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SIMULTANEOUS INDUCTION OF HISTIDINE AND ORNITHINE DECARBOXYLASES AND CHANGES IN THEIR PRODUCT AMINES FOLLOWING THE INJECTION OF *ESCHERICHIA COLI* LIPOPOLYSACCHARIDE INTO MICE

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Abstract—The injection of *Escherichia coli* lipopolysaccharide (LPS) into mice produced simultaneous induction of histidine and ornithine decarboxylases in the liver, lung, spleen and kidney. The time courses of the changes in activities of the two enzymes were similar in all the tissues. After the injection, both activities increased within 1.5 hr, peaked at 4.5 hr and returned to the basal levels within 15 hr. The induction of these enzymes was very sensitive to this agent, i.e. as little as 1 μg/kg of the *E. coli* lipopolysaccharide produced significant increases in these enzyme activities. An increase in the product amines, histamine and putrescine, followed the rise of enzyme activities. The levels of histamine changed more rapidly than those of putrescine. In spite of the increase in putrescine, there was no increase in spermidine and spermine. In the brain and thymus the LPS induced ornithine decarboxylase, but not histidine decarboxylase. In the blood, the histamine level increased without an increase in the activity of histidine decarboxylase. These results are discussed in relation to the actions of lipopolysaccharide. A simple method for the simultaneous assay of the activities of histidine and ornithine decarboxylases without using radioisotope substrates was used in this study.

Lipopolysaccharides (LPS) of the cell walls of Gram-negative bacteria act on various cells and tissues, stimulate non-specific immune responses or the body's defense mechanisms against infections, and cause inflammatory reactions. In the study reported here, the effects of LPS on the metabolism of histamine and polyamines were examined as one approach to understanding the early biochemical changes that follow the injection of LPS. These substances appear to be important during the action of LPS as well as in immune responses.

Histamine has been generally associated with immediate hypersensitivity reactions, and its release from mast cells and basophils has been studied by many investigators. On the one hand, Schayer, Kahlson and their co-workers have observed marked increases in the activity of histidine decarboxylase (HD) in various tissues under many conditions such as rapid growth [1], repair of wounds [1, 2], tissue transplantation [3], inflammatory reactions [4, 5], anaphylaxis [6], and injection of various stimulants including vaccine, immunoadjuvants and LPS [7–9]. They have emphasized the role of newly formed histamine, that is produced by the induced HD, in the regulation of the microcirculatory system [8, 10] or in anabolic processes during rapid growth [1]. There are, however, few available data to answer the question of how extensive histamine accumulation is in tissue when HD activity is enhanced under such conditions. Although there is no detailed study of the problem, there is, however, a recognition that great changes in HD activity may occur without corresponding changes in tissue histamine contents [1].

The induction of ornithine decarboxylase (OD) is a rate-limiting step in polyamine synthesis, and it has been thought to be intimately related to the stimulation of many cell functions including synthesis of nucleic acids and proteins [11]. LPS has been shown to augment the uptake of isotope into RNA and into the protein of the liver [12]. Recently, it was reported that OD was induced in macrophages *in vitro* by LPS and BCG cell walls, and that its induction was one of the early biochemical changes in macrophages stimulated by adjuvants [13]. Therefore, it is of interest to compare the response to LPS of OD in various tissues. There has been no report on the *in vivo* induction of OD by LPS or LPS-like agents.

The present study was designed (a) to provide a detailed study of the time course of induction of HD and OD and of the change in their product amines and (b) to compare the effects of LPS on various tissues in order to consider the significance of these enzyme inductions by LPS. I reported previously a simple method for the determination of histamine, putrescine and polyamines by the use of a small P-cellulose column [14]. In the present study, the method was applied to the assay of activities of both enzymes.

MATERIALS AND METHODS

Materials. LPS prepared from *Escherichia coli* 055:B5 was obtained from Difco Laboratories (Detroit, MI, U.S.A.). The agent was suspended in saline (1 mg/ml) and stored in a freezer. The stock solution was diluted with saline appropriately and injected into mice (in the tail vein, at 9:00 to 11:00 a.m., 0.1 ml/10 g body wt). P-Cellulose powder used for the preparation of enzyme solutions was prepared as follows. P-Cellulose (Brown Co., Berlin, NH, U.S.A.) equilibrated with 0.03 M sodium phosphate buffer (pH 6.2) was washed with water and acetone and dried on a glass filter. Other reagents were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Male ddY mice (18–22 g, 4–5 weeks old) propagated in the Funabashi Farm (Funabashi, Japan) were used. In each experiment, six or nine mice were killed at each time interval, and tissues from three mice were combined.

Preparation of enzyme solutions. Mice were decapitated and the tissues were removed rapidly and stored in a dry-ice box (1–5 days). The first 4–6 drops of blood at the decapitation were also collected in a tube cooled in an ice bath. Tissues from three mice were put together and homogenized with 7–20 vol. of ice-cold 0.02 M sodium phosphate buffer (pH 6.2) containing 20 μM pyridoxal-5'-phosphate and 200 μM dithiothreitol by using a Teflon homogenizer or a glass homogenizer (for lung). Homogenates were centrifuged at 20,000 *g* for 20 min. To remove histamine and putrescine, to each supernatant fraction P-cellulose powder was added (25 mg/ml) and suspended by shaking. Then the suspension was centrifuged at 2,000 *g* for 5 min. The resulting supernatant fraction was used as the enzyme solution.

Amine determinations and enzyme assays. Histamine, putrescine and polyamines in tissues were determined by the method described previously [14]. Using this method, the amines are separated on a small P-cellulose column and determined fluorometrically. In the present study, the method was applied also to the simultaneous assay of HD and OD activities as follows. Enzymatic decarboxylations of both histidine and ornithine were carried out in a single reaction mixture. The reaction mixture contained 0.2 ml of 0.2 M sodium phosphate buffer (pH 6.7), pyridoxal-5'-phosphate (50 nmoles), dithiothreitol (500 nmoles), aminoguanidine sulfate (50 nmoles), L-histidine·HCl (1 μmole), L-ornithine·HCl (1 μmole) and the enzyme solution (0.4 ml), in a final volume of 1.0 ml. The reaction was carried out at 37° for 3 hr and terminated by the addition of 0.4 M $HClO_4$ (2.5 ml). Another reaction mixture was usually used as the blank for the reaction without the addition of substrates, which were added after the addition of $HClO_4$. Histamine and putrescine in the reaction mixture were separated on a P-cellulose column (0.6 × 3 cm) and subjected to reactions with *o*-phthalaldehyde and fluorescamine, respectively, as described previously [14].

RESULTS

Evaluation of the method for the assay of HD and OD activities. The present method for the assay of HD and OD activities is based on the determination of histamine and putrescine, the product amines of these enzymes. In addition to low activities of these enzymes, histamine and putrescine are included in significant amounts in tissue extracts. Therefore, it is necessary to remove these amines from enzyme solutions. For this purpose, tissue extracts were treated with P-cellulose powder as described above. This treatment was very effective in lowering the blank values in the enzyme assays. HD and OD activities were not lost in this treatment. When histamine and putrescine were added to the reaction mixture not containing substrates, no detectable degradation of these amines occurred during the incubation period. Histidine and ornithine did not inhibit OD and HD respectively.

Methods using [carboxyl-^{14}C]histidine and ornithine are currently used for the assay of HD and OD activities. Such methods are more sensitive than the method used in this study, i.e. both activities in most tissues of normal adult mice could not be determined accurately by the latter method because of their low activities. However, the production of $^{14}CO_2$ by pathways other than decarboxylation must be considered in the former methods in some instances [15]. Although there are no available data to compare the present values with those of other investigators because of different conditions of animals and different expressions of activity, the results concerning the method and experiments on LPS indicate that the present method is available for simple assays of both enzymes when they increase markedly. Quantitative determinations could be carried out at least on 0.05 nmole of histamine and 0.5 nmole of putrescine. In addition to the simultaneous assay of HD and OD, another advantage of this method is that the endogenous amines produced by these enzymes can be determined by the same method.

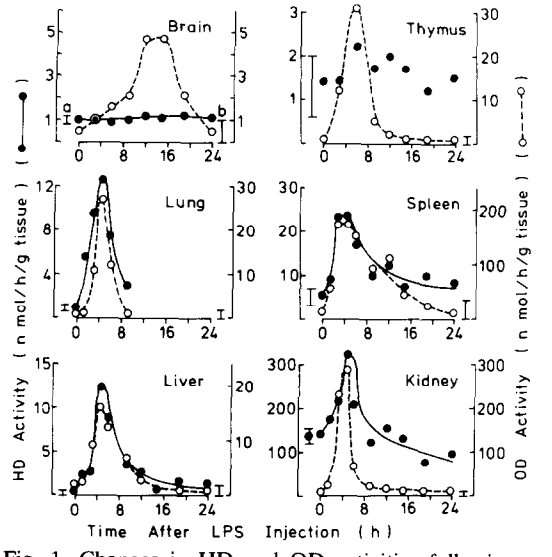

Fig. 1. Changes in HD and OD activities following an injection of LPS. LPS (0.5 mg/kg) was injected at time zero. Each point is a mean derived from two or three experiments (i.e. six or nine mice). Key: (a and b) ranges of HD (left) and OD (right) activities of control mice at 0 (without injection), 6, 12 and 24 hr after the injection of saline.

Changes in HD and OD activities by LPS. HD and OD activities in tissue from untreated mice, except for the kidney, were very low. However, both activities were enhanced markedly by the injection of LPS (Fig. 1). In the lung, liver, spleen and kidney, both activities simultaneously increased within 1.5 hr, peaked at 4.5 hr, and returned to basal levels within 15 hr after the injection.

On the other hand, in spite of the increase in OD activity, there was no increase in HD activity in the brain and thymus. The increase in OD activity in the brain was lower, and its rise was slower, than in other tissues. Neither HD nor OD activity was detected in the blood of treated and untreated mice.

The results on brain appear to have been due to an indirect influence, because little or no entry of LPS into the brain has been shown by Dascombe and Milton [16]. The inability to induce HD in the brain has been demonstrated also by Schayer and Reilly, even after intracerebral injection [17]. Schayer has reported also that the increase in HD activity was very low in the thymus [18] and not present in the blood [8].

In the kidney, the increase in HD activity was not as great as in other tissues. However, its basal level was much higher than those of other tissues. Such high activity was not observed in ddI mice propagated in the mouse center at our university. In this strain, by contrast, its OD activity was very high (300–400 nmoles·hr^{-1}·g^{-1}). Such strain- or sex-dependent differences in HD and OD activities [19] and large variations of HD activity in the kidney of the same strain [20] have been reported also by Rosengren and her co-workers. On the basis of the inverse relationship between HD and OD activities in the kidney, they have postulated that putrescine functionally substitutes for histamine, but the role of these enzymes in the kidney is unknown [19, 20].

Changes in histamine, putrescine and polyamines by LPS. Figure 2 shows the change in histamine and putrescine levels in mouse tissues following LPS injection. Histamine levels were elevated markedly in tissues, except in the brain and thymus. The extent of the histamine increase was greater than that of putrescine. The rise in histamine lagged slightly behind that of HD activity and peaked at 6 hr after LPS injection. The rise of putrescine was much slower than that of OD activity and peaked at 9–15 hr. The elevated histamine levels declined more rapidly than those of putrescine.

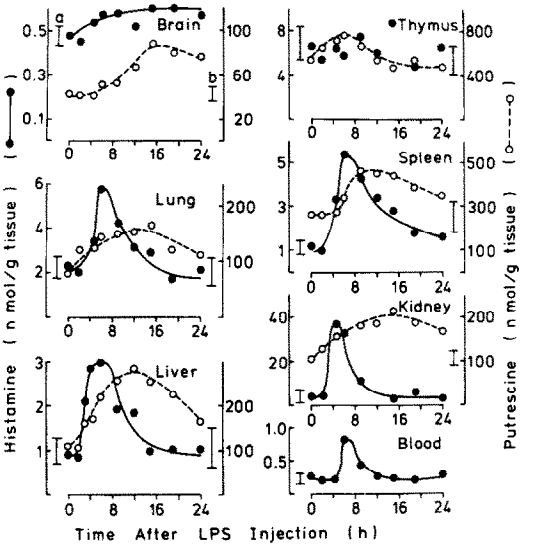

Fig. 2. Changes in histamine and putrescine levels following the injection of LPS. LPS (0.5 mg/kg) was injected at time zero. Each point is a mean derived from two or three experiments (i.e. six or nine mice). Key: (a and b) ranges of histamine (left) and putrescine (right) levels of control mice at 0 (without injection), 6, 12 and 24 hr after the injection of saline.

There was no increase in histamine in the brain and thymus. This corresponded with the lack of increase in HD activity in these tissues.

The increase in histamine in the blood appears to have been due to diffusion from other tissues, because no activity was detected in the blood even after the injection of LPS. On the other hand, in spite of large increases in putrescine in tissues, putrescine was not detected in the blood throughout the experimental period. This result and the slow increases in putrescine levels in tissues suggest that putrescine accumulates in tissues without diffusion and is metabolized slowly there.

There was no significant increase in polyamines or spermidine and spermine in any tissue tested,

Table 1. Dose-dependent effects of LPS on HD and OD activities and on the product amine levels*

Doses of LPS (μg/kg)	Enzyme activities (nmoles/hr/g tissue)						Amine contents in the liver (nmoles/g tissue)	
	Liver		Spleen		Lung			
	HD	OD	HD	OD	HD	OD	Histamine	Putrescine
0	0.6	1.5	4.9	11.4	1.0	0.5	0.8	85
0.25	1.1	1.5	8.3	18.0	1.4	1.5	0.7	90
1.0	2.0	5.4	11.1	18.9	2.2	1.8	0.9	107
5	4.6	8.1	17.3	42.5	3.6	3.0	1.3	106
20	7.5	8.6	24.4	59.1	6.2	3.7	1.6	119
100	10.1	10.3	23.6	125	9.3	10.3	2.6	159
500	12.3	15.9	23.6	173	12.6	26.7	2.9	169

* Mice were killed at 4.5 hr after the injection of LPS. Each value is the mean of two or three experiments (i.e. six or nine mice).

even blood, throughout the experimental period of 24 hr (data not shown).

Dose-dependent effects of LPS on HD and OD activities. Dose-dependent effects of LPS on HD and OD activities in liver, spleen and lung are shown in Table 1. In these tissues, as little as 1 μg/kg of LPS produced significant increases in activities of both enzymes. However, the responses of HD and OD to LPS were different among these tissues. In the liver, both activities were roughly parallel. HD activity in the spleen reached a maximum value at a dose of 20 μg/kg, while OD activity continued to increase at higher doses. OD activity in the lung increased markedly at higher doses of LPS (100–500 μg/kg) rather than at lower doses.

Histamine and putrescine levels in the same liver that was used for enzyme assays are also shown in Table 1. These values were also dose dependent.

DISCUSSION

The finding of OD induction in all tissues tested indicates that the induction that is caused by LPS injection is a common metabolic event in various tissues. In most instances, it is known that the induction of OD produces, subsequently, synthesis of polyamines [11]. In spite of the increase in putrescine, however, there were no significant increases in polyamine levels in any tissues tested. Therefore, the OD induction by LPS appears to be related to biochemical changes other than polyamine synthesis, such as the activation of RNA polymelase I, the enzyme responsible for rRNA synthesis, as suggested by Russell and her co-workers [21–23]. It should be noticed also that the extent of OD induction in the spleen was very high except for the kidney. This seems to be related to the fact that this organ is rich in macrophages, since it has been reported that OD is markedly induced in macrophages by LPS *in vitro* [13].

With respect to HD, the present results are similar to those of Schayer and his co-workers, i.e. marked increases in liver, lung and spleen [9] and little or no increase in brain [17] and thymus [18]. He has reported that, in addition to LPS, various other activators of the reticuloendothelial system (RES) produced increased HD activity in tissues containing large numbers of RES cells [8]. Additionally, most of the conditions known to produce HD induction are related to the stimulation of RES or inflammation as described in the beginning of this paper. These facts appear to mean that HD induction is important during immune responses or changes in the microcirculatory system. Concerning the induction of HD in fetal rat liver, Kahlson and Rosengren [1] suggested a role in rapid growth. However, Russell and McVicker [24] have assumed its relation to hematopoietic function of the liver on the basis of a comparison of HD and OD inductions during fetal development. Bone marrow has a high level of HD activity [25], and the stimulation of hematopoiesis is one of the well known actions of LPS [26]. These facts also suggest a relation between HD and the functions of reticular cells.

In conclusion, OD is inducible in most cells, but the induction of HD may be restricted to certain kinds of cells such as those of the RES. Different responses of these enzymes to LPS in different tissues (Table 1) may be explained by this assumption. With respect to inductions of HD and OD during wound healing in rat skin, Mizutani *et al.* [2] have assumed that these enzymes are derived from different cell types, because the administration of betamethasone delayed the rise in OD activity but not the rise in HD activity.

Induction of OD is known to be caused by the administration of various hormones, amino acids and drugs [11]. None of these substances, however, is known to induce HD. In this sense LPS is a characteristic agent. As little as 1 μg/kg of the agent produced increases in activities of both enzymes. Both activities increased within 1.5 hr and peaked at 4.5 hr after the injection of LPS. This sensitive, rapid and concurrent induction of HD and OD by LPS appears subsequently to affect various functions of many cells or tissues.

Considering the sensitive induction of OD by various stimuli, it is expected that exogeneous stimuli to animals that cause the induction of HD in the tissues may also induce OD, in most instances. In addition to the present finding, inductions of HD and OD during wound healing of rat skin [2] and the recent finding by Watanabe *et al.* that a tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate, induced both enzymes in the skin of mice [27] are examples of the idea. However, the induction mechanism of OD and HD by these stimuli is unknown. Since it has been shown that the rise in OD activity often precedes the increase in DNA, RNA and protein levels [11], and that OD activity increases more rapidly than HD activity in the skin [2, 27], it is to be expected that HD would be induced sequentially depending on the induction of OD. However, the present finding of the lack of a time lag between OD and HD inductions or, rather, more rapid induction of HD in the lung (Fig. 1) appears to indicate that such a relation is unlikely. Schayer and Reilly [28] have reported that the induction of HD does not require RNA synthesis.

Reilly and Schayer [29] reported that [^{14}C]histamine in some tissues of normal mice remained essentially constant throughout an experimental period of 6.5 hr after the injection of a small amount of [^{14}C]histidine. In the present study, however, the pattern of histamine change was essentially the same among tissues, and the levels of histamine changed more rapidly than those of putrescine. Such a rapid decline of elevated histamine was observed also in normal mouse tissues after the administration of *N*-acetylhistamine in my previous study [30]. In addition, the possible diffusion of tissue histamine into the blood was shown (see text). Therefore, it appears that excess histamine is metabolized and/or diffused into blood more rapidly than histamine of normal levels. This concept is analogous to that of "nascent" or "inducible" histamine proposed by Kahlson, Schayer and their co-workers [1, 31].

In contrast to the marked increase in tissue histamine in the present study on mice, Kahlson and Rosengren [1] have reported that, when HD activity increased, histamine excretion in urine increased but histamine levels in tissues did not increase or rather

decreased. The results on mouse skin shown by Watanabe *et al.* [27] are also similar to those of Kahlson and Rosengren. Most of the studies by Kahlson and his co-workers have been carried out on rats. The histamine contents of some rat tissues are extremely high compared with those of mice [30], and in the skin, which is rich in mast cells, its histamine content is very high [27, 32, 33]. Therefore, it is possible that, in such tissues, increases in histamine produced by induced HD are masked by variations of the high levels of histamine.

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