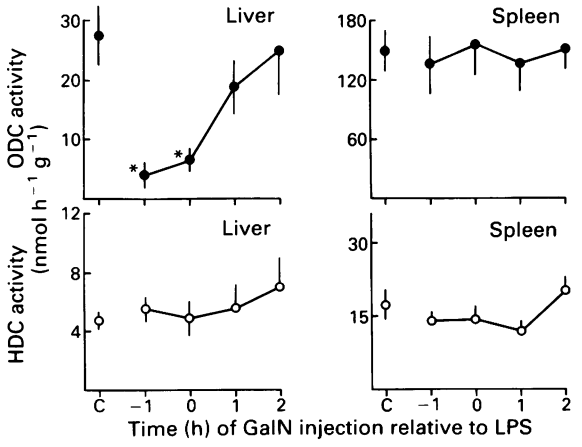

Figure 3 Effects of the injection time of D-galactosamine (GalN) on the induction of ornithine decarboxylase (ODC) and histidine decarboxylase (HDC) by lipopolysaccharide (LPS). GalN (600 mg kg^{-1}) was injected into C57BL/6N mice 1 h before, simultaneously with, or 1 or 2 h after the injection of LPS (50 μg kg^{-1}), and the mice were killed 4.5 h after LPS injection. C: LPS alone (control). Each value is the mean (± s.d., vertical lines) from 4 mice.
*$P<0.05$ vs control.

Effects of uridine on the action of D-galactosamine

Uridine can reverse the depletion of uridine nucleotides by GalN (Decker *et al.*, 1974). As shown in Figure 4, uridine, administered to mice 0.5 h after simultaneous injection of GalN and LPS, diminished the GalN-induced suppression of ODC induction. Moreover, uridine prevented the development of hepatic congestion, i.e., the scores (and incidence) of hepatic congestion in uridine-treated and non-treated mice were 1 ± 0 (0/5) and 2.2 ± 1.3 (3/5), respectively. In another experiment under these conditions, uridine completely abrogated the lethality caused by LPS plus GalN (0/8, 3 days observation). In contrast to its effects on ODC induction in the liver, uridine caused a small but statistically significant suppression of the induction of HDC by LPS in the presence of GalN (Figure 4).

Effects of lipopolysaccharide-pretreatment on the action of D-galactosamine

In order to examine whether prior stimulation of ODC induction would prevent the hepatic congestion and death induced by GalN plus LPS, the effect of LPS pretreatment was tested. In mice that had not received LPS-treatment before injection of GalN plus LPS, the induction of ODC was suppressed (Figure 5) and the score (and incidence) of hepatic congestion were 2.6 ± 1.3 (4/5). However, in mice pretreated with LPS, full ODC activity was induced, and there was no hepatic congestion. This LPS-pretreatment had no clear effect on the HDC induction. In another confirmatory experiment, the LPS-pretreatment again completely abrogated the lethality induced by GalN plus LPS (0/8, 3 days observation).

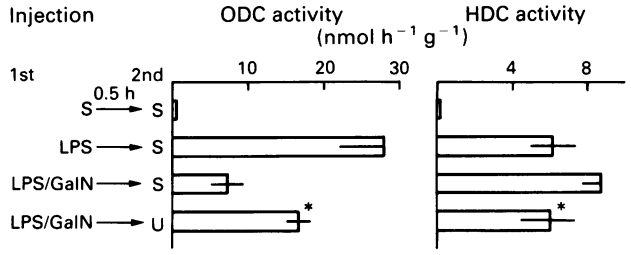

Figure 4 Effects of uridine on the induction of ornithine decarboxylase (ODC) and histidine decarboxylase (HDC) in the liver by D-galactosamine (GalN) plus lipopolysaccharide (LPS). Uridine (U) (500 mg kg^{-1}) or saline (S) was injected into C57BL/6N mice 0.5 h after simultaneous injection of LPS (50 μg kg^{-1}) and GalN (600 mg kg^{-1}). The mice were killed 4.5 h after the second injection. Each value is the mean (± s.d., horizontal lines) from 5 mice. *$P < 0.05$ vs the group injected with saline after LPS plus GalN.

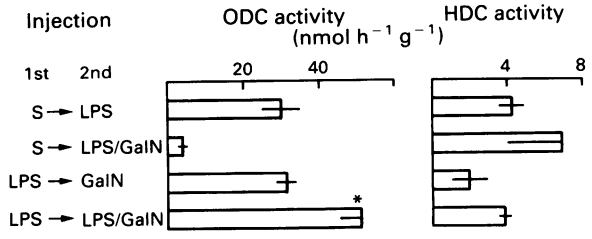

Figure 5 Effects of a prior injection of lipopolysaccharide (LPS) on the induction of ornithine decarboxylase (ODC) and histidine decarboxylase (HDC) in the liver by D-galactosamine (GalN) plus LPS. Saline (S) or LPS (50 μg kg^{-1}) was injected into C57BL/6N mice 2 h before a second injection of LPS (50 μg kg^{-1}), GalN (600 mg kg^{-1}) or their mixture. The mice were killed 5 h after the second injection. Each value is the mean (± s.d. horizontal lines) from 5 mice. *$P < 0.05$ vs the group injected with GalN after LPS.

Effects of putrescine, histamine and inhibitors of ornithine and histidine decarboxylases

The induction of ODC and HDC by LPS resulted in an increase in the levels of putrescine and histamine in the liver (Table 2). GalN inhibited the increase in putrescine but not the increase in histamine.

An injection of putrescine, the product of ODC, into mice given both LPS and GalN prolonged the survival time (Figure 6). In another confirmatory experiment, the development of congestion was also delayed by putrescine, i.e., the scores (and incidence) of hepatic congestion at 5 h after the injection of LPS plus GalN were 1 ± 0 (0/5) in mice given putrescine and 3.0 ± 1.2 (4/5) in mice given saline.

DFMO (Pösö & Pegg, 1982) and FMH (Kollonitsch *et al.*, 1978) are potent irreversible inhibitors of ODC and HDC, respectively. As shown in Table 2, these inhibitors almost completely prevented the increase in putrescine and histamine caused by LPS. However, the administration of DFMO to mice given LPS under these conditions did not produce hepatic congestion and death. In addition, FMH at this dose was ineffective in preventing or in delaying hepatic congestion and death induced by GalN plus LPS, nor did it enhance the protective effect of putrescine (data not shown).

Table 2 Effects of inhibitors of ornithine and histidine decarboxylases on the increase in putrescine and histamine in the liver caused by lipopolysaccharide (LPS)

Treatment	*Amines in the liver* (nmol g^{-1})	
	Putrescine	*Histamine*
Saline + saline	37 ± 4	0.82 ± 0.10
LPS + saline	87 ± 2*	1.69 ± 0.13*
LPS + GalN	47 ± 5	2.35 ± 0.35*
LPS + DFMO	39 ± 2	2.00 ± 0.32*
LPS + FMH	86 ± 6*	0.86 ± 0.06

The mice (C57BL/6N) were killed 5 h after injection of LPS (50 μg kg^{-1}). D-Galactosamine (GalN) (600 mg kg^{-1}) was injected simultaneously with LPS. α-Difluoromethyl ornithine (DFMO) (100 mg kg^{-1}), an inhibitor of ornithine decarboxylase or α-monofluoromethyl histidine (FMH) (50 mg kg^{-1}), an inhibitor of histidine decarboxylase, were injected 1 h after LPS injection. Each value is the mean ± s.d. of 5 or 8 mice.
*$P < 0.05$ vs control.

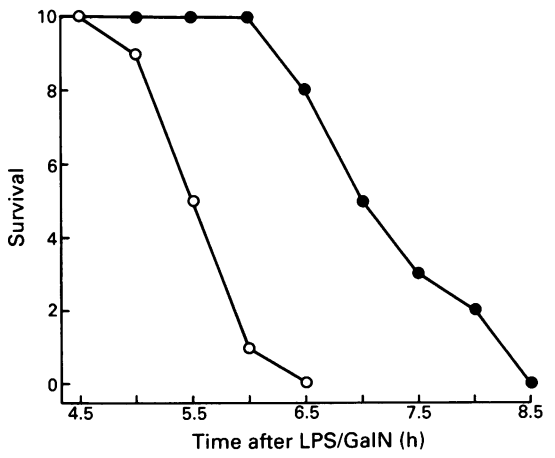

Figure 6 The effect of putrescine on the lethal effect of lipopolysaccharide (LPS) plus D-galactosamine (GalN). Saline (○) or putrescine dihydrochloride (500 mg kg^{-1}) (●) was injected twice into each group of C57BL/6N mice (10 mice/group) at 1.5 h and 4.0 h after simultaneous injection of LPS (50 μg kg^{-1}) and GalN (600 mg kg^{-1}).

Effects of D-galactosamine on the actions of interleukin-1 and tumour necrosis factor

GalN also suppressed the induction of ODC activity in the liver by IL-1α and TNFα (Table 3). Again, neither ODC activity in the spleen nor HDC activity in the liver and spleen were suppressed by GalN. In this experiment, there was no hepatic congestion in the mice given IL-1α plus GalN and the score (and incidence) of hepatic congestion in the mice given TNFα plus GalN was 1.6 ± 0.9 (2/5).

GalN combined with TNFα produced hepatic congestion within 7 h (Table 4). On the other hand, in spite of a higher HDC induction by IL-1α (Table 3), IL-1α in combination with GalN did not produce hepatic congestion (Table 4). In combination with both TNFα and IL-1α, GalN resulted in a high lethality and a high score of hepatic congestion, indicating a synergistic effect between TNFα and IL-1α.

Putrescine, even when administered orally, prolonged the survival time of the mice given GalN in combination with both IL-1α and TNFα (Figure 7). Two of seven mice injected i.p. with putrescine survived in this experiment.

Discussion

LPS, IL-1 and TNF share various biological activities (Dinarello, 1989). As to their effects on the liver, these compounds stimulate the production of various factors involved in the blood coagulation system, acute phase proteins in inflammation and cytokines such as IL-6. They also increase body temperature. They suppress hepatic gluconeogenesis and production of albumin. In addition, we have shown that they induce the enzymes catalyzing the production of polyamines and histamine in the liver. On the other hand, GalN rapidly depletes UTP in the liver, resulting in suppressed RNA synthesis and consequently, may suppress the synthesis of various proteins (Decker & Keppler, 1974). Therefore, the sensitization to LPS or hepatic failure induced by GalN may be due to combined effects on several, but not one single, biochemical event. Wallach *et al.*, (1988) have suggested that the deleterious effects of IL-1, TNF or LPS may be modulated by antagonistic mechanisms; mechanisms

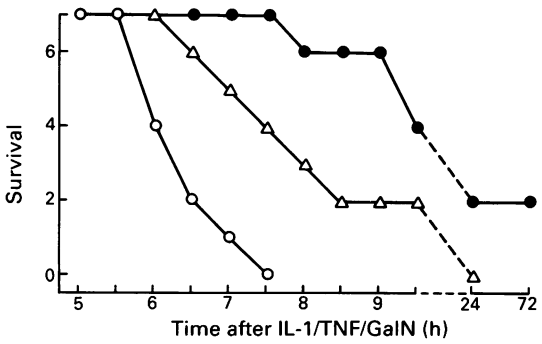

Figure 7 The effect of putrescine on the survival time of mice given D-galactosamine (GalN) in combination with both interleukin-1α (IL-1α) and tumour necrosis factor α (TNFα). Saline (i.p.) (○) or putrescine dihydrochloride (500 mg kg^{-1}) was injected i.p. (●) or given orally (△) twice into each group of C57BL/6N mice (7 mice/group at 2 h and 5 h after injection of GalN (600 mg kg^{-1}) in combination with both IL-1α (20 μg kg^{-1}) and TNFα (20 μg kg^{-1}).

Table 3 Effects of D-galactosamine (GalN) on the induction of ornithine decarboxylase (ODC) and histidine decarboxylase (HDC) by interleukin-1α (IL-1α) or tumour necrosis factor α (TNFα)

Treatment	*ODC activity* (nmol h^{-1} g^{-1})		*HDC activity* (nmol h^{-1} g^{-1})	
	Liver	*Spleen*	*Liver*	*Spleen*
Saline	3 ± 2	3 ± 2	0.2 ± 0.1	1.4 ± 0.7
IL-1α	42 ± 7	109 ± 27	3.0 ± 0.4	9.5 ± 0.5
IL-1α + GalN	13 ± 3*	130 ± 10	3.7 ± 0.4	10.2 ± 0.3
TNFα	30 ± 3	41 ± 2	1.4 ± 0.1	6.4 ± 0.6
TNFα + GalN	14 ± 2*	65 ± 20	1.5 ± 0.2	4.9 ± 0.9

The mice (C57BL/6N) were killed 4.5 h after the injection of IL-1α (100 μg kg^{-1}), TNFα (100 μg kg^{-1}), or a combination of a cytokine with GalN (600 mg kg^{-1}). Each value is the mean ± s.d. of 5 mice.
*$P < 0.05$ vs each cytokine alone.

Table 4 Effects of the combination of interleukin-1α (IL-1α) or tumour necrosis factor α (TNFα) with D-galactosamine (GalN) on hepatic congestion and lethality

Cytokines	(μg kg^{-1})	*GalN* (mg kg^{-1})	*Hepatic congestion*		*Lethality*
IL-1α	500	0	1 ± 0	(0/5)*	0/5
TNFα	500	0	1 ± 0	(0/5)	0/5
IL-1α + TNFα	100 each	0	1 ± 0	(0/5)	0/5
IL-1α	50	600	1 ± 0	(0/5)	0/5
	100	600	1 ± 0	(0/5)	0/5
TNFα	50	600	1.8 ± 1.5	(2/5)	0/5
	100	600	2.4 ± 0.8	(4/5)	0/5
IL-1α + TNFα	10 each	600	3.0 ± 1.2	(4/5)	2/5
IL-1α + TNFα	20 each	600	3.8 ± 0.4	(5/5)	4/5

IL-1α, TNFα or a mixture of the cytokines with or without GalN were injected into C57BL/6N mice. The lethality occurring within 7 h was observed and the scores (and *incidence) of hepatic congestion at death or 7 h after the injection were calculated.

which are suppressed by GalN, but which, in the absence of GalN, are augmented by IL-1 or TNF. However, it is not clear what kinds of deleterious effects or antagonistic mechanisms are involved in the sensitization.

GalN suppressed the induction of ODC in the liver but not in the spleen, and depletion of UTP has been reported to occur predominantly in the liver (Decker & Keppler, 1974). The suppression of ODC induction preceded the development of hepatic congestion. Both the suppression of ODC induction and the development of hepatic congestion were diminished by uridine, the precursor of UTP. Putrescine, the product of ODC, prolonged survival time and delayed the development of hepatic congestion. These results suggest that suppression of ODC induction is necessary to produce the sensitization to LPS by GalN. However, the suppression of ODC induction is not sufficient, because the combination of LPS with an ODC inhibitor did not induce hepatic congestion. Therefore, the induction of ODC seems to be one of the antagonistic mechanisms postulated by Wallach *et al.*, (1988).

It has been reported that the induction of ODC is essential in hepatic regeneration (Luk, 1986) and that putrescine may be involved in the healing or protection from hepatitis induced in rats by GalN alone (Daikuhara *et al.*, 1979; Nishiguchi *et al.*, 1990). Our results indicate that putrescine is also effective in delaying the GalN-induced sensitization to LPS, IL-1 and TNF. However, the detailed role of putresine or the induction of ODC in the antagonistic effects on sensitization is not clear.

In contrast to ODC, the induction of HDC by LPS, IL-1α or TNFα, was not suppressed by GalN. At higher doses, HDC induction was rather augmented by GalN. Histamine is known to be a potent mediator increasing post capillary venule permeability. Therefore, the induction of HDC activity was expected to be one of the deleterious effects described above. However, despite complete inhibition of the LPS-induced increase in histamine by FMH, an inhibitor of HDC, this agent neither prevented the hepatic congestion nor enhanced the protective effect of putrescine. Although IL-1α was more potent than TNFα in inducing HDC (Endo, 1989 and Table 3), its combination with GalN did not produce hepatic congestion (Table 4). Therefore, against our expectations, the present study indicates that induction of HDC is not involved in GalN-induced hepatic congestion or in the sensitization to LPS, TNFα or IL-1α, suggesting that HDC induction is not a deleterious event. Recently, we observed that the injection of LPS, IL-α or TNFα into mice causes the accumulation of platelets, which contain large amounts of 5-hydroxytryptamine, predominantly in the liver (Endo & Nakamura, 1992). The relevance of this phenomenon to the GalN-induced sensitization is now being examined.

The mechanism by which LPS-pretreatment prevented the GalN-induced sensitization to LPS can be explained as follows: there was a time lag of about 2 h before the induction of ODC by LPS (Figure 1), and GalN was ineffective in suppressing the induction of ODC when it was injected 2 h after the LPS injection (Figure 3). These results suggest that although the synthesis of ODC molecules is still low by 2 h after stimulation with LPS, the synthesis of ODC mRNA has been completed in this period, resulting in a full induction of ODC, and this ODC induction contributes to preventing the subsequent sensitization to LPS.

Since the induction of both ODC and HDC by LPS or cytokines is suppressed by cycloheximide, an inhibitor of protein synthesis (Endo, 1983c; 1984; Endo *et al.*, 1985), their induction may depend on *de novo* synthesis of proteins or the ODC and HDC molecules themselves. However, GalN, in spite of suppressing ODC induction in the liver, did not suppress the induction of HDC and, at high doses, enhanced HDC activity in this organ. Since GalN suppresses protein synthesis predominantly in the hepatocytes (Decker & Keppler, 1974), these results suggest that the induction of HDC in the liver may occur in cells other than hepatocytes. We have also shown that the induction of HDC by LPS *in vivo* is enhanced by actinomycin D which inhibits RNA synthesis by intercalating into DNA (Endo, 1983c). The cell types and mechanisms involved in the induction of HDC are of interest. We have evidence that actinomycin D also suppresses ODC induction by LPS preferentially in the liver and produces hepatic congestion (unpublished data).

The cytocidal effects of TNF and IL-1 on tumour cells *in vitro* are known to be markedly potentiated by inhibitors of RNA or protein synthesis or by viral infection (Aderka *et al.*, 1985). The similarity between the cytocidal effects of these cytokines *in vitro* and their lethal effects *in vivo* has been described by Wallach *et al.* (1988). We have demonstrated that the cytocidal effects of IL-1 and TNF on some tumour cells *in vitro* may involve the suppression of ODC induction (Endo *et al.*, 1988). Here we present evidence that the suppression of ODC induction may also be involved in the lethal effects of IL-1 and TNF *in vivo* in the presence of GalN. Finally, the stimulation of IL-1 and/or TNF production by endotoxins (or bacterial infection) in the course of viral infection or during treatment with inhibitors of RNA or protein synthesis might be considered to be an important cause of hepatitis.

We are indebted to Dr Joost J. Oppenheim (Chief of Laboratory of Molecular Immunoregulation, National Cancer Institute, Frederick, MD, U.S.A.), for his review of this manuscript and critical comments.

References

ADERKA, A., NOVICK, D., HAHN, T., FISCHER, D.G. & WALLACH, D. (1985). Increase of vulnerability to lymphotoxin in cells infected by vesicular stomatitis virus and its further augmentation by interferon. *Cell. Immunol.*, **92**, 218–225.

BEUTLER, B. & CERAMI, A. (1987). Cachectin: more than a tumor necrosis factor. *N. Engl. J. Med.*, **316**, 379–385.

DAIKUHARA, Y., TAMADA, F., TAKIGAWA, M., TAKEDA, Y. & MORI, Y. (1979). Changes in polyamine metabolism of rat liver after administration of D-galactosamine. *Gastroenterology*, **77**, 123–132.

DECKER, K. & KEPPLER, D. (1974). Galactosamine hepatitis: Key role of the nucleotide deficiency period in the pathogenesis of cell injury and cell death. *Rev. Physiol. Biochem. Pharmacol.*, **71**, 77–106.

DINARELLO, C.A. (1989). Interluekin-1 and its biologically related cytokines. *Adv. Immunol.*, **44**, 153–205.

ENDO, Y. (1982). Simultaneous induction of histidine and ornithine decarboxylases and changes in their product amines following the injection of *Escherichia coli* lipopolysaccharide into mice. *Biochem. Pharmacol.*, **31**, 1643–1647.

ENDO, Y. (1983a). Induction of histidine decarboxylase in mouse tissues by mitogens *in vivo*. *Biochem. Pharmacol.*, **32**, 3835–3838.

ENDO, Y. (1983b). A simple method for the determination of polyamines and histamine and its application to the assay of ornithine and histidine decarboxylase activities. *Methods Enzymol.*, **94**, 42–47.

ENDO, Y. (1983c). Induction of histidine decarboxylase by mitogens and the effects of actinomycin D and anti-inflammatory agents. *J. Pharmacobio-Dynamics*, **6**, s-55.

ENDO, Y. (1984). Induction of ornithine decarboxylase in mouse tissues following the injection of mitogenic substances. *Biochem. Pharmacol.*, **33**, 2123–2127.

ENDO, Y. (1989). Induction of histidine and ornithine decarboxylase activities in mouse tissues by recombinant interleukin-1 and tumor necrosis factor. *Biochem. Pharmacol.*, **38**, 1287–1292.

ENDO, Y., MATSUSHIMA, K., ONOZAKI, K. & OPPENHEIM, J.J. (1988). Role of ornithine decarboxylase in the regulation of cell growth by IL-1 and tumor necrosis factor. *J. Immunol.*, **141**, 2342–2348.

ENDO, Y. & NAKAMURA, M. (1992). The effect of lipopolysaccharide, interleukin-1 and tumour necrosis factor on the hepatic accumulation of 5-hydroxytryptamine and platelets in the mouse. *Br. J. Pharmacol.*, **105,** 613–619.

ENDO, Y., SUZUKI, R. & KUMAGAI, K. (1985). Induction of ornithine decarboxylase in the liver and spleen of mice by interleukin 1-like factors produced from a macrophage cell line. *Biochim. Biophys. Acta,* **838,** 343–350.

ENDO, Y., SUZUKI, R. & KUMAGAI, K. (1986). Macrophages can produce factors capable of inducing histidine decarboxylase, a histamine-forming enzyme, *in vivo* in the liver, spleen and lung of mice. *Cell. Immunol.*, **97,** 13–22.

GALANOS, C., FREUDENBERG, M.A. & REUTTER, W. (1979). Galactosamine-induced sensitization to the lethal effects of endotoxin. *Proc. Natl. Acad. Sci. U.S.A.*, **76,** 5939–5943.

KOLLONITSCH, J., PERKINS, L.M., PATCHET, A.A., DORDOURAS, G.A., MARBURG, S., DUGGAN, D.E., MAYCOCK, A.L. & ASTER, S.D. (1978). Selective inhibitors of biosynthesis of aminergic neurotransmitters. *Nature,* **274,** 906–908.

LEHMANN, V., FREUDENBERG, M.A. & GALANOS, C. (1987). Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine-treated mice. *J. Exp. Med.*, **165,** 657–663.

LUK, G.D., (1986). Essential role of polyamine metabolism in hepatic regeneration. *Gastroenterology,* **90,** 1261–1267.

NISHIGUCHI, S., KUROKI, T., TAKEDA, T., NAKAJIMA, S., SHIOMI, S., SEKI, S., MATSUI-YUASA, I., OTANI, S. & KOBAYASHI, K. (1990). Effects of putrescine on D-galactosamine-induced acute liver failure in rats. *Hepatology,* **12,** 348–353.

OPPENHEIM, J.J., KOVACS, E.J., MATSUSHIMA, K. & DURUM, S.C. (1986). There is more than one interleukin 1. *Immunology Today,* **7,** 45–56.

PEGG, A.E. & McCANN, P.P. (1982). Polyamine metabolism and function. *Am. J. Physiol.*, **243,** C212–C221.

PÖSÖ, H. & PEGG, A.E. (1982). Effect of α-difluoromethylornithine on polyamine and DNA snythesis in regenerating rat liver. Reversal inhibition of DNA synthesis by putrescine. *Biochim. Biophys. Acta,* **696,** 179–186.

TIEGS, G., WOLTER, M. & WENDEL, A. (1989). Tumor necrosis factor is a terminal mediator in galactosamine/endotoxin-induced hepatitis in mice. *Biochem. Pharmacol.*, **38,** 627–631.

WALLACH, D., HOLTMANN, H., ENGELMANN, H. & NOPHAR, Y. (1988). Sensitization and desensitization to lethal effects of tumor necrosis factor and IL-1. *J. Immunol.*, **140,** 2994–2999.

(Received May 1, 1992
Revised July 20, 1992
Accepted July 23, 1992)