European Journal of Pharmacology, 91 (1983) 493–499
Elsevier

A LIPOPOLYSACCHARIDE AND CONCANAVALIN A INDUCE VARIATIONS OF SEROTONIN LEVELS IN MOUSE TISSUES

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Received 24 November 1983, revised MS received 17 March 1983, accepted 10 May 1983

Y ENDO, *A lipopolysaccharide and concanavalin A induce variations of serotonin levels in mouse tissues*, European J Pharmacol 91 (1983) 493–499

The administration of an *Escherichia coli* lipopolysaccharide (LPS), or an endotoxin into mice produced a variation in tissue serotonin (5HT) levels within 4 5 h 5HT levels in the kidney and lung were decreased by the higher doses of LPS, but those in the liver and spleen were increased even by lower doses of the agent The increase in liver 5HT was most marked Such variations in 5HT levels were also produced by the administration of concanavalin A In vitro experiments using extracts from livers of LPS-treated and non-treated mice indicated that there was no difference in the 5HT formation from 5-hydroxytryptophan between the two groups, but that 5HT formation from tryptophan was higher in the LPS-treated mice The LPS-induced 5HT increase in liver was suppressed by p-chlorophenylalanine (an inhibitor of tryptophan hydroxylase), actinomycin D, cycloheximide and dexamethasone, but not by Ro 4-4602 (an inhibitor of aromatic amino acid decarboxylase), pargyline (an inhibitor of monoamine oxidase) and indomethacin A possible mechanism of the 5HT increase in the liver is discussed on the basis of these results

Serotonin and endotoxin Serotonin Lipopolysaccharide Endotoxin Concanavalin A
Inflammation Mitogens

1. Introduction

It is known that serotonin (5HT) is one of the mediators in immediate hypersensitivity or inflammatory reactions (Gershon and Ross, 1962; Rowley and Benditt, 1956, Nishida et al., 1979) In addition, it has been suggested that this vasoactive amine may also be important in the production of the lesions of delayed-type hypersensitivity (Gershon et al., 1975, Schwartz et al., 1977, Askenase et al., 1980) Many studies have been carried out on the release of 5HT from cells or from inflamed local sites There are, however, few studies on 5HT synthesis in peripheral tissues during inflammatory reactions

Lipopolysaccharides (LPS) of the cell wall of gram-negative bacteria, or endotoxins, stimulate non-specific immune responses and cause inflammatory reactions LPS is also known as a stimulator of B-lymphocyte mitogenesis, i e , B-cell mitogen I reported that this agent produced an increase in histamine, another vasoactive amine believed to be a mediator of inflammation, in various mouse tissues (Endo, 1982) In the present study, I found that LPS also produced an increase in the 5HT levels in the liver and spleen of mice To examine whether such an effect was specific to LPS another immune stimulant, concanavalin A, was tested This agent is used widely as a T-cell mitogen This agent also produced essentially the same effects as LPS on the 5HT levels A possible mechanism for the LPS-induced 5HT increase in the liver is discussed on the basis of the results from in vitro experiments and the effects of various drugs

2. Materials and methods

2.1 Materials

LPS derived from *Escherichia coli* 055 B5, prepared by the Boibin method, was obtained from Difco Lab (Detroit, MI, USA) Actinomycin D was from P-L Biochemical Inc (Milwaukee, Wis., USA) Concanavalin A was from Sigma Chemical Comp (St Louis, MO, USA) Other reagents were purchased from Wako Pure Chemical Ind (Osaka, Japan) Male ddY mice (5-6 weeks old) were purchased from Funabashi Farm (Funabashi, Japan) Male ddI mice (5-7 weeks old) were obtained from the Mouse Center of our University Agents were dissolved or suspended in saline and injected into mice intravenously (i v) or intraperitoneally (i p) (0 05-0 1 ml/10 g body weight) p-Chlorophenylalanine was suspended in saline and dissolved by the addition of 2 M NaOH and the pH of the solution then adjusted to 9 8 with 2 M HCl The concentration of the solution was adjusted to 300 mg/ml with saline for injection into mice

2.2 5HT determination

Mice were decapitated and the tissues used for the fluorometric determination of 5HT as described previously (Tadano et al, 1980) The 5HT levels were expressed as nmol/g tissue for the liver and brain and nmol/organ for the kidney, spleen and lung The method used for the determination of 5HT in this study is based on the purification of 5HT by chromatography using a small P-cellulose column (0 6 × 6 cm) and fluorometric determination using the reaction with o-phthalaldehyde (OPA) In the OPA reaction, several indole substances form fluorescent products as described previously (Tadano et al, 1980) However, acidic and neutral indole substances such as 5-hydroxyindoleacetic acid, 5-hydroxytryptophan (5HTP), melatonin, and N-acetylserotonin were not retained on the P-cellulose column and could be completely separated from 5HT Therefore, the possible substances included in the 5HT fraction and likely to form 5HT-like OPA products are tryptamine and 5-methoxytryptamine These three

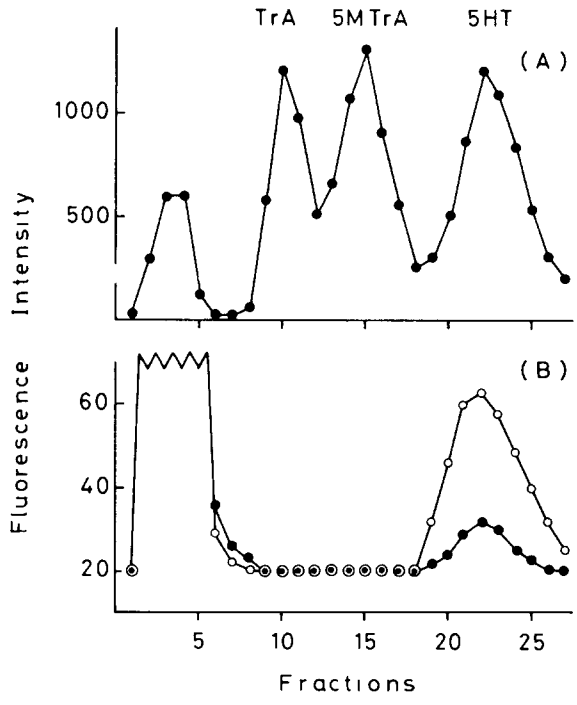

Fig 1 Identification of 5HT by P-cellulose column chromatography The livers of LPS-treated and non-treated mice were homogenized with 10 vol of ice-cold 0 4 M $HClO_4$ containing 2 mM EDTA 2Na The supernatants (3 ml) obtained by centrifugation (5000 × g, 5 min) were neutralized to pH 5-6 with 2 M KOH in an ice bath The neutralized supernatants obtained by centrifugation (5000 × g, 5 min) were used as samples for chromatography P-cellulose columns (1 × 11 cm) were equilibrated with 0 02 M borate buffer (pH 8 5 prepared from 0 02 M H_3BO_3 and 0 02 M Na_2CO_3) After application of each sample to the column elution was carried out with the borate buffer containing both 0 05 M NaCl and 0 25 mM EDTA 2Na Fractions (each 3 ml) were collected and used for the measurement of fluorescence at 348 nm with excitation at 287 nm Control fluorescence intensity was 100 for 0 5 nmol of 5HT (A) Tryptamine (TrA), 5-methoxytryptamine (5MTrA) and 5HT (each 10 nmol) were added to the sample from normal liver, (B) The samples from normal liver (●———●) and from the liver of LPS-treated mice (○———○)

amines could be separated from each other on a larger P-cellulose column (1 × 11 cm) (fig 1A) The results shown in fig 1B indicate that the substance increased by LPS injection was 5HT itself and that there was no detectable amount of tryptamine and 5-methoxytryptamine in the liver of either LPS-treated or non-treated mice

2 3 5HT formation in vitro

2 3 1 From 5HTP

Tissues were homogenized with 10 vol of 20 mM sodium phosphate buffer (pH 7) containing pyridoxalphosphate (0 1 mM) The supernatant (0 2 ml) obtained by centrifugation (20000 × *g*, 20 min) was incubated at 37°C for 30 min in the following reaction mixture (1 ml) phosphate buffer (40 mM), pyridoxalphosphate (0 1 mM), dithiothreitol (0 5 mM), pargyline (1 mM) and 5HTP (1 mM) The reaction was terminated 1 h later by the addition of 0 4 M $HClO_4$ (2 ml)

2 3 2 From tryptophan

Tissues were homogenized with 5 vol of 20 mM tris-HCl buffer (pH 7 5) The supernatant (0 5 ml) obtained by centrifugation (20000 × *g*, 30 min) was incubated at 37°C for 1 h in the following reaction mixture (1 ml) tris-HCl buffer (0.1 M), pyridoxalphosphate (0 05 mM), pargyline (1 mM), dithiothreitol (0 5 mM), 6,7-dimethyl-5,6,7,8-tetrahydropterine (1 mM) and tryptophan (2 mM) The reaction was terminated 1 h later by the addition of 0 4 M $HClO_4$ (2 ml) The 5HT formed in these reaction mixtures was determined as described above

3. Results

3 1 Variation in 5HT levels in mouse tissues after LPS injection

The injection of LPS (0 5 mg/kg i v) into ddY mice produced different effects on the 5HT levels in the various tissues (table 1) The 5HT level in the liver increased markedly and reached a maximum value at about 4 5 h after LPS injection This elevated level was maintained during the experimental period The 5HT levels in the spleen and brain were also increased by LPS, although not so markedly as in the liver. The 5HT increase in the brain appeared to be slow when it was compared with those in the liver and spleen The 5HT levels in the kidney and lung decreased gradually with time The 5HT levels in the blood and thymus were very low, and no variation could be detected

3 2 Effects of various doses of LPS on the 5HT levels

The effects of various doses of LPS on the 5HT levels at 4 5 h after its injection into ddI mice are shown in table 2 As little as 2 μg/kg of LPS produced a significant increase in 5HT in the liver and spleen. When LPS was injected i v 0 2 μg/kg of the agent produced about a 2 fold increase in 5HT in the liver of ddY mice Higher doses of LPS appeared to decrease 5HT in the kidney and lung and even in the spleen The results shown in tables 1 and 2 indicate that the effects of LPS on tissue 5HT levels were the same in ddY and ddI mice In addition, there was also an about 3 fold increase of 5HT in the liver of male Wistar (200-230 g) at 4.5 h after LPS injection (2 mg/kg i p)

3 3 Effects of concanavalin A on the 5HT levels in mouse tissues

To examine whether the effects of LPS on the 5HT levels in mouse tissues are specific to this

TABLE 1

Variation of 5HT levels in tissues of ddY mice after LPS injection (0 5 mg/kg i v) Each value is the mean ± S D of 5 mice [a] There was no significant variation in 5HT in control mice given saline or not injected [b] nmol/g tissue (in the liver and brain) or nmol/organ (in the spleen, kidney and lung) Statistical significances (Student's t-test vs control) are represented as [c] ($P < 0.05$), [d] ($P < 0.01$) and [e] ($P < 0.001$)

Tissues	5HT levels (%) after LPS injection				
	Control [a]	2 h	4 5 h	6 h	12 h
Liver	100 ± 16 (1 9 ± 0 3) [b]	148 ± 48	474 ± 55 [e]	537 ± 37 [e]	473 ± 27 [e]
Spleen	100 ± 5 (1 9 ± 0 1)	122 ± 6 [e]	116 ± 5 [d]	113 ± 3 [d]	109 ± 1 [d]
Brain	100 ± 3 (2 4 ± 0 1)	104 ± 4	121 ± 13 [c]	129 ± 4 [e]	123 ± 2 [c]
Kidney	100 ± 5 (0 19 ± 0 01)	103 ± 8	87 ± 8 [c]	74 ± 6 [e]	58 ± 11 [e]
Lung	100 ± 9 (0 88 ± 0 08)	90 ± 4	80 ± 6 [d]	75 ± 1 [e]	76 ± 1 [c]

TABLE 2

Effects of various doses of LPS and concanavalin A on 5HT levels in ddI mouse tissues. Mice were killed at 4.5 h after the injection of LPS or concanavalin A. [a] nmol/g tissue (in the liver and brain) or nmol/ organ (in the spleen, kidney and lung). Each value is the mean ± S.D. from 5 mice. Statistical significances (Student's t-test vs. control) are represented as [b] ($P < 0.05$), [c] ($P < 0.01$) and [d] ($P < 0.001$).

Treatments	Levels of 5HT (%)				
	Liver	Spleen	Brain	Kidney	Lung
Saline	100 ± 11 (0.92 ± 0.10) [1]	100 ± 18 (1.8 ± 0.2)	100 ± 5 (2.0 ± 0.1)	100 ± 6 (0.31 ± 0.02)	100 ± 18 (0.17 ± 0.03)
LPS 2 μg/kg i.p.	160 ± 23 [c]	146 ± 4 [d]	-	-	105 ± 43
20	311 ± 31 [d]	155 ± 12 [d]	-	-	116 ± 18
200	364 ± 38 [d]	152 ± 4 [d]	-	-	97 ± 11
2000	347 ± 31 [d]	86 ± 11	-	-	61 ± 7 [c]
LPS 1 mg/kg i.v.	374 ± 27 [d]	81 ± 1	111 ± 2 [c]	74 ± 2 [d]	62 ± 8 [c]
Concanavalin A 5 mg/kg i.v.	467 ± 44 [d]	214 ± 21 [d]	108 ± 4 [b]	67 ± 7 [d]	92 ± 13

agent or not, the effects of concanavalin A were examined (table 2). This agent also produced essentially the same effects as LPS on 5HT levels in mouse tissues, i.e., it increased 5HT in the liver and spleen and decreased 5HT in the kidney. In addition, the time course of the 5HT increase in the liver by concanavalin A (5 mg/kg i.v.) was similar to that produced by LPS and shown in table 1 (data not shown).

3.4 5HT formation in vitro

5HT is known to be synthesized through the formation of 5HTP by the hydroxylation of tryptophan and subsequent decarboxylation of 5HTP. Therefore, to find the cause of the LPS-induced 5HT increase in the liver, in vitro 5HT formation from 5HTP and from tryptophan was examined (table 3). 5HT formation from 5HTP in the liver was very rapid, but there was no difference in the rate of formation between LPS-treated and non-treated mice. On the other hand, although, 5HT formation from tryptophan in the liver was very slow, it was significantly higher in LPS-treated mice (about 60%) than in control mice. In the brain, there was no difference in 5HT formation from either 5HTP or tryptophan between the two groups.

TABLE 3

5HT formation in vitro by extracts from the liver and brain of LPS-treated mice. Mice (ddI) were killed at 4 h after LPS injection (0.5 mg/kg, i.v.) and in vitro 5HT formation was examined as described in Materials and methods. Each value is the mean ± S.D. from 5 mice. [a] Significantly different from control ($P < 0.01$, Student's t-test).

Tissue	5HT formation (nmol/h per g tissue)	
	From 5HTP	From Tryptophan
Liver		
Saline	3380 ± 200	1.2 ± 0.2
LPS	3160 ± 390	1.9 ± 0.2 [a]
Brain		
Saline	420 ± 20	10.7 ± 0.1
LPS	430 ± 10	10.9 ± 0.2

3.5 Effects of various inhibitors on the LPS-induced increase in liver 5HT

To investigate the mechanism of the LPS-induced 5HT increase in the liver of mice, the effects of various inhibitors were examined (table 4). p-Chlorophenylalanine is known to deplete 5HT in the brain and some other tissues, possibly by blocking tryptophan hydroxylation (Koe and Weissman, 1966, Welch and Welch, 1968). This agent suppressed the LPS-induced increase in liver 5HT. On the other hand, neither Ro 4-4602, an inhibitor of aromatic amino acid decarboxylase (Burkard et al., 1964), nor pargyline, an inhibitor of monoamine oxidase, were effective. None of

TABLE 4

Effects of drugs on the LPS-induced increase in liver 5HT Inhibition (%) was calculated as follows (100 − (5HT level in drug treatment − control 5HT level)/LPS-induced 5HT level − control 5HT level)) × 100 Drugs were injected (i p) simultaneously with, or before LPS injection (40 μg/kg i p) [a] once per day for 3 days before, [b] 30 min before, [c] simultaneously [d, e] 2 and 1 h before, respectively In each experiment of a to e, saline was injected into the control mice Mice (ddI) were killed at 4 5 h after LPS injection Each value is the mean ± S D from 4-5 mice Statistical significances (Student's t-test vs control) are represented as [f] ($P < 0.01$) and [g] ($P < 0.001$) N S not significant

Drug	Dose (mg/kg)	Inhibition (%)
p-Chlorophenylalanine [a]	150 × 3	67 ± 5 [g]
Ro 4-4602 [b]	50	−8 ± 11 (N S)
Pargyline [b]	75	−4 ± 9 (N S)
Actinomycin D [c]	0 4	7 ± 3 (N S)
	0 8	46 ± 7 [f]
	1 6	52 ± 11 [f]
Cycloheximide [c]	50	75 ± 8 [g]
Dexamethasone [d]	0 8	53 ± 7 [f]
	4	71 ± 11 [g]
	20	82 ± 3 [g]
Quinacrine [e]	10	11 ± 18 (N S)
	50	32 ± 7 [g]
Indomethacin [e]	1	−13 ± 10 (N S)
	3	−22 ± 22 (N S)
	10	0 ± 10 (N S)
	30	−6 ± 19 (N S)

these agents themselves showed any significant effects on the normal level of liver 5HT, although p-chlorophenylalanine decreased brain 5HT by about 40% and pargyline increased it about 2 fold (data not shown) Both actinomycin D and cycloheximide, respectively inhibitors of RNA and protein synthesis, were inhibitory to a significant extent The most potent inhibition was obtained with dexamethasone, a potent anti-inflammatory glucocorticoid and an inhibitor of arachidonic acid release (Hong and Levine, 1976, Flower and Blackwell, 1979, Hirata et al , 1980a, Blackwell et al , 1982) Quinacrine, an inhibitor of arachidonic acid release (Dise et al , 1982), was also inhibitory although its effect was weak Indomethacin, an anti-inflammatory agent and an inhibitor of prostaglandin synthesis, did not show any inhibition

4. Discussion

There are many studies indicating that the release of 5HT from mast cells or other cells is associated with inflammatory reactions The present study demonstrated that inflammatory or mitogenic stimulants, LPS and concanavalin A, caused an increase of 5HT in the liver and spleen of mice and in the liver of rats The increase in liver 5HT by these agents was most marked Such a marked elevation of 5HT in peripheral tissues has not been reported except for the elevation by the administration of 5HTP, a direct precursor of 5HT (Udenfriend et al , 1957) The decrease in 5HT in the kidney and lung by these agents may reflect the release of 5HT from the local sites

LPS and concanavalin A are different mitogens with different chemical structures i e , the former is a B-cell mitogen and the latter is a T-cell mitogen However, both mitogens produced essentially the same effects on 5HT levels in mouse tissues It is known that the presence of macrophages is important for the various mitogen reactions (Rosenstreich and Mizel, 1978, and see the references in Rosenstreich et al , 1976) I have evidence that a lymphocyte stimulating factor derived from macrophages elevates the level of 5HT in mouse liver (unpublished data) Although the meaning of the 5HT increase in the liver and spleen is not clear at present, this phenomenon may be one of immune response or cell-mediated inflammatory reactions

There are few studies on 5HT synthesis in peripheral tissues, possibly because of low activity It is known that the hydroxylation of tryptophan is the rate-limiting step in 5HT synthesis in the brain This is also possible in the liver, because 5HT formation from tryptophan was extremely limited when it was compared with that from 5HTP as in the brain (table 3) The enhancement of 5HT formation in vitro from tryptophan, but not from 5HTP, and the inhibitory effect of p-chlorophenylalanine indicate that the hydroxylation of tryptophan is accelerated in the liver of LPS-treated mice

Although it is expected that an inhibitor of aromatic amino acid decarboxylase would decrease 5HT levels and that an inhibitor of mono-

amine oxidase would elevate them, neither Ro 4-4602 nor pargyline produced significant effects on the normal 5HT level in the liver or on the LPS-induced 5HT level The activity of 5HTP decarboxylation is very high in the liver (table 3) Therefore, partial inhibition of such a high activity by Ro 4-4602 would not affect the total 5HT synthesis The same explanation may also hold for the inefficacy of pargyline, because the activity of monoamine oxidase is known to be very high in the liver In addition, since pargyline elevates 5HT in the brain but not in the liver, it is also possible that the turnover rate of 5HT in the liver is much lower than that in the brain

The activity of tryptophan hydroxylase is known to be affected by a variety of factors (Mandell, 1978) In the present study also it was shown that the LPS-induced increase in liver 5HT was suppressed by various inhibitors such as actinomycin D cycloheximide, dexamethasone and quinacrine These results suggest that the synthesis of RNA and/or protein and the release of arachidonic acid may be included in the process of the acceleration of 5HT synthesis by LPS The release of arachidonic acid through the activation of a phospholipase(s) is shown to be a critical step in the initiation of a variety of cellular responses (see the references in Dise et al , 1982) In the present study, the inhibition by dexamethasone was most potent However, indomethacin, an inhibitor of prostaglandin synthesis, did not suppress the 5HT increase Hirata et al (1980b) have reported that the mitogenesis of T lymphocytes by concanavalin A is triggered by the release of arachidonic acid but that indomethacin does not suppress this mitogenesis Therefore, other metabolites of arachidonic acid might have some effect on the 5HT synthesis induced by mitogens

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 manuscript

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