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Suppression and potentiation of 5-hydroxytryptophan-induced hypoglycaemia by α-monofluoromethyldopa: correlation with the accumulation of 5-hydroxytryptamine in the liver

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1 Experiments were done to examine whether the accumulation of 5-hydroxytryptamine (5-HT) in the liver is responsible for the hypoglycaemia induced in mice by 5-hydroxytryptophan (5-HTP) and lipopolysaccharides (LPS).

2 (±)-α-Monofluoromethyldopa (FMD), a potent irreversible inhibitor of aromatic amino acid decarboxylase, suppressed the 5-HTP-induced accumulation of 5-HT in the liver at a dose of 2 mg kg^{-1} or more, but potentiated the accumulation at a lower dose of 0.4 mg kg^{-1}. Corresponding to these effects, the hypoglycaemic response was prevented by the higher doses of FMD and potentiated by the lower dose. These contrasting effects of FMD were explicable by the amounts of 5-HTP entering the liver.

3 In contrast, FMD did not prevent either the hypoglycaemia or the accumulation of 5-HT in the liver induced by LPS.

4 These results further support the hypothesis that the accumulation of 5-HT in the liver is causally related to the hypoglycaemia induced by 5-HTP and indicate that the LPS-induced 5-HT accumulation in the liver is not derived from stimulation of 5-HT synthesis. It is still not clear whether the accumulation of 5-HT in the liver is involved in the hypoglycaemic response to LPS.

Introduction

On the basis of experiments using carbidopa, it was supposed that the accumulation of 5-hydroxytryptamine (5-HT) in the liver may be a cause of the hypoglycaemia induced by 5-hydroxytryptophan (5-HTP) (Endo, 1985a). Carbidopa is a competitive inhibitor of aromatic amino acid decarboxylase and can suppress the formation of 5-HT from 5-HTP. As shown in a previous study, however, a large dose of the agent (50 to 100 mg kg^{-1}) was required to suppress the formation of 5-HT in the liver from 5-HTP administered to mice (Endo, 1985a). Additionally, the agent has been shown not to be a specific inhibitor, because it also inhibits other enzymes in the tryptophan oxidative pathway at concentrations that are likely to be encountered *in vivo* after administration to experimental animals (Bender, 1980; Smith & Pogson, 1981). In fact, in spite of the protective effect of carbidopa on the tryptophan-induced hypoglycaemia, it has been suggested that 5-HT is unlikely to mediate the hypoglycaemic response to tryptophan (Smith *et al.*, 1980; Lloyd *et al.*, 1982a,b). Therefore, the possibility that metabolites of 5-HTP other than 5-HT may contribute to the development of 5-HTP-induced hypoglycaemia cannot be excluded. In the present study, I tested the effect of α-monofluoromethyldopa (FMD), a potent irreversible inhibitor of aromatic amino acid decarboxylase (Kollonitsch *et al.*, 1978; Jung *et al.*, 1979), on 5-HTP-induced hypoglycaemia.

Various mitogenic substances, including lipopolysaccharides (LPS, endotoxin), cause accumulation of 5-HT predominantly in the liver, and these substances can also produce hypoglycaemia (Endo, 1983; 1984; Endo *et al.*, 1985b). Development of the hypoglycaemic response and the accumulation of 5-HT in the liver correlate well with each other in terms of time course and dose-response. In the present study, therefore, the effects of carbidopa and FMD on these responses induced by LPS were also examined.

Methods

Determination of 5-hydroxytryptamine and blood glucose

Male ddI mice were obtained from the Mouse Centre of this university. The mice were kept under fixed conditions of light and dark (1900 h to 0700 h) and fed *ad libitum*. All injections were made to fed mice (6 to 7 weeks old, 24 to 27 g body weight) between 0900 h to 1200 h. Sampling of tissues and blood and determination of 5-HT and blood glucose were the same as described previously (Endo, 1984).

Assay of 5-hydroxytryptophan decarboxylase activity

Tissues were homogenized in 10 volumes of 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 mM pyridoxal-5'-phosphate by using an Ultra Turrax homogenizer (Janke & Kunkel Co., West Germany). The supernatant obtained by centrifugation (20,000 *g*, 20 min at 4°C) was used as the enzyme solution. The enzymatic reaction was carried out in 1 ml of 0.04 M sodium phosphate buffer (pH 7.0) containing pyridoxal-5'-phosphate (0.04 mM), dithiothreitol (0.5 mM), pargyline (1 mM), 5-HTP (1 mM) and the enzyme solution (0.05 or 0.1 ml). After incubation at 37°C for 1 h, the reaction was terminated by the addition of 2 ml of 0.4 M $HClO_4$ containing 2 mM EDTA 2Na and 0.1% cysteine HCl. The reaction was linear during the incubation period. The 5-HT generated in the reaction mixture was determined as described previously (Tadano *et al.*, 1980).

Chemicals

5-Hydroxy-L-tryptophan (5-HTP) was purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). (±)-α-Monofluoromethyldopa (FMD) was a gift from Merrel Dow Research Institute (Strasburg, France). A lipopolysaccharide (LPS) derived from *Escherichia coli* 055:B5, prepared by the Boivin method, was obtained from Difco, Lab. (Detroit, MI U.S.A.). Other agents were from Wako Chemical Ind. (Osaka, Japan). Agents were dissolved in 0.9% w/v NaCl solution (saline) and injected into mice intraperitoneally (5-HTP and FMD) or intravenously through tail vein (LPS). In combined injections of these agents, the total volume was limited to less than 0.4 ml per mouse.

Results

Effects of α-fluoromethyldopa on 5-hydroxytryptophan decarboxylase activity

To determine appropriate doses of FMD, its effect on the decarboxylation of 5-HTP was examined as follows: various doses of FMD were injected into mice, tissues were removed 3 h later, and 5-HTP decarboxylase activity in the tissues was determined (Table 1). In this experiment, FMD did not show any significant effect on the level of blood glucose. The activity of the enzyme was highest in the liver, and its activity was inhibited almost completely by 2 mg kg^{-1} FMD. On the other hand, the activity in the brain was less affected by the FMD treatment, i.e. about 80% of the activity remained after the treatment with 2 mg kg^{-1} FMD. In order to inhibit the formation of 5-HT selectively in the liver, therefore, this dose was used in the next experiment.

Effects of α-fluoromethyldopa on 5-hydroxytryptophan-induced hypoglycaemia and on accumulation of 5-hydroxytryptamine

As shown in a previous study (Endo, 1985a), 250 mg kg^{-1} 5-HTP produced a profound hypoglycaemia in our mice without death. The treatment of the mice with 2 mg kg^{-1} FMD before the administration of 5-HTP prevented completely the hy-

Table 1 5-Hydroxytryptophan (5-HTP) decarboxylase activity in the tissues of mice treated with α-monofluoromethyldopa (FMD)

FMD (mg kg^{-1})	*5-HTP decarboxylase activity* (nmol h^{-1} g^{-1})				
	Brain	*Liver*	*Kidney*	*Intestine*	*Spleen*
0	435 ± 27 (100)	5544 ± 198 (100)	1200 ± 87 (100)	132 ± 27 (100)	21 ± 2 (100)
0.4	387 ± 24 (89)	669 ± 150* (12)	81 ± 21* (7)	22 ± 2* (16)	17 ± 4 (80)
2	354 ± 18*(81)	90 ± 18*(2)	22 ± 3* (2)	10 ± 4* (7)	11 ± 2* (52)
10	189 ± 21*(43)	<30	<8	6 ± 3 (4)	<3
50	33 ± 18*(8)	<30	<8	<3	<3

Mice were killed at 3 h after the injection of FMD, and 5-HTP decarboxylase activities were determined. Each value is the mean ± s.d. of 3 mice. Values in parentheses are % activity. Significantly different from control, $*P < 0.05$ (Student's *t* test).

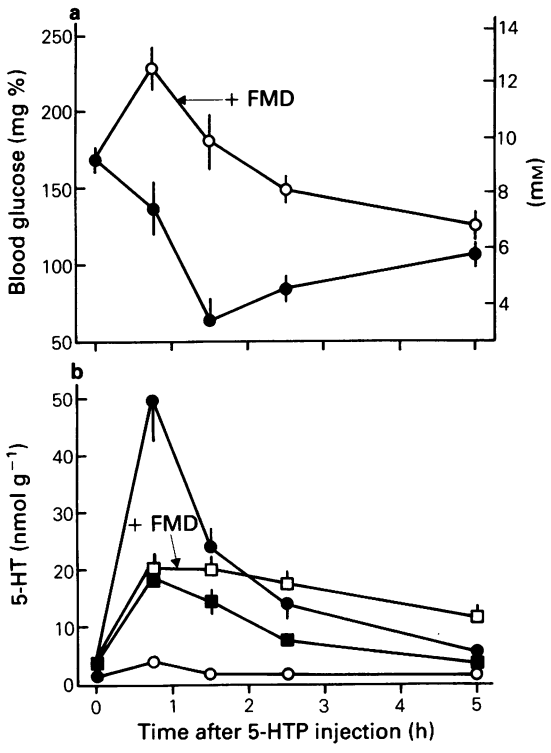

Figure 1 Effects of α-monofluoromethyldopa (FMD) on (a) the hypoglycaemic response to 5-hydroxytryptophan (5-HTP) and (b) on the accumulation of 5-hydroxytryptamine (5-HT) in the liver and brain. FMD (2 mg kg^{-1}) was injected into mice 1.5 h before the injection of 5-HTP (250 mg kg^{-1}). (a) (○) FMD-treated and (●) control (saline injected) mice. (b) Accumulation of 5-HT in the liver, in control (●) and FMD-treated (○) mice, and in the brain in control (■) and FMD-treated (□) mice. Each value is the mean from 4 mice; s.d. shown by vertical lines.

poglycaemia, or rather, produced hyperglycaemia at an early period after the 5-HTP injection (Figure 1). Control mice given saline or FMD alone did not show such a hyperglycaemic response (data not shown). Although the reason is not clear, a similar initial hyperglycaemic effect was also observed in the experiments using carbidopa (Endo, 1985a).

The accumulation of 5-HT in the liver was also entirely suppressed by the FMD treatment throughout the experimental period (Figure 1). In contrast, there was no inhibition of 5-HT formation in the brain, or rather, its elevated level of 5-HT was retained for a longer period, as has been observed in a previous study using carbidopa (Endo, 1985a).

Potentiation of 5-hydroxytryptophan-induced hypoglycaemia by α-fluoromethyldopa

This study produced the unexpected finding that a low dose of FMD sometimes killed the mice and potentiated the hypoglycaemic response to 5-HTP. To investigate this effect, mice were treated with various doses of FMD and given 5-HTP at a dose producing a mild degree of hypoglycaemia (150 mg kg^{-1}, Endo, 1985a). As shown in Figure 2, the treatment with

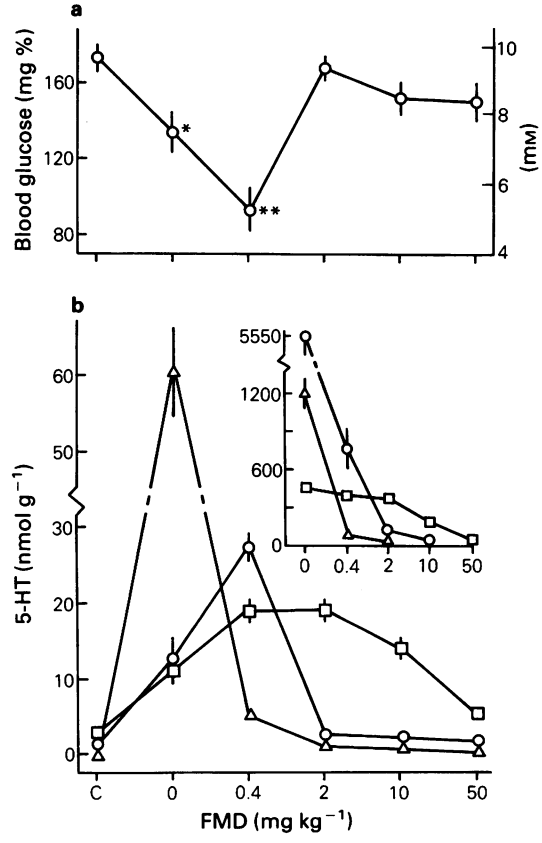

Figure 2 (a) The hypoglycaemic response to 5-hydroxytryptophan (5-HTP). (b) The accumulation of 5-hydroxytryptamine (5-HT) in the brain (□), liver (○) and kidney (△) of mice treated with various doses of α-monofluoromethyldopa (FMD). The FMD was injected into mice 1.5 h before an injection of 5-HTP (150 mg kg^{-1}), and the mice were killed 1.5 h after the 5-HTP injection. C, values from control (saline-injected) mice. Each value is the mean from 4 mice; s.d. shown by vertical lines. *$P < 0.05$ vs control, **$P < 0.05$ vs *. The insert in (b) shows the 5-HTP decarboxylase activities in the brain (□), liver (○) and kidney (△) presented in Table 1.

0.4 mg kg^{-1} FMD potentiated the hypoglycaemic response to 5-HTP, but FMD 2 mg kg^{-1} or more prevented completely the fall in blood glucose.

The levels of 5-HT in the liver, brain and kidney in this experiment are also shown in Figure 2 together with the data on 5-HTP decarboxylase presented in Table 1 for convenience. The accumulation of 5-HT in the liver was potentiated by 0.4 mg kg^{-1} FMD, but it was inhibited completely by 2 mg kg^{-1} or more of FMD. These effects corresponded well to those on blood glucose. In the brain, the elevation of 5-HT was potentiated by FMD in a range 0.4 to 10 mg kg^{-1}.

It is noticeable that in the absence of FMD, 5-HT formation in the kidney was higher than in other tissues, in spite of a lower 5-HTP decarboxylase activity than that in the liver. Both 5-HT formation and 5-HTP decarboxylase activity in the kidney was inhibited completely by 0.4 mg kg^{-1} FMD.

Ineffectiveness of α-fluoromethyldopa on lipopolysaccharide-induced hypoglycaemia and on 5-hydroxytryptamine accumulation in the liver

LPS produces an accumulation of 5-HT in the liver but not in the brain (Endo, 1983). After injection of LPS into mice, the 5-HT level in the liver was elevated within 2 h and reached its maximum level within 3 to 5 h. Corresponding to the 5-HT increase, blood glucose declined (Endo, 1984). However, treatment of mice with FMD, as well as with carbidopa, failed to prevent either the 5-HT increase or the hypoglycaemia induced by LPS, i.e. about a 5 fold increase in 5-HT and a 45% decline in blood glucose at 4.5 h after LPS injection (0.5 mg kg^{-1}) were not affected by treatment with FMD (10, 20 and 40 mg kg^{-1}, 0.5 h before LPS) or carbidopa (90 mg kg^{-1}, 0.5 h after LPS).

Discussion

In previous experiments using carbidopa, a competitive inhibitor of aromatic amino acid decarboxylase, the hypoglycaemic response to 5-HTP corresponded well to the accumulation of 5-HT in the liver but not in the brain (Endo, 1985a). In the present study, the suppressive effects of FMD, a potent irreversible inhibitor of the enzyme, on the accumulation of 5-HT and hypoglycaemia were clearly shown and were essentially the same as those of carbidopa. That is, a close correlation was again observed between the accumulation of 5-HT in the liver and the hypoglycaemic response to 5-HTP.

Additionally and interestingly, a low dose of FMD (0.4 mg kg^{-1}) potentiated both the accumulation of 5-HT in the liver and the hypoglycaemic response to 5-HTP (Figure 2). These effects of FMD can be explained as follows. In the absence of FMD, the kidney was the most active organ for the formation of 5-HT from the 5-HTP administered, in spite of there being lower 5-HTP decarboxylase activity (1200 nmol h^{-1} g^{-1}) in this organ than in the liver (5500 nmol h^{-1} g^{-1}). The low dose of FMD (0.4 mg kg^{-1}) inhibited almost completely the activity in the kidney (only 81 nmol h^{-1} g^{-1} remained) and inhibited entirely the formation of 5-HT in this organ. This inhibitory effect in the kidney may result in an increased amount of 5-HTP entering the liver (and also the brain) which retains a high level of decarboxylase activity (670 nmol h^{-1} g^{-1}) leading to an accelerated production of 5-HT. Therefore, these observations also support the idea that the accumulation of 5-HT in the liver is involved in the hypoglycaemic response to 5-HTP.

It has been suggested that various metabolites of tryptophan, including 5-HT, inhibit hepatic gluconeogenesis at the step catalysed by phosphoenolpyruvate carboxykinase (Smith *et al.*, 1979). In addition, 3-mercaptopicolinic acid, an analogue of quinolinate, has been shown to be a potent inhibitor of the enzyme and to produce hypoglycaemia (DiTullio *et al.*, 1974; Jomain-Baum *et al.*, 1976). Therefore, it remains to be clarified whether the active component of 5-HTP metabolites capable of inducing hypoglycaemia is 5-HT itself or metabolites of 5-HT and/or both. In the case of the metabolites, products from the monoamine oxidase pathway seem to be unlikely candidates, because monoamine oxidase inhibitors markedly potentiate the hypoglycaemic response to 5-HTP (Endo, 1985a).

As described in the Introduction, LPS causes an accumulation of 5-HT in the liver and produces hypoglycaemia. In addition, it has been suggested that a major mechanism underlying LPS-induced hypoglycaemia may be an impairment of hepatic gluconeogenesis (McCallum & Berry, 1973; Filkins & Cornell, 1974). Therefore, it is expected that the accumulation of 5-HT in the liver may be causally related to the hypoglycaemic response to LPS as in the case of 5-HTP. However, neither the accumulation of 5-HT nor the hypoglycaemic response to LPS was prevented by the treatment with FMD or carbidopa. These results indicate that the LPS-induced 5-HT accumulation in the liver is not due to the stimulation of 5-HT synthesis via the decarboxylase of 5-HTP. Results from preliminary experiments using mice treated with reserpine, which depletes amines from platelets and the nervous system, have suggested that the accumulation of 5-HT may be derived from the transfer of 5-HT and/or platelets into the liver from other sites through the circulation. The 5-HT accumulation induced by LPS was markedly diminished in the mice treated with reserpine (2 days before LPS), even though reserpine itself has been shown to be hypoglycaemic (Lernmark,

1971). The causal relationship between the accumulation of 5-HT in the liver and the hypoglycaemic response to LPS is still not clear.

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