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Parallel relationship between the increase in serotonin in the liver and the hypoglycaemia induced in mice by interleukin-1 and tumour necrosis factor

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1. Summary

Endotoxins or lipopolysaccharides (LPS), when injected into mice, increase serotonin (5-hydroxytryptamine; 5HT) in the liver and produce hypoglycaemia. In the present study, it was found that the cytokines produced by macrophages in response to LPS, interleukin-1 (IL-1, 0.1 μg/kg or more) and tumour necrosis factor (TNFα, 100 μg/kg or more), can also induce an increase in liver serotonin and produce hypoglycaemia. The two responses correspond well with each other in terms of time course and dose dependency. These results suggest that the two responses induced by LPS may be mediated by IL-1 and/or TNFα and that the phenomenon of accumulation of 5HT in the liver may be relevant to hypoglycaemia. The hypoglycaemic response to IL-1 was moderate at a wide dose range but was induced by smaller amounts than with insulin.

2. Introduction

Lipopolysaccharides (LPS) are known to produce hypoglycaemia in experimental animals. One mechanism is an impaired hepatic gluconeogenesis [1–3]. LPS itself, however, does not inhibit gluconeogenesis in isolated hepatocytes [3].

Key words: Interleukin-1; Tumour necrosis factor; Lipopolysaccharide; Hypoglycaemia; 5-Hydroxytryptamine; Liver

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Serotonin (5-hydroxytryptamine; 5HT) inhibits gluconeogenesis in isolated hepatocytes [4] by an unknown mechanism. In fact, the accumulation of 5HT in the liver after the injection of its precursor, 5-hydroxytryptophan (5HTP), induces hypoglycaemia, and this hypoglycaemia is prevented by inhibitors of 5HT synthesis from 5HTP [5, 6].

I have previously found that injection of LPS to mice and rats increases 5HT predominantly in the liver [7]. This suggests that the 5HT accumulation induced by LPS may be instrumental for the production of hypoglycaemia through the inhibition of hepatic gluconeogenesis [8].

Additionally, we found that P388D1 cells, a murine macrophage cell line, can produce factors capable of inducing hypoglycaemia and that these factors can also accumulate 5HT in the liver [9]. Although the purification of the macrophage products was not completed, they exhibited interleukin-1 (IL-1) activity, and their molecular weights and isoelectric points were identical to those of IL-1 [9]. To confirm whether IL-1, like LPS, can induce the two responses or not, the effects of highly purified preparations of recombinant IL-1α and IL-1β were examined in the present study. Since LPS is known to stimulate macrophages to produce tumour necrosis factor-α (TNFα) as well as IL-1 [10, 11], the effect of TNFα was also examined.

3. Materials and Methods

Recombinant human IL-1α and recombinant human TNFα were provided by Dainippon Phar-

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maceutical Co. (Osaka, Japan) [12, 13] and recombinant human IL-1β was from Ohtsuka Pharmaceutical Co. (Tokushima, Japan) [14]. Each preparation of these cytokines showed a single band in SDS-polyacrylamide gel electrophoresis. The contamination by endotoxin or LPS in the preparations of IL-1α, IL-1β and TNFα was less than 0.02, 0.1 and 0.04 ng/mg protein, respectively (assayed by the Limulus test). Bovine insulin (24 I.U./mg), pargyline hydrochloride and cyproheptadine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Methysergide hydrogen maleate was provided by Sandoz, Inc. (East Hanover, NJ, U.S.A.). Ketanserin tartrate was obtained from Janssen Pharmaceuticals (Beerse, Belgium). LPS of *Escherichia coli* O55:B5, prepared by Boivin's method, was obtained from Difco Laboratories (Detroit, MI, U.S.A.). Male ddY mice were purchased from Shizuoka Agricultural Association for Laboratory Animals (Shizuoka, Japan).

Mice were kept under a conditioned light (7 a.m. to 7 p.m.)/dark cycle and fed ad libitum. Food was deprived 1 h before experiments. Test samples diluted in sterile saline (0.2–0.3 ml) were injected intraperitoneally (i.p.) or subcutaneously (s.c.) to six-week-old mice (26–28 g) between 10 and 12 a.m. 5HT and blood glucose were determined as described previously [7, 8, 15].

Statistical analysis was made using Student's *t*-test.

4. Results

4.1. *Effects of IL-1 and TNFα*

Before experiments, the lethalities of the recombinant preparations of cytokines were tested. Three groups of mice (5/group) were injected intraperitoneally with 100 μg/kg of IL-1α or IL-1β and 1 mg/kg of TNFα. None of these preparations showed lethality for 24 h at the doses used.

As shown in Fig. 1, both IL-1 and TNFα induced not only the accumulation of 5HT in the liver but also hypoglycaemia in a dose-dependent manner. The potencies of IL-1α and IL-1β were very similar to each other. They were significantly effective ($P<0.01$ vs. control) at a dose as little as 0.1 μg/kg (2.5 ng/mouse) and produced a maximum effect at 10 μg/kg. On the other hand, a large dose

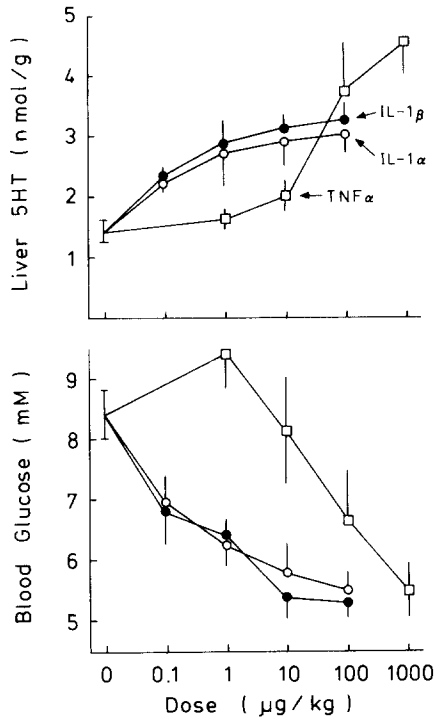

Fig. 1. Dose-dependent induction of 5HT accumulation in the liver and hypoglycaemia by IL-1 and TNFα. Mice were killed 4 h after i.p. injection of various doses of IL-1α, IL-1β and TNFα. Each value is the mean ± S.D. of 4–8 mice.

(100 μg/kg) of TNFα was required to produce a significant effect ($P<0.01$). It is noticeable that the accumulation of 5HT and the hypoglycaemic response induced by these cytokines corresponded well with each other with regard to dose dependency.

Although these responses are highly sensitive to LPS [8], the injection of 1 ng/kg of LPS (an amount more than 10 times higher than that contained in 1 mg of the cytokine preparations: see Materials and Methods) did not induce these responses, indicating that the effects of the cytokine preparations were not due to contaminating LPS.

It is also noticeable that the increase in 5HT was specific in the liver, because IL-1 (50 μg/kg, i.p.) and TNFα (500 μg/kg, i.p.), like LPS [7], produced no such marked elevation of 5HT in other tissues such as brain, lung, kidney and intestine (data not shown).

The accumulation of 5HT in the liver and the decline in blood glucose occurred simultaneously after a lag time of about 1 h and reached maximum levels

within 4.5 h after the injection of IL-1β or TNFα (Fig. 2). The effects of IL-1β were transient, while those of TNFα were longer-lasting. Both the accumulation of 5HT and the hypoglycaemic response had a corresponding time course.

4.2. *Effects of inhibitors of 5HT synthesis and degradation and of antagonists of 5HT receptors on the actions of LPS and cytokines*

In my previous study, it was shown that α-monofluoromethyldopa, a potent irreversible inhibitor of aromatic amino acid decarboxylase, inhibits both the hypoglycaemia and the 5HT accumulation in the liver induced by 5HTP [6]. However, the agent (10 mg/kg, i.p.) injected 0.5 h before injections of IL-1α, TNFα or LPS (100 μg/kg each, i.p.) inhibited neither the 5HT increase nor the hypoglycaemia induced by these stimulators.

The liver is known to be rich in monoamine oxidase, which metabolises 5HT. Pargyline, a potent irreversible inhibitor of this enzyme, potentiates both the 5HT increase in the liver and the hypoglycaemic response induced by 5HTP [5]. However, this agent (100 mg/kg, i.p., injected 1 h before LPS injection) also failed to show any effect on 5HT accumulation in the liver or on hypoglycaemia induced by LPS (100 μg/kg, i.p.).

In addition, antagonists of 5HT receptors such as methysergide, cyproheptadine (5 mg/kg each, i.p.) and ketanserine (0.5 mg/kg, s.c.), each were injected immediately after LPS or IL-1α (100 μg/kg each, i.p.), were also ineffective in suppressing hypoglycaemia (data not shown).

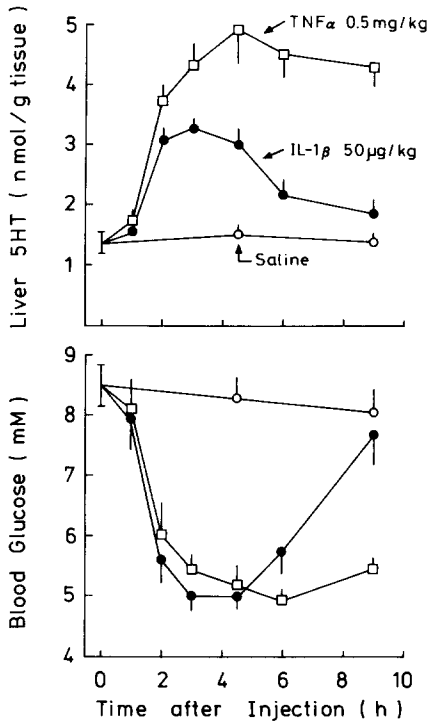

Fig. 2. Time course of 5HT accumulation in the liver and hypoglycaemia induced by IL-1 and TNFα. Mice were killed at indicated time intervals after i.p. injection of IL-1β (50 μg/kg) or TNFα (500 μg/kg). Each value is the mean ± S.D. of 4 mice.

4.3. *Comparison of hypoglycaemic responses induced by IL-1, TNFα and insulin*

The present study indicates that both IL-1 and TNFα are hypoglycaemic agents. Therefore, the effect of these cytokines was compared to that of insulin. Since insulin is usually injected subcutaneously, all the agents were given by that route (Fig. 3). The effect of TNFα was reduced in comparison to that induced by intraperitoneal injection (Fig. 1). On the other hand, IL-1β decreased blood glucose significantly ($p<0.01$) at 1 μg/kg, and this hypoglycaemic effect was moderate in its extent and almost constant at between 10 and 200 μg/kg. In contrast, insulin produced a profound hypoglycaemia at 50 μg/kg or more. Convulsions occurred in some mice at a dose of 200 μg/kg or more, and some of the mice died

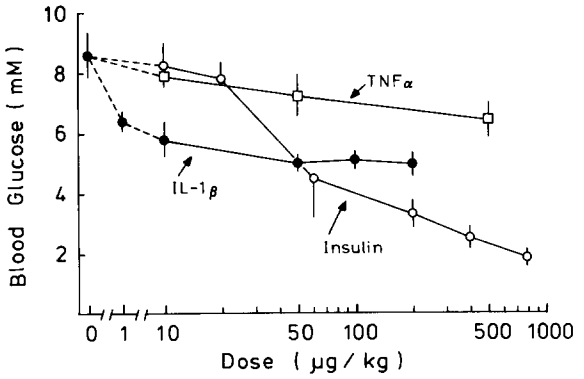

Fig. 3. Comparison of hypoglycaemic responses induced by IL-1β, TNFα and insulin. Mice were killed at 4 h after s.c. injections of various doses of IL-1β and TNFα. Mice were killed at 1.5 h after s.c. injections of various doses of insulin. Each value is the mean ± S.D. of 4–8 mice.

within 2 h after the injection of insulin. In addition, the hypoglycaemic response to insulin occurred rapidly; its maximum effect was seen within 2 h after injection.

5. Discussion

By using highly purified preparations of IL-1α and IL-1β, our previous finding [9], that IL-1-like factors can induce both hypoglycaemia and accumulation of 5HT in the liver, was confirmed. Recently, other investigators have also reported the hypoglycaemic action of IL-1 [16–18]. In addition to IL-1, the present study demonstrates that TNFα, at high doses, can also induce both responses. The two responses induced by IL-1 and TNFα corresponded well with each other in the terms of time course and dose dependency, as observed in LPS, various other inflammatory agents and IL-1-like factors [8, 9].

The major mechanisms underlying the LPS-induced hypoglycaemia have been suggested to be impaired hepatic gluconeogenesis [1–3, 19] and increased glucose utilisation in peripheral tissues [20]. Although these two events are characteristic of hyperinsulinaemia, there is disagreement as to the contribution of insulin [1, 17, 18, 21–23]. LPS decreases blood glucose even in diabetes induced by alloxan or streptozotocin [1, 22, 23]. IL-1 was also shown to have such an anti-diabetic effect on alloxan-induced diabetes and genetically diabetic, insulin-resistant mice [18].

As described in the Introduction, we proposed the hypothesis that 5HT accumulation in the liver may inhibit hepatic gluconeogenesis and contribute to produce hypoglycaemia [8, 9]. Therefore, it was expected that the inhibition of 5HT accumulation would prevent hypoglycaemia induced by LPS, IL-1 and TNFα as observed in the 5HTP-induced hypoglycaemia [5, 6]. However, neither 5HT accumulation nor hypoglycaemia, induced by LPS or these cytokines, was prevented by α-monofluoromethyldopa, a potent irreversible inhibitor of 5HT synthesis. This result indicates that the 5HT increase induced by LPS or the cytokines is not due to stimulation of 5HT synthesis. In addition, pargyline, an inhibitor of 5HT degradation, was also ineffective on the two responses induced by LPS, indicating that the 5HT increase in the liver is also not due to inhibition of its degradation.

My recent studies indicated that the increase in 5HT in the liver induced by LPS may be due to an accumulation of platelets in this organ (submitted for publication). Therefore, it may be expected that 5HT could be released from the accumulated platelets. However, various types of antagonist of 5HT receptors such as methysergide ($5HT_1$ and $5HT_2$ antagonist), ketanserine ($5HT_2$ antagonist) and cyproheptadine (antagonist to both histamine and 5HT) failed to suppress the hypoglycaemia induced by LPS and IL-1. Although these results do not necessarily contradict the idea that 5HT may be involved in the induction of hypoglycaemia induced by LPS and the cytokines, it is also possible that the accumulation of platelets in the liver by itself, or a local release of their constituents at the site, may derange liver function and result in an impaired gluconeogenesis.

Although the mechanism of the hypoglycaemic effect of LPS is still not clear, LPS is known to stimulate macrophages to produce IL-1 and TNFα [10, 11]. The present results, therefore, provide evidence that hypoglycaemia and accumulation of 5HT in the liver induced by LPS may be mediated by IL-1 and/or TNFα. The hypoglycaemic response to IL-1 is moderate in a wide range of its dose but it is induced by smaller amounts than with insulin. The hypoglycaemic response to IL-1 is markedly different from that of insulin in extent and time course, but differences between the mechanisms of these agents in their hypoglycaemic effects are not unexpected.

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