Biochemical Pharmacology, Vol. 33, No. 13, pp. 2123–2127, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00

INDUCTION OF ORNITHINE DECARBOXYLASE IN MOUSE TISSUES FOLLOWING THE INJECTION OF MITOGENIC SUBSTANCES

ENHANCEMENT BY ACTINOMYCIN D

YASUO ENDO
Department of Pharmacology, School of Dentistry, Tohoku University, Sendai 980, Japan

(*Received* 21 *April* 1983; *accepted* 9 *December* 1983)

Abstract—Experiments were carried out to study the mechanism of the induction of ornithine decarboxylase (ODC) in mouse tissues by the injection of a lipopolysaccharide (LPS). In addition to LPS, various mitogenic substances, such as concanavalin A, pokeweed mitogen, polyI:polyC and a phorbol diester, induced ODC in the liver and the spleen of mice at 4.5 hr after injection. Non-mitogenic immuno-stimulants or inflammatory agents, such as zymosan, carrageenan, *N*-acetylmuramyl-L-alanyl-D-isoglutamine, glycogen, D-galactosamine and interferon, did not induce the enzyme. ODC induction by LPS in C3H/HeJ mice, the lymphocytes and/or macrophages of which are known to be less responsive to LPS, was much less than in C3H/He and ddI mice. ODC induction by LPS was suppressed by dexamethasone and cycloheximide. Actinomycin D did not suppress ODC induction by LPS but, rather, enhanced it. These results suggest that (1) lymphocytes and/or macrophages may participate in the induction of ODC by mitogenic substances as well as by LPS, (2) ODC may be induced by mitogenic substances without the synthesis of RNA, and (3) the translation of existing RNA may be accelerated by actinomycin D.

Ornithine decarboxylase (ODC*) is induced in various tissues and cells in response to a variety of stimuli [1, 2]. The enzyme is a rate-limiting enzyme in the synthesis of polyamines, which have been implicated as regulators of cellular metabolism, proliferation and differentiation [1–3].

Various inflammatory stimuli also induce ODC *in vivo*, e.g. injury [4], parial hepatectomy [5], administration of inducers of hepatitis [6] and carcinogens [7], and application of a tumor promoter [8, 9].

In experiments *in vitro*, it has been shown that lectins or mitogens [10–14], an antigen [15], and viral infection [16] also induce ODC in cultured cells. ODC is induced in lymphocytes by concanavalin A (Con A) [10] and phytohemagglutinin (PHA) [11–13] and in macrophages by lipopolysaccharides (LPS) [14]. Con A, PHA and LPS are used widely, instead of specific antigens, to stimulate lymphocytes.

I reported previously that the injection of a lipopolysaccharide into mice rapidly induces ODC in various tissues [17]. The ODC activity induced in the spleen by the LPS was very high. In addition to inflammatory actions, LPS is known to be a stimulator of macrophages and B-cells (B-cell mitogen). Con A and PHA are stimulators of T-cells (T-cell mitogen).

LPS induction of ODC in mouse tissues may be due to the mitogenic activity of LPS. This possibility was tested in this study by using other mitogenic substances and non-mitogenic immuno-stimulants or inflammatory agents.

MATERIALS AND METHODS

Materials. A lipopolysaccharide derived from *Escherichia coli* 055:B5, prepared by the Boibin method, was obtained from Difco Laboratories (Detroit, MI, U.S.A.). Pokeweed mitogen (PWM) was from E-Y Laboratories (San Mateo, CA, U.S.A.) A synthetic double-stranded polyI:polyC (pIpC) and actinomycin D (Act D) were from P-L Biochemicals, Inc. (Milwaukee, WI, U.S.A.). Carrageenan (Seakem No 202) was supplied by Marine Colloid Inc. (Springfield, NJ, U.S.A.). Zymosan (from *Saccharomyces cerevisiae*), concanavalin A (Con A), 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Mouse interferon (β) was a gift from Professor K. Kumagai of this school (Department of Microbiology). Other reagents were purchased from Wako Pure Chemical Ind. (Osaka, Japan).

Agents, except for TPA, were dissolved or suspended in saline and injected into mice intravenously (i.v.) or intraperitoneally (i.p.) (0.05 to 0.1 ml/10 g body wt). TPA was dissolved in ethanol, mixed with 6 vol. of saline, and injected (i.p., 0.1 ml/mouse).

* Abbreviations: ODC, ornithine decarboxylase; LPS, lipopolysaccharide; Con A, concanavalin A; PHA, phytohemagglutinin; PWM, pokeweed mitogen; pIpC, polyI:polyC; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; MDP, *N*-acetylmuramyl-L-alanyl-D-isoglutamine; and Act D, actinomycin D.

Table 1. ODC induction by various stimulants in the liver and spleen of mice*

Stimulants	Dose (mg/kg)	ODC activity Liver (nmoles/hr/g)	Spleen (nmoles/hr/spleen)
Saline		<0.5	<0.5
LPS	0.5	24 ± 4	24 ± 4
Con A	5	7 ± 3	17 ± 4
PWM	5	23 ± 3	18 ± 2
pIpC	5	16 ± 3	4 ± 1
TPA†	0.4	18 ± 9	7 ± 1
MDP	5	<0.5	<0.5
Zymosan	5	<0.5	<0.5
Carrageenan	5	<0.5	<0.5
Glycogen	100	<0.5	<0.5
D-Galactosamine	400	<0.5	<0.5
Interferon	(2.7×10^5 (units/mouse)	<0.5	<0.5

* All stimulants were injected into mice (ddI, 6-weeks-old) intravenously, and the mice were killed at 4.5 hr later. Each value is the mean ± S.D. of five mice.
† Injected intraperitoneally.

Animals. C3H/HeJ mice were donated by Dr. R. Yoshida (Department of Medical Chemistry, Kyoto University, Kyoto, Japan) and propagated in our laboratory under conventional conditions. Male ddI mice were obtained from the Mouse Center of our university. C3H/He, BALB/c and BALB/c-nu/nu mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka. Japan). The latter two strains of mice were raised under specific pathogen-free conditions. Male mice (5- to 7-weeks-old) of these strains were used for experiments.

Assay of ODC activity. ODC activities were determined by measuring the formation of putrescine from L-ornithine as described previously [17, 18].

RESULTS

ODC induction by various mitogenic substances. In many *in vivo* systems, induction of ODC has been shown to be rapid, with the activity reaching a maximum within 3–6 hr after various stimuli. The LPS-induced ODC activities in mouse tissues also peak at 3–6 hr after LPS injection [17]. Therefore, ODC activity was determined at 4.5 hr after the injection of various mitogenic or non-mitogenic substances (Table 1). Con A, PWM, pIpC and TPA induced ODC in both the liver and the spleen, as did LPS. All of these substances are known to stimulate lymphocytes, i.e. mitogens [19–23].

On the other hand, the agents without mitogenic activity did not induce ODC. MDP is a minimum unit of structure with adjuvant activity of BCG vaccine [24]. LPS also has adjuvant activity. Zymosan, carrageenan and glycogen are used as experimental inducers of inflammatory reactions or immunostimulants [25, 26]. D-Galactosamine, an inducer of experimental hepatitis, has been reported to induce ODC in rat liver at the dosage used in this experiment, but the induction of ODC by this agent occurs more than 10 hr after its injection [6]. Although LPS and pIpC are known to be inducers of interferon, interferon itself did not induce ODC. It is possible that higher doses of these agents may induce ODC at 4.5 hr or later after their injection; even so, it is clear that these agents had very little ability to induce ODC when compared with mitogenic substances.

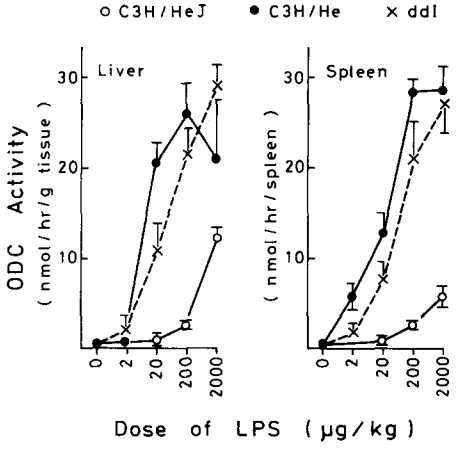

Fig. 1. ODC induction in C3H/HeJ, C3H/He and ddI mice by LPS. Mice were killed 4.5 hr after the intraperitoneal injection of LPS. Each value is the mean ±S.D. of three mice.

ODC induction in C3H/HeJ and nude mice. In *in vitro* experiments, lymphocytes and/or macrophages from C3H/HeJ mice have been shown to be much less responsive than other strains of mice to LPS, in various immunological tests [27–29]. To test ODC induction in C3H/HeJ mice, they were injected with various doses of LPS. The C3H/HeJ mice were shown to be much less responsive than C3H/He and ddI mice to LPS induction of ODC (Fig. 1). There was not such a marked difference in ODC induction by Con A in C3H/HeJ mice (data not shown).

It is known that nude mice with degenerated thymus lack T-cells. If T-cells were critically important in ODC induction by mitogens, mitogens would not induce ODC in nude mice. Injection of both Con A and LPS, however, induced ODC in the nude mice as well as the normal control mice (Table 2).

Table 2. Con A and LPS induction of ODC in nude mice*

	ODC activity	
	Liver (nmoles/hr/g)	Spleen (nmoles/hr/spleen)
BALB/c-nu/nu		
Saline	<0.5	<0.5
Con A	27 ± 5	13 ± 1
LPS	20 ± 1	15 ± 1
BALB/c		
Saline	<0.5	<0.5
Con A	8 ± 1	12 ± 3
LPS	32 ± 1	15 ± 3

* Mice were killed 4.5 hr after the intravenous injection of Con A (20 mg/kg) or LPS (0.5 mg/kg). Each value is the mean ± S.D. of three mice.

Effects of Act D, cycloheximide and dexamethasone on ODC induction in ddI mice by LPS. Since, of all the mitogenic substances tested, LPS was the most potent inducer of ODC, this agent was used to induce ODC in the following experiments. In preliminary experiments, Act D (1 mg/kg, i.p.) and LPS (200 μ/kg, i.p.) injected simultaneously were very toxic; some mice died within 6 hr after the injection. Therefore, lower doses of LPS were used in the following experiments.

Act D injected with LPS enhanced ODC activity dose dependently in both liver and spleen (Table 3). Act D alone, at the doses tested, did not induce ODC in these tissues. The effect of Act D was marked at low LPS doses, i.e. the ODC activities induced in the liver and the spleen by 2 μg/kg of LPS (i.p.) were very low (Fig. 1), but the combination of LPS with Act D (0.5 mg/kg, i.p.) produced ten to twenty times higher ODC activities (data not shown). Following injection of LPS (40 μg/kg, i.p.), ODC activity in the spleen increased at 2 hr, peaked at about 4 hr, and then decreased. This time course of ODC induction was not changed by Act D (0.8 mg/kg) (data not shown). Mixing of the two enzyme solutions, that had been prepared from tissues of mice treated with LPS alone and in combination with Act D, resulted in simple additive ODC activities (data not shown). When putrescine was added to the ODC reaction mixture in the absence of ornithine, there was no variation in the amount of the putrescine (data not shown).

Cycloheximide inhibited ODC induction by LPS almost completely even at a high dose of LPS (200 μg/kg, i.p.) (Table 3).

Glucocorticoids are known to induce ODC in the liver and the kidney of rats [2]. Dexamethasone (10 mg/kg, i.p.), however, did not induce ODC in the liver and the spleen of mice, at least at 4.5 hr after injection (data not shown). On the contrary, this agent suppressed LPS induction of ODC in these tissues, especially in the spleen (Table 3).

DISCUSSION

In addition to LPS, various mitogenic substances, i.e. Con A, PWM, pIpC and TPA, but not non-mitogenic immuno-stimulants or inflammatory agents (carrageenan, zymosan, D-galactosamine, glycogen, MDP and interferon), induced ODC in the liver and the spleen of mice. Con A is a typical T-cell mitogen, LPS and pIpC are B-cell mitogens, and PWM is a T-cell-dependent B-cell mitogen

Table 3. Effects of actinomycin D, cycloheximide and dexamethasone on the LPS-induced increase in ODC activity*

Drug treatment (mg/kg, i.p.)	ODC activity (%) Liver	P†	Spleen	P
Actinomycin D‡				
I 0	100 ± 11 (19 ± 2.1)		100 ± 24 (12 ± 2.9)	
II 0.4	142 ± 16	vs I, <0.01	147 ± 27	vs I, <0.05
III 0.8	168 ± 26	vs II, <0.5	187 ± 40	vs II, <0.5
IV 1.6	189 ± 32	vs III, <0.5	307 ± 80	vs III, <0.05
Cycloheximide§				
I 0	100 ± 18 (25 ± 4.5)		100 ± 19 (21 ± 4.0)	
II 200	<2		5 ± 1	vs I, <0.001
Dexamethasone‖				
I 0	100 ± 9 (20 ± 1.8)		100 ± 13 (15 ± 2.0)	
II 0.8	64 ± 14	vs I, <0.01	33 ± 3	vs I, <0.001
III 4	26 ± 10	vs II, <0.001	23 ± 7	vs II, <0.05
IV 20	17 ± 2	vs III, <0.001	18 ± 7	vs III, <0.05

* Mice (ddI) were treated with LPS alone or with a combination of drugs and were killed 4.5 hr after the injection of LPS. Each value is the mean ± S.D. of five mice. The values in parentheses are absolute values of ODC activities in the liver (nmoles/hr/g) and spleen (nmoles/hr/spleen).

† Statistical significance was determined using Student's *t*-test of the difference of unpaired means.

‡ Injected simultaneously with LPS (40 μg/kg, i.p.).

§ Injected simultaneously with LPS (200 μg/kg, i.p.).

‖ Injected at 2 hr before LPS (40 μg/kg, i.p.).

[19–22]. TPA is also known to stimulate lymphocytes [23]. Induction of ODC in the skin by direct application of TPA, which was used as a promoter of skin tumor, has been studied by many investigators (see for example 8 and 9). This agent has also been shown to induce ODC in the brain, liver and lung of rats by i.p. injection [9].

In C3H/HeJ mice, the lymphocytes and/or macrophages of which are known to be much less responsive to LPS [27–29], LPS induction of ODC was much less than in control mice (C3H/He and ddI). Of all the mitogenic substances tested, LPS was the most potent inducer of ODC in ddI mice. LPS is known to be a potent stimulator of macrophages. Macrophages have been shown to be required for the activation of lymphocytes by various mitogens including Con A [30, 31], and a factor produced by macrophages has been proposed to trigger various immune responses [32, 33].

The results described above suggest that macrophages and/or lymphocytes may participate in the induction of ODC by mitogenic substances as well as by LPS, although they do not rule out direct interaction of mitogens with the cells in which ODC was induced. Induction of ODC in nude mice, which lack T-cells, by LPS and Con A may have been due to the participation of macrophages. Recently, I found that a factor(s) derived from a macrophage cell line and from mitogen-stimulated spleen cells induced ODC more rapidly than did LPS and Con A (unpublished data), suggesting the participation of hormone-like substances or lymphokines in ODC induction by mitogens. The suppression of ODC induction by dexamethasone, a potent inhibitory agent of immune responses or inflammation, appears also to support the possibility described above.

A characteristic property of ODC induction by LPS was observed in this study. Although cycloheximide inhibited induction of ODC by LPS as reported in other systems, Act D enhanced it. The time course of increase and decrease in ODC activity induced in the spleen by LPS was not changed by Act D. Taken together these results and those obtained by experiments using enzyme solutions that had been prepared from tissues of mice treated with LPS alone and in combination with Act D, it appears that the synthesis of ODC itself is enhanced by Act D, and that induction of ODC by LPS may not require synthesis of RNA. Since it has been reported that Act D stimulates the template activity of mRNA by changing its conformation [34, 35], accelerated translation of existing RNA may have been one of the mechanisms of the enhanced induction of ODC by Act D. Such an effect of Act D on the induction of ODC, termed "superinduction", has been reported in a few *in vitro* systems [36, 37]. In many *in vivo* systems, ODC induction is known to be inhibited by Act D [1, 2]. Therefore, the mechanism of LPS induction of ODC appears to be different than that of certain other inducers. Recently, Sasaki *et al.* [38] reported that ODC induction in the liver by a factor from tumor ascites fluid is also enhanced by Act D under limited conditions. I found that ODC induction by a factor(s) derived from a macrophage cell line was also enhanced by Act D (unpublished data).

Acknowledgements—I am grateful to Professor Y. Ogura of this laboratory for supporting this study and to Mr. T. Kikuchi for help in the preparation of the manuscipt.

REFERENCES

1. C. W. Tabor and H. Tabor, *A. Rev. Biochem.* **45**, 285 (1976).
2. D. R. Morris and R. H. Fillingame, *A. Rev. Biochem.* **43**, 303 (1974).
3. T. Takano, M. Takigawa and F. Suzuki, *J. Biochem., Tokyo* **93**, 591 (1983).
4. A. Mizutani, H. Inoue and Y. Takeda, *Biochim. biophys. Acta* **338**, 183 (1974).
5. D. H. Russell and S. H. Snyder, *Molec. Pharmac.* **5**, 253 (1969).
6. Y. Daikuhara, F. Tamada, M. Takigawa, Y. Takeda and Y. Mori, *Gastroenterology* **77**, 123 (1979).
7. W. J. Ball, Jr., J. S. Salser and M. E. Balis, *Cancer Res.* **36**, 2686 (1976).
8. M. Takigawa, A. K. Verma, R. C. Simsiman and R. K. Boutwell, *Biochem. biophys. Res. Commun.* **105**, 969 (1982).
9. R. A. Weiner and C. V. Byus, *Biochem. biophys. Res. Commun.* **97**, 1575 (1980).
10. R. H. Fillingame and D. R. Morris, *Biochemistry* **12**, 4479 (1973).
11. J. E. Kay and A. Cooke, *Fedn. Eur. Biochem. Soc. Lett.* **16**, 9 (1971).
12. J. E. Kay and V. J. Lindsay, *Expl. Cell Res.* **77**, 428 (1973).
13. H. Korpela, E. Hölttä, T. Hovi and J. Jänne, *Biochem. J.* **196**, 733 (1981).
14. W. K. Nichols and F. H. Prosser, *Life Sci.* **27**, 913 (1980).
15. T. Watanabe, T. Kishimoto, T. Miyake, Y. Nishizawa, H. Inoue, Y. Takeda and Y. Yamamura, *J. Immun.* **115**, 1185 (1975).
16. S. Don and U. Bachrach, *Cancer Res.* **35**, 3618 (1975).
17. Y. Endo, *Biochem Pharmac.* **31**, 1643 (1982).
18. Y. Endo, *Meth. Enzym.* **94**, 42 (1983).
19. S. M. Wolff, *J. infect. Dis.* **128** (Suppl.), S259 (1973).
20. M. F. Greaves and G. Janossy, *Transplantn Rev.* **11**, 87 (1972).
21. R. G. Keightley, M. D. Cooper and A. R. Lawton, *J. Immun.* **117**, 1538 (1976).
22. I. Scher, D. M. Strong, A. Ahmed, R. C. Knudsen and K. W. Sell, *J. exp. Med.* **138**, 1545 (1973).
23. J. E. Nagel, F. J. Chrest and W. H. Adler, *Clin. expl Immun.* **49**, 217 (1982).
24. L. Chedid, F. Audibert and A. G. Johnson, *Prog. Allergy* **25**, 63 (1978).
25. J. L. Humes, R. J. Bonney, L. Pelus, M. E. Dahlgren, S. J. Sadowski, F. A. Kuehl, Jr. and P. Davies, *Nature, Lond.* **269**, 149 (1977).
26. K. Sugio and S. Tsurufuji, *Br. J. Pharmac.* **73**, 605 (1981).
27. L. M. Glode, I. Scher, B. Osborne and D. L. Rosenstreich, *J. Immun.* **116**, 454 (1976).
28. J. L. Ryan, L. M. Glode and D. L. Rosenstreich, *J. Immun.* **122**, 932 (1979).
29. M. Nowakowski, P. J. Edelson and C. Bianco, *J. Immun.* **125**, 2189 (1980).
30. D. L. Rosenstreich, J. J. Farrar and S. Dougherty, *J. Immun.* **116**, 131 (1976).
31. D. L. Rosenstreich and S. B. Mizel, *Immun. Rev.* **40**, 102 (1978).
32. S. B. Mizel, *Immun. Rev.* **63**, 51 (1982).
33. J. J. Farrar, W. R. Benjamin, M. L. Hilfiker, M. Howard, W. L. Farrar and J. F. Farrar, *Immun. Rev.* **63**, 129 (1982).

34. L. Leinwand and F. H. Ruddle, *Science* **197**, 381 (1977).
35. S. Toku, Y. Nabeshima and K. Ogata, *J. Biochem., Tokyo* **93**, 361 (1983).
36. D. A. Goldstein, O. Heby and L. J. Marton, *Proc. natn. Acad. Sci. U.S.A.* **73**, 4022 (1976).
37. Y. Yamasaki and A. Ichihara, *J. Biochem., Tokyo* **81**, 461 (1977).
38. K. Sasaki, A. Kashiwagi, K. Imamura and T. Tanaka, *J. Biochem., Tokyo* **92**, 1591 (1982).