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POSSIBLE ROLE OF ENDOGENOUS HISTAMINE IN MEDIATION OF LPS-INDUCED SECRETION OF CORTICOSTERONE IN MICE

SEIJI SUZUKI and KIWAO NAKANO

Department of Nutritional Regulation, Research Institute for Biochemical Regulation, Nagoya University, Chikusa, Nagoya 464, Japan

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Abstract—*Escherichia coli* lipopolysaccharide (LPS) induced a strong secretion of corticosterone in C3H/HeN mice with a concomitant increase in the splenic histidine decarboxylase activity. Treatment of the mice with α-fluoromethyl histidine, a suicide substrate for the enzyme, markedly attenuated both the secretion and the increase. In C3H/HeJ mice, LPS provoked little corticosterone release and induction of the enzyme. However, these mice responded to tetradecanoyl phorbol acetate with a large increase in both this secretion and enzyme activity. Injection of LPS produced a comparable increase in the serum histamine and corticosterone level and activity of histidine decarboxylase in various tissues of genetically mast-cell-deficient W/W^v mice and in closely related +/+ mice. These results suggest that secretion of corticosterone caused by LPS is mediated by histamine produced through induction of histidine decarboxylase in non-mast cells.

Secretion caused by an endotoxin lipopolysaccharide (LPS) of corticosterone (CS) in mice is mediated by histamine (Hm) produced peripherally through induction of histidine decarboxylase (HDC, *l*-histidine carboxy lyase EC 4.1.1.17). The evidence is (1) LPS- or histamine-induced secretion of CS was abrogated by treatment of rats beforehand with an H_1-antihistaminic drug, promethazine, (2) injection of histamine *per se* caused a marked increase in the serum CS levels, (3) there was a close relationship between the degree of the LPS-induced CS release and that of an increase in splenic HDC activity, and (4) C3H/HeJ mice, which were less sensitive to LPS, were made sensitive by adoptive transfer of spleen cells from C3H/HeN mice in both inducibility of the enzyme and CS release [1, 2].

Although mast cells have HDC at an extraordinarily high level [3], it has been repeatedly argued that a rapid increase in HDC activity is associated with "inducible HDC" in cells other than mast cells [4, 5]. Taguchi *et al.* [6] found, using genetically mast-cell-deficient W/W^v mice, that the increase in HDC activity in the skin of the mice after application of tetradecanoylphorbol acetate (TPA) is due to induction of the enzyme in non-mast cell. Hm has been associated with the pathogenesis of various inflammatory diseases including endotoxemia and is a modulator of immunocompetence [9–11]; it has multiple immunosuppressive effects through induction of T-suppressor cells, which bear H_2-receptors [12, 13]. However, almost all this information was from studies on the effects of Hm injected into animals or added *in vitro*. Thus, details about the source of the amine and the regulation of its production are unknown. The purposes of our study were: (1) to see if HDC is pivotal in the LPS-provoked secretion of CS, (2) to define the kind of cells responsible for the induction of the enzyme, and (3) to obtain further information on the source of Hm and the mechanism controlling its production.

MATERIALS AND METHODS

Mice. C3H/HeJ mice were donated by Dr T. Masuda (Institute for Immunity, Kyoto University School of Medicine, Kyoto) and propagated in our laboratory under the usual conditions. C3H/HeN mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Shizuoka. C57BL/6J-W^v/+ mice were donated by Dr H. Nishimura of the Hamamatsu University School of Medicine, Hamamatsu, Shizuoka. WB-W/+ mice were supplied by Dr N. Wakasugi (Nagoya University School of Agriculture, Nagoya). W/W^v and +/+ mice were raised in our laboratory from the parent strains described above. Mice were used at 3–6 months of age.

LPS. A Westphal preparation of *Escherichia coli* (055 : B5), control 704089, was purchased from Difco Laboratories, Detroit, MI. LPS was dissolved in 0.9% NaCl saline and injected intraperitoneally (i.p.) into the mice at doses noted in the figures in the paper. Mice were killed after 120 min unless otherwise noted.

α-Fluoromethylhistidine (α-FMH). To examine HDC in LPS-induced CS release, three groups of C3H/HeN mice, each containing 4 animals, were starved overnight. At 10.00 a.m. the next morning the first group of the mice were injected i.p. with α-FMH (25 mg/kg/ml 0.9% NaCl saline, kindly supplied by Merck & Co., Inc., Rahway, NJ). The second and third group of mice received an equal volume of saline. Four hours later the first group of

animals were given LPS (25 μg/kg/ml saline) i.p. Control animals were given an equal volume of saline. All mice were killed by decapitation 2 hr afterwards. The trunk blood was collected for analysis of serum CS. The spleen and liver were removed for the assay of HDC.

TPA. TPA was dissolved in ethanol, mixed with 5 vol. of 0.9% NaCl saline, and injected i.p. (0.5 mg/kg) into C3H/HeN and C3H/HeJ mice. Control animals received an equal volume of the vehicle. The mice were killed by decapitation 4.5 hr afterwards and the trunk blood, spleen, liver and lung were removed for analyses of serum CS and HDC.

Measurement of serum. Mice were killed by decapitation, and trunk blood was collected in a centrifuge tube. After having been kept in ice for about an hour, the blood was centrifuged and the serum obtained was analyzed for CS by the competitive protein-binding method described previously [1]. Briefly, 0.1 ml of the serum was mixed with an equal volume of H_2O, incubated at 70° for 10 min to dissociate CS from its binding protein and centrifuged at 3000 *g* for 10 min. To each 0.1 ml of the supernatant fraction 4 ml of dichloromethane was added in a glass-stoppered centrifuge tube. The content was shaken vigorously for 1 min to extract CS, then centrifuged at 1000 *g* for 5 min. After the upper layer was aspirated, the dichloromethane layer was washed with 0.5 ml of 1 N NaOH, followed by 0.1% acetic acid and then three times with H_2O. An aliquot of the dichloromethane extract was used for the analysis for CS by the competitive protein-binding method described previously [1].

HDC. Mice were decapitated and the spleen, liver, lung, and stomach were removed rapidly, frozen in liquid nitrogen and stored at −80° before assay. A preliminary study showed that the enzyme was stable for at least 7 days under the condition. The tissues were homogenized with 7–20 vol. of ice-cold 0.02 M sodium phosphate buffer (pH 6.2) containing pyridoxal-5′-phosphate (20 μM) and dithiothreitol (200 μM) in a Teflon homogenizer or glass homogenizer (for stomach). To remove Hm and putrescine contained in the tissue phosphocellulose powder was added to each supernatant fraction (25 mg/ml). Then the supernatant was centrifuged at 2000 *g* for 5 min. The resulting supernatant fraction was used for the enzyme assay. One ml of the reaction mixture contained 0.2 ml of 0.2 M sodium phosphate buffer (pH 6.7), 50 nmoles of pyridoxal-5′-phosphate, 500 nmoles of dithiothreitol, 50 nmoles of aminoguanidine sulphate, 1 μmole of L-histidine HCl and 0.4 ml of enzyme solution. The reaction was carried out at 37° for 18 hr and terminated by adding 2.5 ml of 0.4 M $HClO_4$. A preliminary study showed that the enzyme reaction proceeded linearly for at least 24 hr. Hm formed was purified using phosphocellulose column (0.6 × 3 cm) and determined fluorometrically using *o*-phthalaldehyde as described previously [1].

Tissue Hm levels. Hm in the tissues and blood was measured fluorometrically using *o*-phthalaldehyde [1].

Statistical procedures. Turkey's *q*-test was used after analysis of variance to estimate the significance of differences between groups [14].

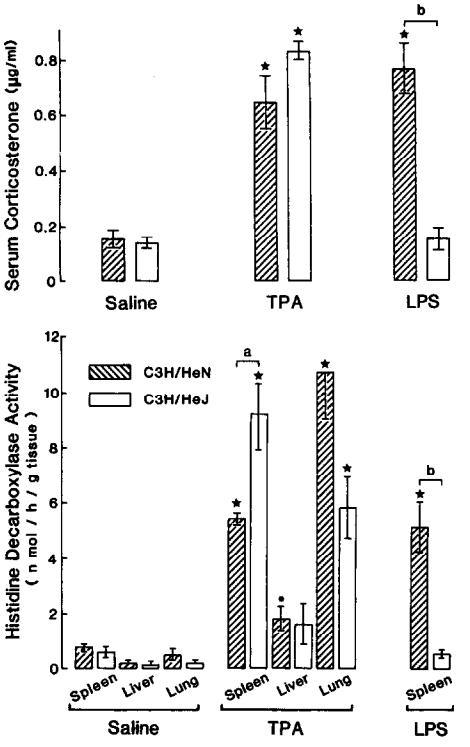

Fig. 1. Effects of *E. coli* LPS and tetradecanoylphorbol acetate (TPA) on serum corticosterone levels and on HDC activity in the spleen, liver and lung of C3H/HeN (hatched bars) and C3H/HeJ (open bars) mice. Mice were injected i.p. with 25 μg/kg of LPS or 0.5 mg/kg of TPA and killed 2 hr or 4.5 hr later, respectively. Control mice received an equal volume of a vehicle. Values are mean ± SE (indicated by bars) of 4 mice. Statistical difference between the control and the stimulated values, $^*P < 0.05$; $\bigstar P < 0.01$. Statistical differences between C3H/HeN and C3H/HeJ mice $^aP < 0.05$; $^bP < 0.01$.

RESULTS

LPS- and TPA-induced secretion of CS in C3H/HeN and C3H/HeJ mice

Figure 1 illustrates the effects of LPS (25 μg/kg) and TPA (0.5 mg/kg) on serum CS levels in C3H/HeN and C3H/HeJ mice. As shown in the upper part of the figure, LPS-injection caused a strong rise in the serum CS levels in C3H/HeN mice; C3H/HeJ mice responded far less to the LPS. Injection of LPS markedly increased the splenic HDC activity in C3H/HeN mice; it had no effect on C3H/HeJ mice (Fig. 1, bottom). C3H/HeJ mice responded to TPA as much as C3H/HeN mice did, by a large increase in serum CS levels and in the splenic HDC activity.

Effects of α-FMH on LPS-induced CS release

The effects of treatment of mice with α-FMH, a suicide substrate for the enzyme, before giving LPS on the LPS-provoked CS release in C3H/HeN mice were examined. Such treatment significantly attenuated the LPS-induced CS secretion (Fig. 2). The increase caused by LPS in splenic and hepatic HDC was completely abolished by treatment with this drug.

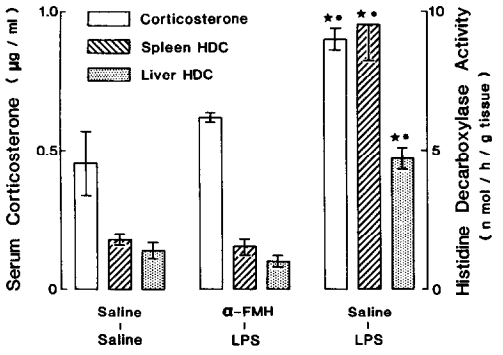

Fig. 2. Effects of treatment with α-fluoromethylhistidine (α-FMH) on the LPS-induced corticosterone release and on increase in HDC activity in the spleen and liver. A group of C3H/HeN mice were starved overnight and injected i.p. with 25 mg/kg of α-FMH. Other groups of mice were injected with an equal volume of 0.9% NaCl saline. Four hours later two groups of mice were given 25 μg/kg of LPS i.p. The remaining mice received an equal volume of saline. All mice were killed 2 hr afterwards. Values are mean ± SE for 4 mice per group. Statistical differences between α-FMH-LPS and Saline-LPS group: *P < 0.01. Statistical differences between saline-saline and saline-LPS groups: ★P < 0.01.

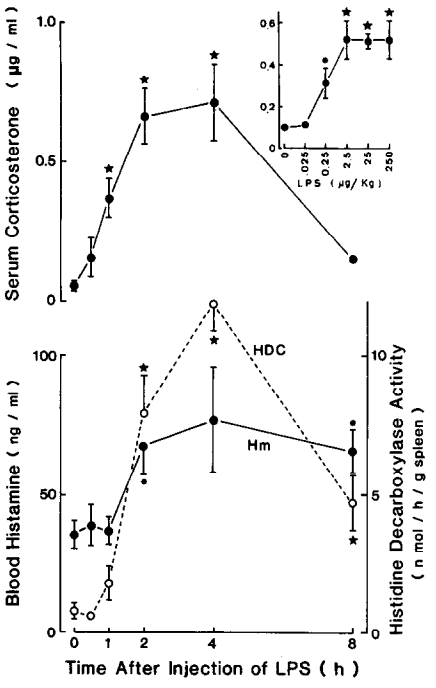

Fig. 3. Effects of LPS on serum levels of corticosterone, histamine and splenic HDC activity as a function of dose of LPS (upper panel, mice were killed 2 hr after the injection of LPS) and time after the injection of LPS (lower panel, 25 μg/kg) in +/+ mice. Each point represents the mean ± SE (bars) of 4 mice per group. Statistical differences between saline-treated and stimulated groups: *P< 0.05; ★P < 0.01.

LPS-caused CS release in W/W^v and +/+ mice

To find if mast cells are involved in the LPS-caused induction of HDC, we then examined the effects of LPS on genetically mast-cell-deficient W/W^v mice [8]. The basal level of Hm was significantly less in all tissues of W/W^v mice examined than that in the closely related +/+ mice (Table 1). In particular, the Hm level of the epidermis of W/W^v mice was less than 1/30 of that of +/+ mice, supporting the suggestion that W/W^v mice lack mast cells in this tissue [8]. We first examined the effects of LPS on the serum levels of CS in +/+ mice as a function of dose and time after its injection. The serum CS level increased promptly in these mice upon injection of LPS (Fig. 3, middle). It reached a plateau 2 hr later, began to decline after 4 hr, and was close to the base-line 8 hr afterwards. Hence, in all later studies, mice were killed 2 hr after the injection of LPS. Figure 3 shows changes in the blood Hm levels and the splenic HDC activity as a function of time after the LPS-injection. Activity of the enzyme began to increase 30 min after the injection of LPS, reaching a maximum 4 hr later, at sixteen times the base-line level; then it declined. The blood Hm levels increased significantly, after the latent period of 1 hr, reaching a plateau after 2 hr and remained around this level up to 8 hr. The top of Fig. 3 depicts the effect of LPS on the serum CS levels of these mice as a function of dose. LPS injection caused a strong, dose-dependent increase in the serum CS. Based on these results, we used a dose of 25 μg/kg as the standard in the later studies. We then evaluated the effect of LPS injection on W/W^v mice. It caused an increase in the serum CS levels comparable to that observed with +/+ mice (Fig. 4A). It also caused a large increase in the activity of HDC in the spleen, lung, and liver

Table 1. Effect of LPS injection on tissue histamine levels

Mouse strain	+/+			W/W^v		
	Histamine (μg/g)			Histamine (μg/g)		
Tissues	Saline (A)	LPS (B)	(B)/(A)	Saline (A)	LPS (B)	(B)/(A)
Lung	1.65 ± 0.18	1.01 ± 0.08*	0.61	0.95 ± 0.11[a]	0.74 ± 0.05[a]	0.78
Stomach	7.27 ± 0.42	7.83 ± 0.35	1.08	3.91 ± 0.27[c]	3.17 ± 0.37[c]	0.81
Dermis	2.03 ± 0.27	1.96 ± 0.24	0.97	0.85 ± 0.14[b]	0.69 ± 0.05[c]	0.81
Epidermis	17.7 ± 4.1	12.0 ± 2.1	0.68	0.61 ± 0.09[b]	0.59 ± 0.08[c]	0.97

[a]P < 0.05, [b]P < 0.01, [c]P < 0.001, as compared to +/+ strain.
*P < 0.05 as compared to saline control.

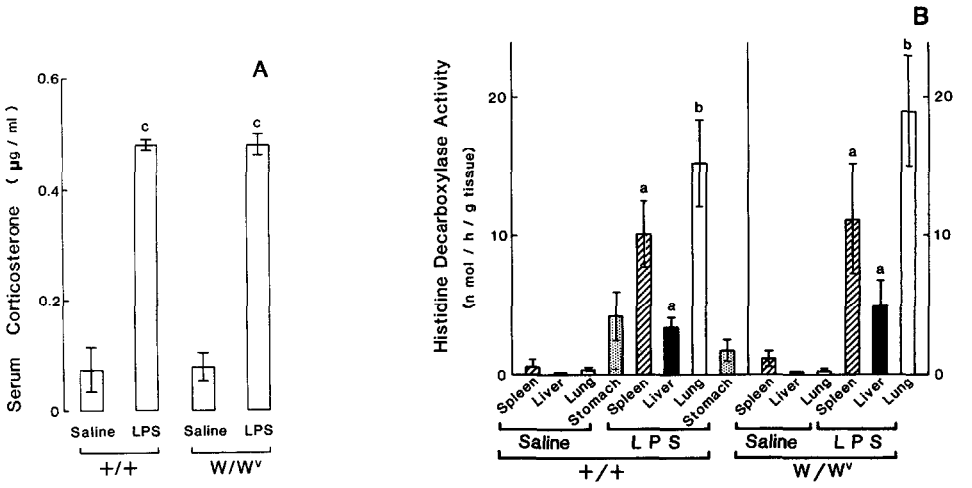

Fig. 4. Effects of LPS-injection on serum corticosterone levels (A) and HDC activity in the spleen, liver, lung and stomach (B) of +/+ and W/W^v mice. Mice were injected i.p. with 25 μg/kg of LPS and killed 2 hr later. Values are mean ± SE for 4 mice per group. Statistical differences between saline-treated and LPS-treated group: $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.001$.

of W/W^v mice, the magnitude being close to that observed with +/+ mice. However, HDC activity in the stomach did not change upon injection of LPS. The amount of Hm in the lung decreased significantly in the +/+ mice 2 hr after injection of LPS, but there was no such change in the stomach, dermis and epidermis (Table 1). A similar tendency was seen in the tissue Hm levels of the W/W^v mice; differences between the control and the group treated with LPS were not significant.

DISCUSSION

The results obtained from this study gave further support to the view that the LPS-induced secretion of CS is mediated by Hm produced through induction of HDC. Thus, (1) treatment of mice with α-FMH, a specific inhibitor of HDC, significantly attenuated the LPS-induced CS release, (2) C3H/HeJ mice responded to LPS by smaller increase of the serum CS levels and induction of HDC, and (3) these mice were fully sensitive to TPA, resulting in a significant rise in secretion of CS with a concomitant increase in the splenic HDC activity. These results are consistent with those obtained in our previous study: (1) there is a close relationship between the extent of the LPS-induced increase in the serum CS levels and the increase in the splenic HDC activity, and (2) C3H/HeJ mice are rendered sensitive to LPS by an adoptive transfer of the spleen cells from sensitive C3H/HeN mice [2]. It was also shown here that LPS administration produced an abrupt concomitant rise in the serum CS and Hm and HDC activity in various tissues of W/W^v mice (Figs. 3 and 4). It should be noted that the serum CS levels began to rise earlier than the Hm levels did (Fig. 3). This early rise of the serum CS levels might be due to the effect of other pro-inflammatory substances produced in response to LPS, e.g. eicosanoids or kinins. This view may be supported by the findings that the LPS-induced CS secretion was not completely abrogated by treatment of H_1-antihistaminic drug [1] or by α-FMH (Fig. 2). The residual presence of a small but measurable CS secretion suggests possible involvement of factors other than Hm.

The next problem is whether mast cells are involved in the LPS-provoked increase in tissue HDC activity. As mentioned above, these cells have a very high level of this enzyme [3]. The results obtained here showed that mast cells are not pivotal in the process since the genetically mast-cell-deficient W/W^v mice responded to LPS as efficiently as normal +/+ mice in both the increase in the serum CS levels and induction of HDC in diverse tissues. It is likely, therefore, that non-mast cells are responsible for the LPS-induced increase in HDC activity. It is possible that the blood Hm we measured was contained in or released from, preformed stores within circulating basophils and not synthesized *de novo*. However, this appears to be unlikely since LPS-injection caused no sign of basophilia: the population of basophils was no more than 0.08% of whole leukocytes even in the blood of the LPS-treated C57BL+/+ mice (data not shown), which is not sufficient to account for a big increase in the blood Hm content. It is known that basophils are not essentially detectable in the blood of this species of animals [15].

Another problem is to discover the cell type of these non-mast cells. Discussion can be focused, for convenience, on spleen cells since C3H/HeJ mice became sensitive by adoptive transfer of spleen cells alone from C3H/HeN mice [2]. This finding is consistent with the observation of others. Thus, Taguchi *et al.* [6] found that TPA-induced increase in HDC activity in the skin of W/W^v mice is abolished by prior X-irradiation and restored by later transfer of bone marrow cells. Endo [7] showed that a variety of substances with mitogenic activity such as LPS, concanavalin A and TPA can induce HDC in the liver, spleen and lung of mice, while other inflammatory agents without mitogenicity such as zymosan and carrageenan can not. It is likely, therefore, that

immune cells other than mast cells are associated with the LPS-provoked increase in HDC activity. Macrophages are possible candidates since low responsiveness of C3H/HeJ mice to LPS is considered to be due to a genetic defect in macrophages [7]. We obtained evidence supporting this view; whole body X-irradiation of C3H/HeN mice caused less marked decrease in population of the cells containing LPS-inducible HDC than the cells which were responsive to T or B lymphocyte specific mitogens (Suzuki and Nakano, unpublished results). Macrophages are more radioresistant than other immune cells [16].

In spite of a large increase in HDC activity in various tissues examined, the Hm level in these tissues did not increase but rather tended to decrease after LPS injection in both W/W^v and +/+ mice (Fig. 4, Table 1). These results are consistent with the finding that the concentration of Hm in the skin decreases after application of TPA in spite of a marked increase in HDC activity [6], confirming the view that Hm synthesized by "inducible HDC" is not stored in the tissue, but released and metabolized immediately [4–6].

Bacterial endotoxin causes severe inflammatory reactions as exemplified by a syndrome of disseminated intravascular coagulation, thrombocytemia [17], and pulmonary and mesenteric vasoconstriction [18]. Hm has been associated with pathogenesis of various inflammatory diseases including endotoxemia [9]. Glucocorticoids inhibit the function of various immune cells such as macrophages [18–21], neutrophils [22, 23], mast cells and basophils [24–26], and lymphocytes [27–29], thus causing their anti-inflammatory effects and probably preventing endotoxemia [30]. These findings together with the results obtained here suggest that endotoxin stimulates production of toxic Hm, leading to secretion of CS, which in turn contributes to attenuation of this proinflammatory substance. Presumably, this is the physiological meaning of the LPS-induced secretion of CS.

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