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GLUCOCORTICOIDS SUPPRESS AND OESTROGENS ENHANCE THE LIPOPOLYSACCHARIDE-INDUCED INCREASE IN PUTRESCINE AND N^1-ACETYLSPERMIDINE IN MOUSE LIVER

HIROYUKI SUGIMOTO,[1]* KOEI HAMANA,[2] SHIGERU MATSUZAKI,[1] TAKAYUKI ARAI[3] and SHOJI YAMADA[3]

[1]Department of Physiology, Institute of Endocrinology, [2]College of Medical Care and Technology and [3]1st Department of Internal Medicine, School of Medicine, Gunma University, Maebashi 371, Japan

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Summary—Previously we reported that administration of lipopolysaccharide (LPS) to mice increased the hepatic levels of putrescine (PUT) and N^1-acetylspermidine (N^1-acetyl-SPD). In the current study, we examined the *in vivo* effects of some steroid hormones on the LPS-induced increase in PUT and N^1-acetyl-SPD. Corticosterone, hydrocortisone and dexamethasone suppressed the LPS-induced increase in PUT and N^1-acetyl-SPD in mouse liver in a dose-dependent manner, dexamethasone being the most effective among them. On the other hand, oestrone and oestradiol-17β enhanced the LPS-induced increase in PUT and N^1-acetyl-SPD in a dose-dependent manner. Oestradiol-17α and 16β-ethyl-oestradiol, as an inactive oestradiol isomer and an antioestrogen, respectively, likewise enhanced the increase in PUT and N^1-acetyl-SPD concentrations induced by LPS. 16α-hydroxy-oestradiol (oestriol), 16α-hydroxyestrone, 2-hydroxyoestradiol, 2-hydroxyoestrone, progesterone, testosterone, diethylstilboestrol and nonsteroidal antioestrogens such as tamoxifen and nafoxidine had no effect on the increase. Oestradiol-17β enhanced and corticosterone had little effect on the carbon tetrachloride-induced increase in PUT and N^1-acetyl-SPD. These results suggest that glucocorticoids suppress the increase by preventing the immunological injury by Kupffer cells on hepatocytes and that the stimulatory effect of oestrogens may not be associated with their oestrogenic activities mediated by the oestrogen receptor system.

INTRODUCTION

Lipopolysaccharide (LPS) is known to induce hepatic damage under various experimental conditions [1]. Since LPS activates macrophages to produce oxygen radicals *in vitro* [2], LPS-induced hepatic damage may be due to the increase in oxygen radicals released from Kupffer cells *in vivo* [1, 3]. Parmacological doses of steroids, especially glucocorticoids and oestrogens, attenuate the toxicity of LPS treatment. In fact, glucocorticoids have been shown to prolong the survival of patients and experimental animals with endotoxin shock [4, 5]. Glucocorticoids suppress the oxygen radical release from macrophages [6, 7] and this effect may be involved in the suppression of LPS toxicity. On the other hand, oestrogens induce immunosuppression by inhibiting interleukin-2 production from splenic lymphocytes [8], without decreasing superoxide generation by polymorphonuclear leukocytes [9].

LPS induces an increase in putrescine (PUT) and N^1-acetyl-spermidine (N^1-acetyl-SPD) in mouse liver *in vivo* [10, 11], while in primary culture of adult rat hepatocytes, it has little effect on polyamine concentrations *in vitro* [3]. These results raise the possibility that the accelerated retroconversion of polyamines after administration of LPS *in vivo* is associated with the activation of immunoreactive cells, especially Kupffer cells. We thought it of interest to investigate the correlation between polyamine metabolism and steroid hormones. The purpose of this study was to test the effects of glucocorticoids, oestrogens and antioestrogens on the LPS-induced increase in PUT and N^1-acetyl-SPD.

EXPERIMENTAL

Animals and treatments

Seven-week-old male ddY mice were kept in standard plastic cages and allowed free access to a usual diet before administration of LPS and

*To whom correspondence should be addressed.

Table 1. Effect of glucocorticoids on the hepatic levels of PUT and N^1-acetyl-SPD in mice treated with or without LPS

	No.	Polyamines (nmol/mg DNA)			
		PUT	SPD	SPM	Acetyl-SPD
Control	11	6.5 ± 0.6	476 ± 11	599 ± 19	2.2 ± 0.3
LPS	18	20.0 ± 1.5[a]	537 ± 21	583 ± 17	15.5 ± 2.5[a]
LPS + corticosterone					
(50 μmol)	17	15.0 ± 1.1[b]	464 ± 18	553 ± 19	9.7 ± 0.6[b]
(25 μmol)	10	17.8 ± 1.9	456 ± 22	501 ± 15	10.4 ± 1.8
(5 μmol)	8	20.1 ± 1.3	563 ± 28	584 ± 12	18.9 ± 4.3
LPS + dexamethasone (5 μmol)	18	14.0 ± 1.1[b]	464 ± 24	519 ± 19	9.1 ± 1.3[b]
LPS + hydrocortisone (50 μmol)	10	17.9 ± 2.1	414 ± 28	478 ± 12	9.3 ± 1.5[b]
Corticosterone (50 μmol)	9	5.7 ± 0.6	529 ± 25	658 ± 21	1.2 ± 0.5
Dexamethasone (50 μmol)	12	7.4 ± 1.9	542 ± 45	629 ± 36	2.0 ± 0.4
Hydrocortisone (50 μmol)	11	8.1 ± 0.9	498 ± 46	641 ± 47	3.0 ± 0.7

Each steroid (5–50 μmol/kg) was administered to mice with or without LPS (30 mg/kg). SPM, spermine. [a] $P < 0.01$ compared with controls. [b] $P < 0.05$ compared with LPS-treated group.

steroid hormones. All the animals were given only water for the last 8 h before sacrifice.

LPS (30 mg/kg), dissolved in saline solution (0.2 ml), and all the steroid hormones, dissolved in olive oil (0.1 or 0.2 ml), were injected intraperitoneally (i.p.) and the animals were killed at 4, 8 and 16 h thereafter. Steroid hormones were injected i.p. at a dose of 0.5, 5, 25 or 50 μmol/kg simultaneously with or without LPS. Some steroid hormones were first administered at a dose of 50 μmol/kg simultaneously with or without LPS and then at a dose of 25 μmol/kg 1, 2 and 3 h before sacrifice.

Carbon tetrachloride (CCl_4) (0.2 mmol/kg), dissolved in olive oil (0.1 ml), was injected i.p. with corticosterone or oestradiol-17β (50 μmol/kg) and animals were killed 6 h thereafter.

Assay of polyamines

Mouse livers (100 mg) were homogenized in 1 ml of cold 5% perchloric acid ($HClO_4$) and then centrifuged at 2000 rpm for 10 min at 4°C. Polyamines in $HClO_4$ extracts were analysed by HPLC on a column (4.6 × 80 mm) of cation-exchange resin (Kyowa Seimitsu, 62210FK) as described previously [12,13]. DNA in the pellets was extracted in 1 ml of 5% $HClO_4$ at 90°C for

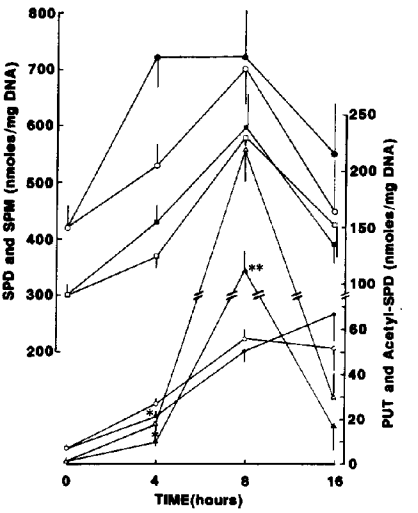

Fig. 1. Time course of hepatic levels of polyamines after administration of LPS with or without corticosterone. Corticosterone (50 μmol/kg), administered simultaneously with LPS (30 mg/kg), suppressed the LPS-induced increase in PUT at 4 h and N^1-acetyl-SPD both at 4 and 8 h. ○—PUT; ○—SPD; □—SPM; △—N^1-acetyl-SPD in LPS-treated mice. ●—PUT; ●-SPD; ■—SPM; ▲—N^1-acetyl-SPD in LPS and corticosterone-treated mice. All values are mean ± SEM of at least 6 animals per group. *$P < 0.02$, **$P < 0.001$ compared with LPS-treated group. SPM, spermine.

Table 2. Effect of oestrone on the hepatic levels of PUT and N^1-acetyl-SPD in mice treated with or without LPS

	No.	Polyamines (nmol/mg DNA)			
		PUT	SPD	SPM	Acetyl-SPD
Control	5	3.1 ± 0.2	319 ± 11.7	476 ± 12.3	<0.5
LPS	8	20.1 ± 0.7[a]	494 ± 24.1	741 ± 33.5	11.0 ± 1.0[a]
LPS + oestrone (50 μmol)	8	22.1 ± 2.2	516 ± 26.5	792 ± 32.0	18.8 ± 2.7[b]
LPS + oestrone (5 μmol)	8	24.1 ± 2.3	629 ± 46.8	1035 ± 47.8	15.4 ± 1.7[b]
LPS + oestrone (0.5 μmol)	8	21.8 ± 1.5	510 ± 33.1	899 ± 27.6	8.3 ± 0.6
Oestrone (50 μmol)	8	2.8 ± 0.2	341 ± 17.0	484 ± 19.1	<0.5

Each steroid (0.5–50 μmol/kg) was administered to mice with or without LPS (30 mg/kg). SPM, spermine. [a] $P < 0.001$ compared with controls. [b] $P < 0.05$ compared with LPS-treated group.

Table 3. Effect of oestrogens on the hepatic levels of PUT and N^1-acetyl-SPD in mice treated with or without LPS

	No.	Polyamines (nmol/mg DNA) PUT	SPD	SPM	Acetyl-SPD
Control	8	7.8 ± 1.0	471 ± 41	569 ± 19	3.3 ± 0.5
LPS	11	35.9 ± 3.0[a]	727 ± 40	866 ± 28	28.9 ± 4.7[a]
LPS + oestrone	7	44.3 ± 1.4[c]	687 ± 29	847 ± 32	64.0 ± 9.4[b]
LPS + 16α-hydroxyoestrone	7	26.3 ± 1.6[c]	683 ± 32	769 ± 37	24.8 ± 3.2
LPS + 2-hydroxyoestrone	9	34.3 ± 3.1	559 ± 23	804 ± 35	17.5 ± 2.5
LPS + oestradiol-17β	11	46.1 ± 3.0[c]	678 ± 44	801 ± 26	82.7 ± 20.8[c]
LPS + 16α-hydroxyoestradiol	11	34.5 ± 4.0	700 ± 52	863 ± 20	20.7 ± 2.6
LPS + 2-hydroxyoestradiol	10	33.6 ± 2.3	695 ± 37	923 ± 43	26.3 ± 4.1
Oestrone	7	5.4 ± 0.4	431 ± 22	435 ± 20	4.7 ± 0.7
16α-Hydroxyoestrone	5	11.5 ± 1.0	663 ± 69	632 ± 54	4.9 ± 0.8
2-Hydroxyoestrone	5	10.2 ± 0.8	509 ± 46	561 ± 19	5.9 ± 0.7
Oestradiol-17β	9	12.9 ± 2.2	427 ± 22	558 ± 28	8.2 ± 2.1
16α-Hydroxyoestradiol	12	7.6 ± 1.2	438 ± 39	518 ± 25	4.3 ± 1.4
2-Hydroxyoestradiol	3	7.3 ± 1.2	412 ± 19	436 ± 27	2.3 ± 2.3

Each steroid was first administered at the dose of 50 μmol/kg simultaneously with or without LPS (30 mg/kg) and then at a dose of 25 μmol/kg at 1, 2 and 3 h before sacrifice. SPM, spermine. [a] $P < 0.001$ compared with controls. [b] $P < 0.01$, [c] $P < 0.05$ compared with LPS-treated group.

15 min and assayed according to Burton [14]. The polyamine concentrations were expressed as nmol/mg DNA. N^1-Acetyl-SPD and N^8-acetyl-SPD were distinguished from each other by using specific acetylpolyamine deacetylases and hydrolysis with 6 N HCl [10].

Materials

Lipopolysaccharide of *Salmonella* origin was obtained from DIFCO Lab. (Detroit, MI, U.S.A.). 16β-Ethyl-oestradiol, nafoxidine and tamoxifen were from Takeda Co. Ltd, Upjohn Co. Ltd (Kalamazoo, MI, U.S.A.) and ICI Pharmaceutical Co. Ltd, respectively. All other hormones tested were obtained from Sigma (St Louis, MO, U.S.A.). CCl_4 and olive oil were from Wako Chemicals (Osaka, Japan).

Statistical analysis

All the values were expressed as mean ± SEM. Group means between LPS-treated groups, and LPS + steroid hormones-treated groups were compared by Student's *t*-test or Cochran–Cox test after analysis of variance to determine the significance of difference between individual means. In all cases, a probability of error of < 0.05 was selected as the criterion for statistical significance.

RESULTS

Effects of glucocorticoids on LPS-treated mice

The administration of corticosterone, hydrocortisone or dexamethasone suppressed the LPS-induced increase in hepatic PUT and N^1-acetyl-SPD (Table 1). Corticosterone suppressed the increase in a dose-dependent manner (Table 1). Corticosterone at 5 μmol increased N^1-acetyl-SPD levels slightly in LPS-treated mice but not significantly.

Corticosterone suppressed the increase in PUT at 4 h and N^1-acetyl-SPD both at 4 and 8 h (Fig. 1). The suppression of the increase in PUT and N^1-acetyl-SPD by corticosterone in LPS-treated mice was not observed at 16 h.

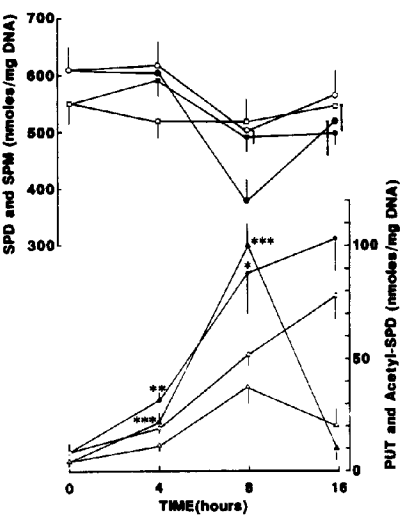

Fig. 2. Time course of hepatic levels of polyamines after administration of LPS with or without oestradiol-17β. Oestradiol-17β (50 μmol/kg), administered simultaneously with LPS (30 mg/kg), enhanced the LPS-induced increase in PUT and N^1-acetyl-SPD both at 4 and 8 h. ○—PUT; ○—SPD; □—SPM; △—N^1-acetyl-SPD in LPS-treated mice. ●—PUT; ●—SPD; ■—SPM; ▲—N^1-acetyl-SPD in LPS and oestradiol-treated mice. All values are mean ± SEM of at least 6 animals per group. *$P < 0.05$, **$P < 0.02$, ***$P < 0.001$ compared with LPS-treated group. SPM, spermine.

Table 4. Effect of progesterone, testosterone, diethylstilboestrol, oestradiol-17α and antioestrogens on the hepatic levels of PUT and N^1-acetyl-SPD in mice treated with or without LPS

	No.	Polyamines (nmol/mg DNA)			
		PUT	SPD	SPM	Acetyl-SPD
Control	5	10.1 ± 1.5	814 ± 75	660 ± 30	<0.5
LPS	11	26.9 ± 1.4[a]	794 ± 36	580 ± 14	17.7 ± 2.1[a]
LPS + progesterone	11	42.6 ± 4.9[b]	807 ± 41	641 ± 18	19.4 ± 2.5
LPS + testosterone	10	26.4 ± 2.7	654 ± 64	522 ± 40	12.5 ± 1.9
LPS + diethylstilboestrol	12	27.2 ± 1.2	857 ± 33	711 ± 26	15.5 ± 2.3
LPS + oestradiol-17α	12	35.9 ± 2.3[c]	1047 ± 64	774 ± 33	39.6 ± 5.1[c]
LPS + 16β-ethyl-oestradiol	10	44.0 ± 5.2[b]	1014 ± 76	713 ± 25	55.8 ± 11.7[b]
LPS + tamoxifen	12	18.4 ± 2.1[c]	698 ± 62	503 ± 30	21.7 ± 3.3
LPS + nafoxidine	12	27.3 ± 3.1	864 ± 33	647 ± 20	20.0 ± 2.4
Progesterone	5	10.3 ± 1.9	784 ± 67	685 ± 59	<0.5
Testosterone	5	8.0 ± 0.6	780 ± 40	638 ± 37	<0.5
Diethylstilboestrol	3	6.8 ± 1.1	618 ± 57	582 ± 33	<0.5
Oestradiol-17α	4	9.3 ± 0.7	639 ± 64	577 ± 19	<0.5
16β-Ethyl-oestradiol	4	10.8 ± 2.2	1030 ± 179	812 ± 116	0.6 ± 0.1
Tamoxifen	4	11.8 ± 2.9	968 ± 153	761 ± 78	1.5 ± 0.7
Nafoxidine	4	7.8 ± 0.7	646 ± 33	623 ± 42	<0.5

Each steroid (50 μmol/kg) was administered to mice with or without LPS (30 mg/kg). SPM, spermine. [a] $P < 0.001$ compared with controls. [b] $P < 0.05$, [c] $P < 0.01$ compared with LPS-treated group.

Effects of oestrogens on LPS-treated mice

Oestrone enhanced the LPS-induced increase in PUT and N^1-acetyl-SPD in mouse liver in a dose-dependent manner (Table 2).

When injected 4 times, oestrone and oestradiol-17β enhanced the LPS-induced increase in PUT and N^1-acetyl-SPD (Table 3). 16α-Hydroxyoestrone, 2-hydroxyoestrone, 16α-hydroxyoestradiol and 2-hydroxyoestradiol showed no effect on the increase (Table 3).

Oestradiol-17β enhanced significantly the increase in hepatic PUT and N^1-acetyl-SPD in LPS-treated mice both at 4 and 8 h (Fig. 2). The enhancement of increases in hepatic PUT and N^1-acetyl-SPD by the oestrogen in LPS-treated mice was not detected at 16 h.

Effects of progesterone, testosterone, diethylstilboestrol, oestradiol-17α and antioestrogens on LPS-treated mice

Progesterone, testosterone, diethylstilboestrol, tamoxifen and nafoxidine had little effect on the LPS-induced increase in PUT and N^1-acetyl-SPD in mouse liver (Table 4). On the other hand, both oestradiol-17α and 16β-ethyl-oestradiol were effective in enhancing the increase (Table 4).

Effects of corticosterone and oestradiol-17β on CCl_4-treated mice

Corticosterone had little effect on the CCl_4-induced increase in PUT, while it enhanced the increase in hepatic N^1-acetyl-SPD in CCl_4-treated mice (Table 5). Oestradiol-17β enhanced the increase in PUT and N^1-acetyl-SPD in CCl_4-treated mouse liver (Table 5).

DISCUSSION

The *in vivo* administration of radical-producing substances, e.g. LPS and paraquat, the latter of which is known to produce superoxide anion in every tissue [15], leads to an increase in hepatic PUT and N^1-acetyl-SPD in mice [10]. In primary culture of adult rat hepatocytes, paraquat enhances the increase in PUT and N^1-acetyl-SPD, and superoxide dismutase suppresses the increase [3]. LPS has little effect on polyamine metabolism in this culture system in which Kupffer cells were almost absent [3]. The

Table 5. Effect of corticosterone and oestradiol on the hepatic levels of PUT and N^1-acetyl-SPD in mice treated with or without CCl_4

	No.	Polyamines (nmol/mg DNA)				
		PUT	SPD	SPM	Acetyl-SPD	Acetyl-SPM
Control	3	10.2 ± 5.1	572 ± 27	687 ± 53	5.1 ± 0.5	<0.5
CCl_4	11	27.2 ± 2.7[a]	507 ± 34	657 ± 28	93.1 ± 19.2[b]	9.4 ± 2.5[b]
CCl_4 + corticosterone	12	27.9 ± 2.9	566 ± 32	691 ± 31	173.5 ± 24.5[d]	14.9 ± 2.4
CCl_4 + oestradiol-17β	12	61.2 ± 4.5[c]	461 ± 35	626 ± 27	196.9 ± 25.4[e]	22.0 ± 4.4[d]
Corticosterone	7	7.9 ± 0.8	616 ± 53	706 ± 36	5.2 ± 0.6	<0.5
Oestradiol-17β	9	16.9 ± 2.8	519 ± 27	675 ± 34	12.5 ± 3.3	<0.5

Each steroid (50 μmol/kg) was administered to mice with or without CCl_4 (0.2 mmol/kg). SPM, spermine; Acetyl-SPM, N^1-acetylspermine. [a] $P < 0.05$, [b] $P < 0.01$ compared with controls. [c] $P < 0.001$, [d] $P < 0.05$, [e] $P < 0.01$ compared with CCl_4-treated group.

LPS-induced increase in PUT and N^1-acetyl-SPD in mouse liver *in vivo* appears to follow the activation of immunoreactive cells, especially Kupffer cells [11], and to be stimulated by a certain factor released from them [3, 10].

Glucocorticoids are potent anti-inflammatory agents that are effective in ameliorating certain forms of shock [16, 17]. The main actions of LPS in endotoxin shock are the activation of macrophages, especially in the liver [1], and the production of some active oxygens [2]. The present study has shown that corticosterone, hydrocortisone and dexamethasone suppress the LPS-induced increase in PUT and N^1-acetyl-SPD in mouse liver. Among glucocorticoids, dexamethasone was the most potent in suppressing the increase. These results apparently suggest that the suppression may be correlated with the anti-inflammatory potency of glucocorticoids. Glucocorticoids inhibit the release of superoxide anion from human monocytes [6]. Endotoxin infusion induces the release of factors from lung lymph which are capable of stimulating normal sheep neutrophils to aggregate, migrate and release superoxide [18]. Pretreatment with glucocorticoids reduces such activity 3–4 h after endotoxin infusion [18].

The administration of CCl_4 induces the increase in PUT and N^1-acetyl-SPD in mouse liver [10]. Corticosterone did not suppress the CCl_4-induced increase, as shown in this study. It is reported that CCl_4 is converted by cytochrome *P*-450 to produce radicals in hepatocytes [19] and that it may directly enhance the polyamine metabolism in hepatocytes [10]. These results suggest that glucocorticoids suppress the release of oxygen radicals from Kupffer cells but not in hepatocytes and then inhibit the increase of PUT and N^1-acetyl-SPD in mouse liver.

Corticosterone suppressed the increase in PUT at 4 h and N^1-acetyl-SPD within 8 h after LPS treatment, but not at 16 h. It has been reported that LPS overcomes glucocorticoid inhibition of superoxide release in human monocytes [6]. The release of endotoxin-induced factor which in turn releases superoxide anion from neutrophils is suppressed by glucocorticoids within 4 h [18]. Thus, glucocorticoids exert their suppressive effect at an early stage of endotoxin shock.

Some of the oestrogens tested enhanced the increase of PUT and N^1-acetyl-SPD in mouse liver after administration of LPS or CCl_4. Both oestradiol-17β and oestrone were effective but not 16α-hydroxyoestradiol (oestriol). Unexpectedly, oestradiol-17α and 16β-ethyl-oestradiol, which are known as an inactive oestradiol isomer and an antioestrogen [20], respectively, had nearly the same effect as oestradiol-17β. On the other hand, diethylstilboestrol, a non-steroidal potent oestrogen, had no effect. Progesterone, testosterone and non-steroidal antioestrogens such as tamoxifen [21] and nafoxidine [22] had no such effect. These results indicate that the effect of steroids on the enhancement of LPS-induced increase in PUT and N^1-acetyl-SPD require on oestratriene structure containing a hydroxy group at C-3 and 17β- or 17α-hydroxy group or keto at C-17 in the steroids. Oestradiol-17β and oestrone are metabolized by the hydroxylation at C-16α or C-2 *in vivo* and 16α-hydroxyoestrogens and catecholoestrogens such as 2-hydroxyoestradiol and 2-hydroxyoestrone decrease the oestrogenic activity [23]; 2 or 16α-hydroxylation of the steroids results in loss of the effect. There is no direct correlation between the effect in question and the oestrogenic potency of the oestrogens and of the antioestrogens. These findings suggest that the modulation of polyamine metabolism by oestrogens is not mediated by a steroid receptor system and that oestrogens enhance that effect by a certain mechanism unrelated to the usual oestrogenic hormone actions.

It is reported that steroids with a phenolic structure in ring A, such as oestrogens, have substantial activities in protecting model membrane from phospholipid peroxidation [24]. Catecholoestrogens, 2-hydroxyoestrone, 2-hydroxyoestradiol and 2-hydroxyoestriol are more potent as antioxiants of phospholipid peroxidation than α-tocopherol [25]. The results of our present study show that the effect of oestrogens on the LPS-induced change in polyamine metabolism is not correlated with the antioxidative activity of oestrogens *in vitro*.

The pharmacological doses of oestrogens often influence the immunological function [8]. It has been suggested that oestrogens modulate T-cell maturation by interfering with normal thymus hormone production [8], and decrease interleukin-2 production by splenic lymphocytes [8] and myeloperoxidase in polymorphonuclear cells [9]. Diethylstilboestrol-injected rats were shown to exhibit a greater degree of hepatocellular injury following subsequent endotoxin administration as compared to non-treated animals [26]. In these experiments, rats were repeatedly injected with oestrogens for

several days before administration of LPS. In the present study, oestrogens were injected simultaneously with LPS. However, it remains to be clarified how oestrogens enhanced the polyamine metabolism so drastically after LPS treatment and whether or not these effects of oestrogens are beneficial to hepatocytes and hosts.

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