Br. J. Pharmac. (1974), **50**, 575-580

THE HYPOGLYCAEMIC EFFECT OF 5-HYDROXYTRYPTOPHAN

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1 In nialamide-treated mice, L-5-hydroxytryptophan produced a dose-dependent hypoglycaemic response which was independent of whether the animals had been fed or fasted.

2 This response was accompanied by a head-twitching response which was also dose-dependent.

3 The hypoglycaemic response was not accompanied by an elevation in plasma immunoreactive insulin levels and was fully manifest in alloxan diabetic animals.

4 5-Hydroxytryptophan did not increase the *in vitro* glucose uptake of adipose tissue or skeletal muscle, either on incubation of the tissues in contact with the drug or on incubation of tissues removed from mice injected with the drug.

5 Both the hypoglycaemic and head-twitching responses were prevented by pretreatment with methysergide or cyproheptadine and augmented by mepyramine treatment.

Introduction

A possible role of 5-hydroxytryptamine (5-HT) in the regulation of insulin secretion was investigated by several authors (Feldman & Lebovitz, 1970; Lundquist, Ekholm & Ericson, 1971). Lundquist *et al.* (1971) showed that the precursor of 5-HT, 5-hydroxytryptophan (5-HTP), apart from inhibiting insulin secretion under certain conditions, produced hypoglycaemia in mice pretreated with a monoamine oxidase inhibitor (MAOI). He tentatively suggested that this response was mediated through stimulation of peripheral glucose utilization by 5-HT formed intracellularly from 5-HTP. In this paper, results are presented to provide additional information concerning this hypoglycaemic response.

Methods

All experiments were done on male albino mice weighing 30-35 g. The mice referred to as 'fasted' were deprived of food for 18-20 h before use but allowed free access to water.

Blood sampling

Blood was obtained from the femoral vein under light ether anaesthesia, within 30 s of onset of anaesthesia. One sample only was removed from each mouse.

Drug administration

Nialamide (80 mg/kg) was given intraperitoneally 20 h and 2 h before blood sampling. 5-HTP was injected into a tail vein 1 h before blood sampling. Methysergide, cyproheptadine and mepyramine were injected subcutaneously in the scruff of the neck.

Glucose uptake by skeletal muscle

Intact muscle tissue (rectus abdominis) was removed from mice killed by cervical dislocation and incubated in Krebs bicarbonate buffer in 2 ml conical flasks sealed with Parafilm. The flasks were gassed with 95% O_2 : 5% CO_2 and placed in a shaking water bath at 37° C for 60 minutes. Glucose uptake (mg/g wet weight) was determined from the disappearance of glucose from the medium during incubation, relative to control flasks containing no tissue.

Glucose uptake by adipose tissue

Epididymal fat pads were removed and two fat pads per incubation flask were treated in a similar way to muscle, but incubated for 2 hours.

Glucose determination

Plasma glucose was determined in 10 μl plasma by a glucose oxidase method using a Beckman RIIC

Glucose Analyser. In a few experiments whole blood glucose was determined by a microcolorimetric copper reduction technique (Haslewood & Strookman, 1939). The latter method was always employed for the determination of glucose in incubation media. None of the drugs or drug combinations employed interfered with the determination of glucose in aqueous solution by either method. The drug concentrations used in these 'recovery' experiments were those likely to be encountered in the plasma following the distribution in the extracellular fluid of the largest dose administered.

Plasma immunoreactive insulin (IRI) determination

Plasma IRI was determined in 50 μl undiluted plasma by the double antibody method of Hales & Randle (1963) as modified by the Radiochemical Centre, Amersham. Human insulin was used as a reference standard. This method is valid for mouse plasma (Foy & Furman, 1971). 5-HTP did not influence the recovery of human insulin from protein buffer solution and it was assumed that the recovery of mouse insulin from plasma would be similarly unaffected.

Alloxan diabetes

Alloxan diabetes was produced by injection of alloxan (80 mg/kg i.v.) 3 days before use. Only mice exhibiting glycosuria (detected with Clinistix; Ames) were selected for experiments.

Drugs used

The following drugs were used: nialamide (Pfizer); L-5,hydroxytryptophan monohydrate (Grade B Sigma); 5-hydroxytryptamine creatinine sulphate (British Drug Houses); methysergide maleate (Sandoz); cyproheptadine hydrochloride (Merck, Sharp & Dohme); mepyramine maleate (May & Baker); alloxan monohydrate (British Drug Houses). Doses are expressed in terms of the active molecule.

All drugs were dissolved in 0.9% w/v NaCl solution except methysergide maleate (dissolved in water). Nialamide was solubilized with drops of 1 N HCl. Control solutions consisted of the appropriate solvent. The experimental design was similar to that used by Foy & Furman (1973).

Statistical significance was determined by Student's t test, significance being accepted where $P < 0.05$.

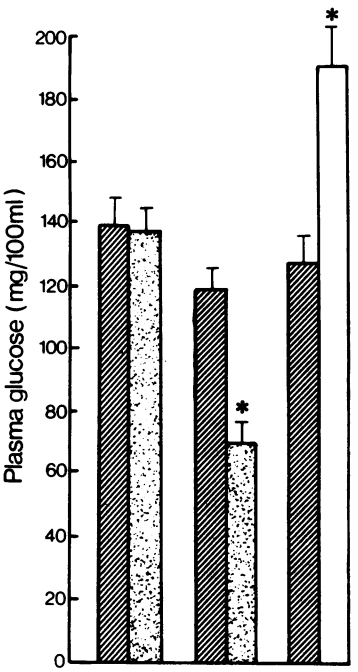

Fig. 1 The effect of the administration of 5-hydroxytryptophan (5-HTP; 60 mg/kg i.v. 1 h before blood sampling; stippled columns) or 5-hydroxytryptamine (5-HT; 50 mg/kg i.v. 1 h before blood sampling; open column) on the plasma glucose concentration of fed mice. Four groups (middle and right hand pairs of columns) of mice received in addition nialamide (80 mg/kg i.p.) at 20 h and 2 h before blood sampling. Each column represents the mean (±s.e.) of 10 observations. * Indicates a statistically significant difference between drug-treated mice and controls: $P < 0.05$. Hatched columns, control.

Results

Plasma glucose

In normal, fed mice, 5-HTP (up to 60 mg/kg i.v.) had no effect on plasma glucose. However, if the mice were pretreated with nialamide (80 mg/kg, 20 h and 2 h before blood sampling) 5-HTP produced a significant hypoglycaemic response at 1 h after injection (Figure 1). Under the same conditions, 5-HT (50 mg/kg) produced a significant hyperglycaemic response (Figure 1).

Figure 2 shows the plasma glucose response to various doses of 5-HTP in nialamide-treated fasted mice. An increasing hypoglycaemic effect was obtained with increasing doses of 5-HTP between 4 and 60 mg/kg. 5-HT (50 mg/kg) was without effect on plasma glucose in fasted mice. Similar results were obtained when whole blood glucose

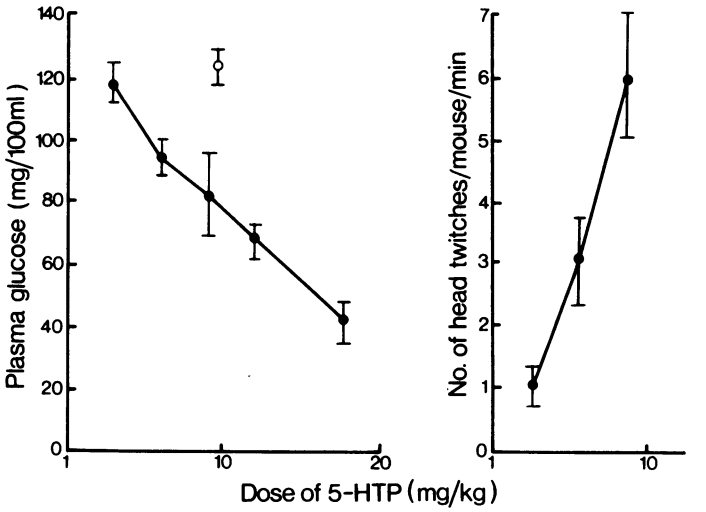

Fig. 2 Relation between various doses of 5-hydroxytryptophan (5-HTP) and the degree of hypoglycaemia or the number of head twitches observed in fasted, nialamide-treated mice (nialamide, 80 mg/kg administered i.p. 20 h and 2 h before blood sampling or measurement of head twitches). Plasma glucose was determined 1 h after the injection of 5-HTP and head-twitches were counted 30 min after injection. (o) Control. Each point represents the mean (±s.e.) of 10 observations.

was determined. Glucose was not detectable (with Clinistix) in the urine of any non-diabetic mice receiving 5-HTP. Although the hypoglycaemic response to 5-HTP was always obtainable, quantitative variations in the response were encountered between experiments performed on different days. Comparisons are therefore only made between experiments performed on the same day and at the same time of the day.

Central nervous system effects of 5-hydroxytryptophan

Injection of 5-HTP in nialamide-treated mice, but not in normal mice, produced side-to-side head movements, head twitching, tremor and, very rarely, clonic convulsions. Head twitching was observed with the lowest dose employed (2 mg/kg). This started within 5 min of injection at a time when the blood glucose was still unaltered. Tremor was seen with doses of 8 mg/kg and above, and convulsions only with the largest dose. Figure 2 shows the relationship between the dose of 5-HTP injected and the number of head twitches observed per minute. With doses of 5-HTP of 8 mg/kg and above, head twitching could not be accurately measured because of the marked tremor present. Intravenously injected 5-HT (50 mg/kg) produced none of the above effects.

Plasma insulin

Plasma IRI concentrations in fasted nialamide-treated mice were not increased but rather decreased by 5-HTP (8 mg/kg i.v.) 5 or 30 min after the injection (Table 1).

Table 1 Effect of 5-hydroxytryptophan (5-HTP) on plasma immunoreactive insulin (IRI) concentrations and plasma glucose concentrations of nialamide pretreated mice

	Plasma glucose		*Plasma IRI*	
	5 min after injection	*30 min after injection*	*5 min after injection*	*30 min after injection*
Control ($n = 6$)	137 ± 5	133 ± 5 $P < 0.05$	6 ± 1	11 ± 2
5-HTP (8 mg/kg i.v.) ($n = 6$)	149 ± 11	86 ± 3	3 ± 1	5 ± 1

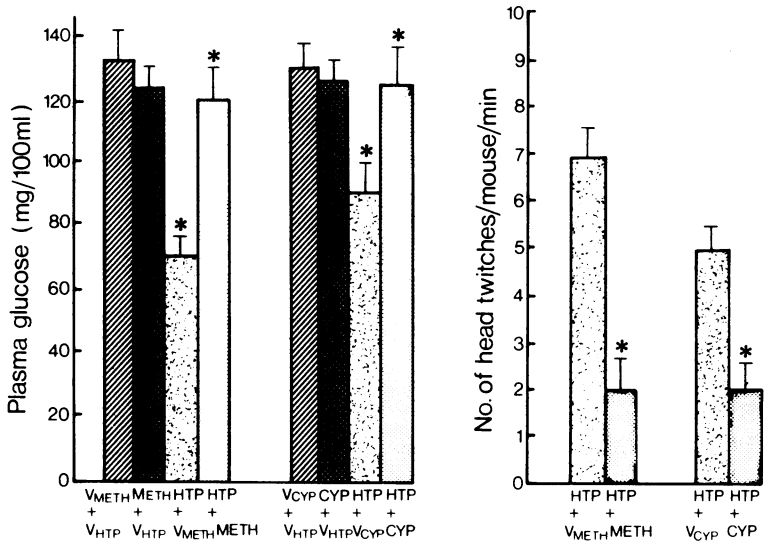

Fig. 3 The effect of methysergide (METH) or cyproheptadine (CYP) on the hypoglycaemia and head-twitching produced by 5-hydroxytryptophan (HTP) in nialamide-treated, fasted mice. Nialamide 80 mg/kg was administered intraperitoneally 20 h and 2 h before blood sampling. Methysergide, 0.1 mg/kg or cyproheptadine 0.1 mg/kg, was administered subcutaneously 90 min before blood sampling. 5-HTP, 4 mg/kg, was injected intravenously 1 h before blood sampling. V_{METH}, V_{CYP} and V_{HTP} refer to the appropriate control vehicles administered by the appropriate routes. Each column represents the mean (±s.e.) of 10 observations. * Indicates a statistically significant difference between drug and appropriate control treatment ($P < 0.05$).

Table 2 Effect of 5-hydroxytryptophan (5-HTP) on the *in vitro* glucose uptake by the rectus abdominis muscle and epididymal adipose tissue

Treatment	*No. of observations*	*Glucose uptake (mg/g wet weight)* *Muscle*	*Adipose tissue*
(a) Incubation of tissue from nialamide-treated mice with (5-HTP)			
Control	5	3.3 ± 0.4	2.2 ± 0.2
5-HTP (80 μg/ml)	5	3.1 ± 0.3	2.0 ± 0.3
Control	6	4.2 ± 0.4	2.8 ± 0.3
5-HTP (300 μg/ml)	6	3.7 ± 0.3	2.6 ± 0.3
(b) Incubation of tissues from nialamide-treated mice receiving 5-HTP or control solution			
Control	6	2.9 ± 0.3	2.6 ± 0.3
5-HTP (16 mg/kg i.v.)	6	2.5 ± 0.3	2.4 ± 0.2
Control	6	3.5 ± 0.4	1.9 ± 0.2
5-HTP (60 mg/kg i.v.)	6	3.9 ± 0.4	2.3 ± 0.3

Alloxan diabetic mice

In alloxan diabetic, nialamide-treated mice, 5-HTP (60 mg/kg i.v.) produced a significant hypoglycaemic response (blood glucose of control mice: 414 ± 33 mg/100 ml; blood glucose of 5-HTP-treated mice: 214 ± 27 mg/100 ml, $P < 0.001$).

Glucose uptake by skeletal muscle or adipose tissue

The addition of 5-HTP (80 μg/ml; 3.6×10^{-4} M or 300 μg/ml; 1.35×10^{-3} M) to the incubation medium did not affect the *in vitro* glucose uptake by the rectus abdominis muscle or epididymal adipose tissue removed from nialamide-treated mice (Table 1). Furthermore, the glucose uptake of muscle and adipose tissue removed from nialamide-treated mice injected with 5-HTP (16 or 60 mg/kg i.v.) 30 min before death was not significantly different from that of tissues removed from nialamide-treated mice receiving saline injections (Table 2).

Modification of 5-hydroxytryptophan-induced hypoglycaemia by drugs

The hypoglycaemia induced by 5-HTP (4 mg/kg i.v.) was completely prevented by pretreatment of the mice with methysergide or cyproheptadine (each 0.1 mg/kg s.c.) 30 min before the 5-HTP injection. Under these conditions the antagonists themselves were without effect on the plasma glucose (Figure 3). When thus administered, methysergide and cyproheptadine significantly reduced the head-twitching response to 5-HTP (4 mg/kg i.v.) measured at 30 min after 5-HTP injection (Figure 3).

Mepyramine (16 or 35 mg/kg s.c.) administered to nialamide-treated mice 16 or 35 mg/kg produced a significant hypoglycaemic response (Fig. 4) and also produced a head-twitching-tremor syndrome similar to that produced by 5-HTP. An augmentation of the effect of 5-HTP by mepyramine in nialamide-treated mice is suggested by the fact that 5-HTP (2 mg/kg) produced a significant hypoglycaemic response when administered with mepyramine (9 mg/kg). When the same dose of 5-HTP was administered alone, no hypoglycaemic response was obtained (Figure 4). A few experiments have also indicated that the head-twitching response to 5-HTP is augmented by mepyramine.

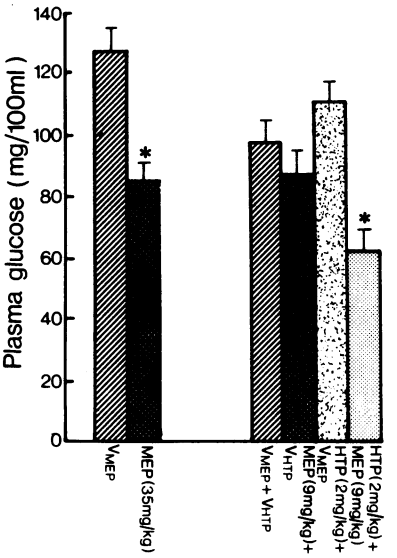

Fig. 4 The effect of mepyramine (MEP) 35 mg/kg subcutaneously, 90 min before blood sampling on the plasma glucose concentrations of nialamide-treated, fasted mice (nialamide 80 mg/kg i.p. 20 h + 2 h before blood sampling) and the interaction between mepyramine (9 mg/kg s.c.) and 5-hydroxytryptophan (5-HTP) (2 mg/kg i.v.) in producing hypoglycaemia in nialamide-treated fasted mice. Mepyramine was injected immediately after 5-HTP. V_{MEP} and V_{HTP} refer to the appropriate control vehicles administered by the appropriate routes. Each column represents the mean (±s.e.) of nine observations. * Indicates a statistically significant difference between drug and control treatments ($P < 0.05$).

Discussion

These results extend the observation of Lundquist *et al.* (1971) that 5-HTP produces a dose-dependent, hypoglycaemic effect in mice.

The production of this response only in MAOI-treated animals (present study and Lundquist *et al.*, 1971), its prevention by a decarboxylase inhibitor (Lundquist *et al.*, 1971) and by small doses of cyproheptadine and methysergide (present study), support the idea (Lundquist *et al.*, 1971) that the response is mediated by 5-HT formed from 5-HTP in the tissues.

The hypoglycaemic response occurred in fed and fasted animals, eliminating the possible involvement of an effect upon the gastrointestinal absorption of glucose. In the experiments of Lundquist *et al.* (1971), mice were allowed free access to food before and throughout experiments.

Hypoglycaemia may be produced by several mechanisms, including increased peripheral glucose utilization, increased glucose utilization by the CNS, decreased glucose release or synthesis by the liver, and inhibition of the proximal tubular reabsorptive mechanism for glucose. These effects may arise from direct actions of the drug on the

tissues concerned or, in some cases, from a drug-induced alteration in hormone secretion. An enhancement of insulin secretion, for example, would increase peripheral glucose utilization and decrease hepatic glucose production. That an enhanced insulin secretion is not involved in 5-HTP-induced hypoglycaemia is suggested by the production of the response in the alloxan diabetic animal and the failure of serum IRI to rise even at early times (5 and 30 min) after drug injection. This confirms the finding of Lundquist *et al.*, although these authors only measured IRI levels 1 h after 5-HTP injection thus leaving open the possibility of the responses being mediated by an early but transient increase in insulin secretion. The apparent lack of involvement of the β cells of the islets of Langerhans in the production of hypoglycaemia by 5-HTP, differentiates this effect from that produced by tryptophan (Mirsky, Perisutti & Jinks, 1957) which is only hypoglycaemic in the presence of functional β cell tissue.

The *in vitro* glucose uptake by muscle or adipose tissue was not influenced by a large concentration of 5-HTP, neither on incubation of the tissues from nialamide-treated mice with 5-HTP in the incubation medium nor on incubation of tissues removed from nialamide and 5-HTP-treated animals. This suggests that the hypoglycaemic response is not mediated by a direct effect on peripheral glucose utilization. However, it is possible that 5-HTP increased glucose uptake by muscle and adipose tissue *in vivo* or stimulated glucose utilization by some other structure such as the CNS.

The involvement of the CNS in the production of the response cannot be excluded in view of the closeness of the dose-response relationship of the 5-HTP-induced hypoglycaemia and the 5-HTP-induced head-twitching, an effect shown to be mediated through the CNS (Corne, Pickering & Warner, 1963). Both effects were readily antagonized by cyproheptadine and methysergide. Furthermore, intravenously administered 5-HT, which penetrates into the CNS only poorly, either had no effect on plasma glucose (fasting mice) or produced hyperglycaemia (fed mice) but never produced hypoglycaemia. These results could also indicate that the hypoglycaemic effect is mediated through the accumulation of 5-HT in some intracellular compartment in peripheral tissues in which a blood and cell barrier for 5-HT exists. The mechanism by which mepyramine produces head-twitching and hypoglycaemia in nialamide-treated mice and augments the effects of 5-HTP is not known. Mepyramine and certain other antihistamines have been shown to augment some central effects of MAOI believed to be mediated through increased 5-HT levels in the brain (Sinclair, 1972) and one such antihistamine (chlorpheniramine) has been shown to block the neuronal uptake of 5-HT (Carlsson & Lindquist, 1969).

The importance of the CNS in the production of hypoglycaemia by 5-HTP remains to be determined, as does the role of altered utilization or hepatic production of glucose.

I am grateful to Messrs J. Boswell and T. McCluskey for technical assistance to Pfizer Ltd, May & Baker Ltd, and Merck, Sharp and Dohme Ltd, for gifts of drugs.

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(Received November 3, 1973)