Proc. Natl. Acad. Sci. USA
Vol. 75, No. 8, pp. 3998–4000, August 1978
Medical Sciences

Induction of pulmonary indoleamine 2,3-dioxygenase by intraperitoneal injection of bacterial lipopolysaccharide

(lung/endotoxin)

RYOTARO YOSHIDA AND OSAMU HAYAISHI

Department of Medical Chemistry, Kyoto University Faculty of Medicine, Kyoto 606, Japan

Contributed by Osamu Hayaishi, June 8, 1978

ABSTRACT **Indoleamine 2,3-dioxygenase [indoleamine: oxygen 2,3-oxidoreductase (decyclizing)] activity in the supernatant fraction (30,000 × *g*, 30 min) of the mice lung homogenate increased approximately 30- to 50-fold after an intraperitoneal administration of bacterial lipopolysaccharide. In all other tissues tested, no significant increase in enzyme activity was observed. The effect appeared to be specific for the lipopolysaccharide fraction because glycogen and zymosan were almost ineffective under the same experimental conditions. In the lung, the enzyme activity increased almost linearly during the first 24 hr after a single injection of the lipopolysaccharide fraction (20 μg per mouse). The enzyme activity started to decrease after 48 hr and reached a normal value after about 6 days. The increase in enzyme activity was completely abolished by cycloheximide or actinomycin D. Other enzymes in the lung such as β-glucuronidase, acid phosphatase, and monoamine oxidase did not change significantly with this treatment.**

Indole 2,3-dioxygenase [IDOase; indole:oxygen 2,3-oxidoreductase (decyclizing)] is a novel hemoprotein that catalyzes the oxygenative ring cleavage of various indoleamine derivatives such as tryptophan, 5-hydroxytryptophan, and serotonin (1) and utilizes the superoxide anion both *in vitro* (2–4) and *in vivo* (5). It was originally found in the rabbit intestine (6), and subsequent studies demonstrated its wide distribution in various tissues of mammals (7). In order to investigate the biological significance of this enzyme, the levels and regulatory mechanisms of the IDOase activity were examined in various organs under various physiological and pathological conditions. In this paper we describe a dramatic increase in the IDOase activity in the mouse lung after a single intraperitoneal injection of bacterial endotoxin. This increase appears to be specific for the lipopolysaccharide (LPS) fraction and is probably due to *de novo* synthesis of the enzyme protein.

MATERIALS AND METHODS

Chemicals. DL-[*ring*-2-^{14}C]Tryptophan (35 Ci/mol) was purchased from Schwarz/Mann, and the separation of D- and L-[*ring*-2-^{14}C]tryptophan was performed by cellulose column chromatography with a solvent system of n-butyl alcohol/pyridine/water, 4:1:1 (vol/vol). Catalase was a product of Boehringer Mannheim and was dialyzed to remove a thymol preservative. Methylene blue, ascorbic acid, glycogen, and L-tryptophan was obtained from Wako Pure Chemical Ind. Cycloheximide, actinomycin D, and zymosan A (from *Saccharomyces cerevisiae*) were from Sigma. LPS prepared by the Westpahl method (Difco, Detroit, MI) was derived from either *Escherichia coli* 055:B5 or *Salmonella abortus equi*.

Animals. Male Slc:ICR mice weighing 31 ± 2 g were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Mice were raised in an air-conditioned room at 25° and 50% humidity in the Institute of Laboratory Animals, Kyoto University.

Stimulants. LPS (100 μg/ml) derived from either *E. coli* or *S. abortus equi*, zymosan A (250 μg/ml), and 1% glycogen were suspended in a nonpyrogenic isotonic NaCl solution and stored at 4° in screw-capped bottles. Each of 0.2 ml of a suspension was administered intraperitoneally.

Inhibitors. Actinomycin D was dissolved in 95% ethanol (1 mg/ml) and then diluted in nonpyrogenic saline to a desired concentration. Each mouse was usually given 15 μg in 0.2 ml of solution. Cycloheximide was dissolved in nonpyrogenic saline (25 mg/ml) and was given at a dose of 0.2 ml per mouse. All injections were given intraperitoneally.

Enzyme Assays. All mice were sacrificed by dislocation of the neck. The tissues were rapidly removed and frozen on dry ice. Except for epididymis, the tissues were homogenized with 2 vol of ice-cold 0.14 M KCl/0.02 M potassium phosphate, pH 7.0, by using a Polytron homogenizer. The epididymis was homogenized with 10 vol of the same buffer. Homogenates were centrifuged at 30,000 × *g* for 30 min. The resulting supernatants were used as the enzyme source. The IDOase activity was assayed as described (8). Tryptophan 2,3-dioxygenase [L-tryptophan:oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11] activity was determined by the method of Feigelson and Greengard (9). β-Glucuronidase, acid phosphatase, and monoamine oxidase activities were determined by the methods of Levry and Marsh (10), Schmidt (11), and Guilbault *et al.* (12), respectively.

Miscellaneous Determination. Protein concentration was determined by the method of Lowry *et al.* (13) with bovine serum albumin as a standard.

RESULTS

Glycogen, zymosan, and LPS derived from *S. abortus equi* or from *E. coli* were tested for their ability to increase the IDOase activity in various tissues of mice. Both preparations of LPS caused a 30- to 50-fold increase in the IDOase activity in the lung, but glycogen and zymosan were almost ineffective (Table 1). Significant but much less stimulation was observed in the heart, spleen, and colon. In the epididymis, where the specific activity was by far the highest, the increment in the enzyme activity was only less than 2-fold. In the brain and liver, the enzyme activity was essentially unchanged or even somewhat decreased. Because the tryptophan cleaving activity in the liver is known to be due to tryptophan dioxygenase (14), these results are interpreted to mean that the hepatic tryptophan dioxyge-

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: IDOase, indoleamine 2,3-dioxygenase; LPS, lipopolysaccharide.

Table 1. Effect of various treatments on indoleamine 2,3-dioxygenase activity

	Relative specific activity (nmol per hr/mg protein)*				
	0.9% NaCl	Glycogen	Zymosan	LPS (*E. coli*)	LPS (*S. abortus equi*)
Lung	1.0(0.06 ± 0.01)	1.9(0.13 ± 0.02)	2.4(0.17 ± 0.02)	30.0(2.10 ± 0.02)	44.0(3.10 ± 0.01)
Heart	1.0(0.04 ± 0.01)	1.0(0.04 ± 0.01)	0.8(0.03 ± 0.00)	5.8(0.23 ± 0.04)	1.3(0.05 ± 0.01)
Spleen	1.0(0.03 ± 0.00)	1.3(0.04 ± 0.01)	0.7(0.02 ± 0.00)	3.3(0.10 ± 0.02)	3.7(0.11 ± 0.02)
Colon	1.0(5.87 ± 0.52)	0.6(3.57 ± 0.31)	0.2(2.12 ± 0.18)	2.9(17.3 ± 0.78)	3.8(22.3 ± 1.20)
Kidney	1.0(0.03 ± 0.00)	1.3(0.04 ± 0.01)	0.7(0.03 ± 0.00)	2.3(0.07 ± 0.02)	1.7(0.05 ± 0.01)
Intestine	1.0(4.61 ± 0.38)	0.8(3.58 ± 0.32)	0.4(1.84 ± 0.12)	1.5(6.9 ± 0.58)	3.1(14.2 ± 1.10)
Epididymis	1.0(248.9 ± 9.3)	0.8(195.1 ± 7.1)	0.7(179.3 ± 7.0)	1.5(378.9 ± 13.4)	1.3(313.7 ± 10.7)
Thymus	1.0(0.23 ± 0.01)	1.0(0.23 ± 0.01)	0.7(0.16 ± 0.01)	1.5(0.32 ± 0.01)	1.0(0.23 ± 0.01)
Stomach	1.0(15.7 ± 0.72)	0.6(10.0 ± 0.56)	0.8(11.9 ± 0.57)	1.0(16.2 ± 0.77)	1.0(15.5 ± 0.68)
Brain	1.0(0.06 ± 0.01)	0.5(0.03 ± 0.00)	0.2(0.01 ± 0.00)	0.8(0.05 ± 0.01)	0.7(0.04 ± 0.00)
Liver	1.0(13.6 ± 0.08)	0.8(11.4 ± 0.09)	0.8(10.3 ± 1.01)	0.4(5.40 ± 0.04)	0.5(6.80 ± 0.08)

Mice were sacrificed 24 hr after intraperitoneal injection of LPS (from *E. coli* or *S. abortus equi*), glycogen, zymosan, or saline.
* Relative to saline treatment. Data in parentheses represent the mean ± SEM of actual enzyme activity for five mice.

nase activity is not elevated but is lowered by LPS. A similar observation was reported for rats by Berry and Smythe (15).

Groups of mice were injected intraperitoneally with increasing amounts of LPS (2.5–250 μg per mouse). Epididymis had the highest IDOase activity among the tissues so far tested and therefore was used as a control. At 24 hr later, a dose-dependent increase in the specific activity of IDOase was observed in both the lung and the epididymis, reaching a plateau in the range of about 20 μg per mouse (Fig. 1), a dose almost identical to that used for previous immunological studies (16).

The time course of the changes in the IDOase activity in the lung and the epididymis was determined after a single injection of bacterial LPS derived from *E. coli* (20 μg per mouse), glycogen (2 mg per mouse) (data not included), or 0.9% NaCl (0.2 ml). LPS caused an approximately 30-fold increase in the pulmonary IDOase activity within 24 hr after a single intraperitoneal administration (Fig. 2). The activity started to decrease in about 48 hr and normal values were reached in about 6 days. In epididymis, a similar but lesser change in the IDOase activity was observed although the plateau was maintained between 48 and 72 hr.

In order to determine if the observed increase in enzyme activity was specific for IDOase, typical lysosomal enzymes such as β-glucuronidase and acid phosphatase, as well as monoamine oxidase, another enzyme involved in the metabolism of biogenic amines, were examined. As shown in Table 2, none of these enzymes was stimulated by LPS.

The increase in enzyme activity may be due to (*i*) net protein synthesis, (*ii*) activation of the preexisting IDOase molecules, or (*iii*) the removal of inhibitors. When cycloheximide or actinomycin D was administered to mice together with LPS, the increase in the IDOase activity was completely abolished during the first 4 hr (Fig. 3); thereafter, the enzyme activity started to increase, presumably because these agents were excreted, as reported by Berry *et al.* (17). Preliminary evidence indicated the presence of a weak inhibitor(s) of the IDOase activity in several tissues, such as lung and heart, but no activators in various tissues so far tested.

DISCUSSION

A dramatic induction of pulmonary IDOase by an intraperitoneal injection of LPS was observed. The LPS fraction of the cell wall of Gram-negative bacteria is an inflammatory agent and causes nonspecific immune responses (18). Zymosan, crude yeast cell wall preparations consisting chiefly of protein–carbohydrate complexes, and glycogen are also inflammatory agents (19, 20). Similar cell infiltrations were observed in the peritoneal cavity after the intraperitoneal administration of LPS (*E. coli*), glycogen, or zymosan, but the latter two agents were almost ineffective in the induction of IDOase activity. Furthermore, lysosomal enzymes did not increase after the LPS treatment, although Martini (21), Weissmann and Thomas (22), and Janoff *et al.* (23) strongly implicated lysosomes as a primary target of the LPS action. It has been demonstrated that LPS accumulates in the lung, liver, and kidney when administered intraperitoneally (24). However, whether or not the dramatic

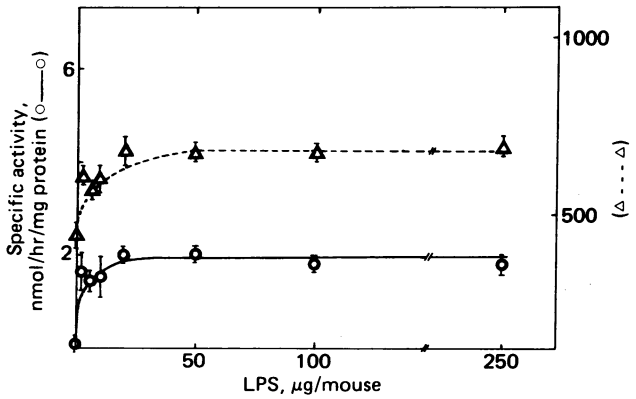

FIG. 1. Effect of LPS on IDOase activity in the lung (○—○) and epididymis (△- - -△) in mice. Each point represents the mean ± SEM for five mice. Mice were sacrificed 24 hr after intraperitoneal injection of LPS.

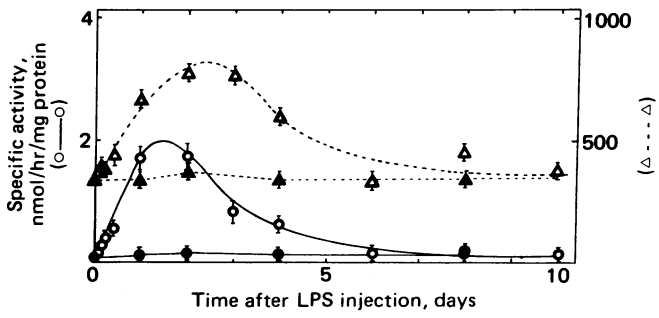

FIG. 2. Time course of the increase of IDOase activity in the lung (○—○) and the epididymis (△- - -△) from LPS-treated mice (20 μg per mouse). Each point represents the mean ± SEM for five mice. Also shown are activities in the lung (●—●) and the epididymis (▲—▲) of control mice.

Table 2. Effect of LPS on various enzyme activities in the lung

Treatment	Relative specific activity* IDOase, nmol/min	β-Glucuronidase, nmol/min	Acid phosphatase, nmol/min	Monoamine oxidase, nmol/hr
0.9% NaCl	1.0(0.09 ± 0.02)	1.0(2.28 ± 0.32)	1.0(8.74 ± 0.32)	1.0(0.11 ± 0.01)
Glycogen	1.7(0.15 ± 0.07)	1.0(2.17 ± 0.21)	0.9(8.27 ± 0.32)	1.0(0.11 ± 0.01)
LPS (*E. coli*)	29.6(2.66 ± 0.34)	0.7(1.70 ± 0.06)	0.9(7.63 ± 0.16)	0.6(0.06 ± 0.00)

Mice were sacrificed 24 hr after intraperitoneal injections of the various stimulants.
* Relative to saline treatment. Data in parentheses represent mean ± SEM of actual enzyme activity, expressed per mg of protein, for five mice.

induction of pulmonary IDOase reported in this paper was due directly to the inflammatory processes or to secondary or adventitious biochemical changes is not known at present.

IODase appears to be involved in the metabolism of serotonin and other indole derivatives and has been demonstrated to utilize the superoxide anion (2–5). Monoamine oxidase, another enzyme involved in the metabolism of biogenic amines, was decreased, rather than increased, by LPS. On the other hand, Crapo (25) recently reported an increase in pulmonary superoxide dismutase activity after rats were exposed to 85% oxygen atmosphere for 5 days, but the increment was on the order of 50%. Preliminary experiments in our laboratory indicated that viral infection of mice (Sendai virus or influenza virus) also causes a similar induction of pulmonary IDOase, suggesting that the enzyme may be closely related to the inflammatory process in some way.

We are indebted to M. Ohara for critical readings of this manuscript. This work was supported in part by research grants from the Naito Foundation, Nippon Shinyaku Co., Ltd., and the Intractable Disease Division, Public Health Bureau, the Ministry of Health and Welfare, Japan, and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

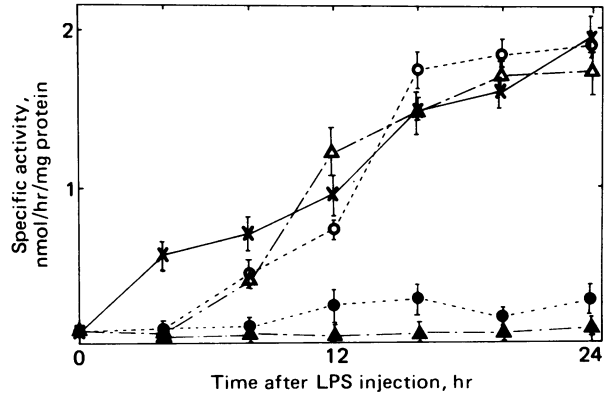

FIG. 3. Effect of actinomycin D and cycloheximide on the increase in pulmonary IDOase activity induced by LPS treatment. Each point represents the mean ± SEM for five mice. ×—×, LPS (20 μg per mouse); ●- - -●, cycloheximide (5 mg per mouse); ▲-·-▲, actinomycin D (25 μg per mouse); ○- - -○, LPS plus cycloheximide; △-·-△, LPS plus actinomycin D. Inhibitors were administered simultaneously with LPS at zero time.

1. Hirata, F. & Hayaishi, O. (1972) *Biochem. Biophys. Res. Commun.* **47**, 1112–1119.
2. Hirata, F. & Hayaishi, O. (1971) *J. Biol. Chem.* **246**, 7825–7826.
3. Hirata, F. & Hayaishi, O. (1975) *J. Biol. Chem.* **250**, 5960–5966.
4. Hayaishi, O., Hirata, F., Ohnishi, T., Henry, J. P., Rosenthal, I. & Katoh, A. (1977) *J. Biol. Chem.* **252**, 3548–3550.
5. Taniguchi, T., Hirata, F. & Hayaishi, O. (1977) *J. Biol. Chem.* **252**, 2774–2776.
6. Yamamoto, S. & Hayaishi, O. (1967) *J. Biol. Chem.* **242**, 5260–5266.
7. Hayaishi, O. & Hirata, F. (1973) *Abstract of 9th International. Congress of Biochemists, Stockholm*, p. 323.
8. Hayaishi, O., Hirata, F., Fujiwara, M., Ohnishi, T. & Nukiwa, T. (1975) *Proceedings 10th FEBS Meeting*, pp. 131–144.
9. Feigelson, P. & Greengard, O. (1961) *J. Biol. Chem.* **236**, 153–157.
10. Levry, G. A. & Marsh, C. A. (1960) *The Enzymes* **4**, 397–407.
11. Schmidt, G. (1961) *The Enzymes* **5**, 37–47.
12. Guilbault, G. G., Montalvo, J. & Smith, R. (1969) *Anal. Chem.* **41**, 600–605.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
14. Feigelson, P. & Greengard, O. (1962) *J. Biol. Chem.* **237**, 1903–1907.
15. Berry, L. J. & Smythe, D. S. (1964) *J. Exp. Med.* **120**, 721–732.
16. Cohn, Z. A. & Benson, B. (1964) *J. Exp. Med.* **121**, 153–169.
17. Berry, L. J., Smythe, D. S. & Colwell, L. S. (1966) *J. Bacteriol.* **92**, 107–115.
18. Milner, K. C., Rudbach, J. A. & Ribi, E. (1971) in *General Characteristics in Microbial Toxins*, eds. Weinbaum, G., Kaidis, S. & Ajl, S. T. (Academic, New York), Vol. 4, pp. 1–66.
19. Humes, J. L., Bonney, R. J., Pelus, L., Dahlgren, M. E., Sadowski, S. J., Kuehl, F. A. & Davies, P. (1977) *Nature (London)* **269**, 149–150.
20. Nowotny, A., Behling, U. H. & Chang, H.-L. (1975) *J. Immunol.* **115**, 199–203.
21. Martini, E. (1959) *Experientia* **15**, 182–183.
22. Weissmann, E. & Thomas, L. (1962) *J. Exp. Med.* **116**, 433–450.
23. Janoff, A., Weissmann, E., Zwifach, B. W. & Thomas, L. (1962) *J. Exp. Med.* **116**, 451–466.
24. Barnes, F. W., Lupter, J. H. & Henry, S. S. (1952) *Yale J. Biol. Med.* **24**, 384–400.
25. Crapo, J. D. (1977) in *Superoxide and Superoxide Dismutase*, eds. Michelson, A. M. & Fridvich, I. (Academic, New York), pp. 231–238.