Tryptophan and the Control of Plasma Glucose Concentrations in the Rat

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(Received 3 May 1977)

1. Injection of L-tryptophan (750 mg/kg body wt.) led to pronounced hypoglycaemia in fed and 48 h-starved rats. 2. The hypoglycaemic effect is blocked by pretreatment with *p*-chlorophenylalanine, compound MK-486 [Carbidopa: L- α -(3,4-dihydroxybenzyl)- α -hydrazinopropionic acid monohydrate] or methysergide, and potentiated by pargyline. 3. 5-Hydroxy-L-tryptophan is more potent and induces a more rapid hypoglycaemia than does tryptophan. Other tryptophan metabolites were not associated with hypoglycaemia. 4. Adrenalectomy increases, and acute experimental diabetes strongly decreases, the sensitivity of rats to tryptophan induction of hypoglycaemia. Diabetic animals were also insensitive to 5-hydroxytryptophan. 5. Metabolite concentration changes in the livers from tryptophan-treated 48 h-starved and diabetic animals were consistent with a rapid inhibition of gluconeogenesis. This did not correlate with the hypoglycaemic response. 6. Tryptophan treatment was associated with a significant increase in the plasma [β -hydroxybutyrate]/[acetoacetate] ratio; there were no changes in the plasma concentrations of urea, triacylglycerol, non-esterified fatty acids and glycerol. 7. These observations suggest that the hypoglycaemic action of tryptophan is mediated through formation of intracellular 5-hydroxytryptamine, and is unrelated to the inhibition of gluconeogenesis. It is unlikely that this increased synthesis of 5-hydroxytryptamine involves directly either the adrenal glands or the central nervous system.

Gullino *et al.* (1955) first showed that administration of tryptophan to intact rats resulted in hypoglycaemia. This has been generally confirmed in several other studies (Mirsky *et al.*, 1956; Gagliardino *et al.*, 1971; McDaniel *et al.*, 1973), although there are considerable variations between individual reports, particularly with respect to the time-course and intensity of the hypoglycaemic response, and in the conclusions about the mechanism of this effect.

It has been accepted that tryptophan itself is unlikely to be the direct cause of hypoglycaemia. Mirsky *et al.* (1956, 1957) claimed that several indolic and other metabolites could also elicit decreases in blood glucose concentrations, and ascribed these actions to alterations in circulating insulin availability. In contrast, the demonstration by Lardy and his group (Ray *et al.*, 1966; Veneziale *et al.*, 1967) that glucose synthesis in the isolated perfused liver is inhibited by tryptophan and metabolites derived through the 'kynurenine' pathway (Scheme 1) stimulated the suggestion that hypoglycaemia in the whole animal may be caused by impaired gluconeogenesis, this impairment in turn arising from increases in hepatic quinolinate concentrations (Ray *et al.*, 1966; McDaniel *et al.*, 1973). This hypothesis is supported by observations that quinolinate administration *in vivo* produces changes in hepatic

metabolites similar to those seen after tryptophan treatment (Williamson *et al.*, 1971; Spydevold *et al.*, 1974), although Mirsky *et al.* (1957) had previously found that quinolinate did not affect blood glucose concentrations in intact rats.

In a study of the role of intracellular monoamines in the regulation of insulin secretion in the mouse, Lundquist *et al.* (1971) found that administration of low doses of 5-hydroxytryptophan to fed mice pretreated with a monoamine oxidase inhibitor elicited a profound hypoglycaemia. This effect is abolished by inhibitors of aromatic L-amino acid decarboxylase (Scheme 1) and by antagonists of 5-hydroxytryptamine action (Furman, 1974). This suggests that intracellular 5-hydroxytryptamine may be the active agent in promoting hypoglycaemia under these conditions. The effect of tryptophan itself, however, was differentiated from that of 5-hydroxytryptophan in that only the latter compound was found to be effective in the alloxan-diabetic animal (Furman, 1974).

Carbohydrate metabolism and its control processes vary from one mammalian species to another (Hanson, 1974; Elliott & Pogson, 1977) and due care should be taken in extrapolating from the rat, or any other single species, to man. Nevertheless it is of interest that, in limited experiments, human volun-

Scheme 1. Pathways of tryptophan metabolism in the rat

Enzymes: (1) tryptophan 5-mono-oxygenase (EC 1.14.16.4); (2) aromatic L-amino acid decarboxylase (EC 4.1.1.28); (3) monoamine oxidase (EC 1.4.3.4); (4) tryptophan aminotransferase (EC 2.6.1.27); (5) tryptophan dioxygenase (EC 1.13.11.11). $\Rightarrow$. Inhibition by the agent named; MAOI, monoamine oxidase inhibitors (including pargyline); ClPhe, *p*-chlorophenylalanine.

teers exhibited responses to tryptophan basically similar to those in the rat (Ajdukiewicz *et al.*, 1968; Fürst *et al.*, 1971).

In the present paper we report a systematic investigation of the mechanism of tryptophan-induced hypoglycaemia in the rat and present a unifying hypothesis consistent with other studies. Some of these data have been presented in preliminary form elsewhere (Smith & Pogson, 1976).

Materials and Methods

Animals

All rats (males, 200–250 g) were of the Sprague-Dawley CSE/ASH strain (Scientific Products Farm, Ash, Canterbury, Kent, U.K., now Charles River U.K. Ltd., Manston, Kent, U.K.) and were inbred in this Laboratory. Mice (males, 40–50 g), Porton ASH/XP strain, were from the same supplier. All animals were allowed food (no. 1 maintenance diet;

Cooper Nutritional Products, Witham, Essex, U.K.) and water *ad libitum* unless otherwise specified.

Adrenal glands were removed by means of a midline dorsal incision under diethyl ether anaesthesia. Adrenalectomized animals were given 1% (w/v) NaCl in place of water and were left for 5–8 days before use.

Diabetes was induced in two ways. In most experiments animals were injected intravenously with a solution of alloxan [60 mg (rats) or 80 mg (mice)/kg body wt.] in 0.9% (w/v) NaCl. These animals were used after 48 h. In some experiments, rats received a similar dose of streptozotocin in 0.9% NaCl; these were used after 3 days. In all cases diabetes was confirmed by glycosuria (Clinistix; Ames Co., Stoke Poges, Slough, Bucks., U.K.) and subsequent measurement of plasma glucose.

Experimental protocol

Animals were brought to the laboratory immediately before experiments, and were kept together in

the same place over the time course of each experiment.

L-Tryptophan for injection was prepared as follows. Sufficient solid to yield a final concentration of 40 mg/ml was added to 0.9% NaCl containing 0.1% (w/v) Tween 80. The suspension was heated until all solid had dissolved (near boiling), and was then allowed to cool at room temperature. The resulting suspension of microcrystalline tryptophan was stored at 4°C. L-Tryptophan was administered by intraperitoneal injection through a 16-gauge needle under light diethyl ether anaesthesia. Controls received a similar volume of 0.9% NaCl, containing 0.1% Tween 80, under the same conditions.

Tryptamine and 5-hydroxy-L-tryptophan were injected as solutions in 0.9% NaCl; 5-hydroxytryptamine (as the creatinine sulphate) was injected as a suspension in 0.9% NaCl, and quinolinate and indol-3-ylacetate as neutralized aqueous solutions.

Pargyline (75 mg/kg body wt.), compound MK-486 (68 mg/kg body wt.) and methysergide maleate (0.25 mg/kg body wt.) were given by intraperitoneal injection of solutions in 0.9% NaCl at stated times (see the Results and Discussion section) before injection of tryptophan or NaCl. *p*-Chlorophenylalanine was dissolved in NaOH and reprecipitated with HCl to give a microcrystalline suspension (31.3 mg/ml). This was injected intraperitoneally at a dosage of 313 mg/kg body wt.

All experiments were performed with appropriate simultaneous control animals.

Materials

L-Tryptophan, 5-hydroxy-L-tryptophan, 5-hydroxytryptamine creatinine sulphate, indol-3-ylacetic acid, tryptamine, Tween 80, Tris, glucose oxidase (type II) and peroxidase were from Sigma (London) Chemical Co., Kingston, Surrey, U.K. Quinolinic acid, alloxan and *p*-chlorophenylalanine were from Koch-Light Laboratories, Colnbrook, Bucks, U.K. Norit GSX and PCS solubilizer for insulin assays were from Norit Clydesdale Co., Glasgow, Scotland, U.K., and Hopkin and Williams, Chadwell Heath, Essex, U.K. respectively. ¹²⁵I-labelled insulin was obtained from The Radiochemical Centre, Amersham, Bucks, U.K. Rat insulin standard and guinea-pig anti-insulin serum were from Novo Industri AB, Bagsvaerd, Copenhagen, Denmark, and Wellcome Reagents, Beckenham, Kent, U.K. respectively.

Phosphoenolpyruvate (monocyclohexylammonium salt) was prepared as described by Clark & Kirby (1966). Pargyline (*N*-benzyl-*N*-methylprop-2-ynylamine) was from Abbott Laboratories, Queenborough, Kent, U.K. Compound MK-486 [Carbidopa; L- α -(3,4-dihydroxybenzyl)- α -hydrazinopropionic acid monohydrate] and methysergide [*N*-

1-(hydroxymethyl)propyl-1-methyl-(4)-lysergamide] maleate were kindly supplied by Merck, Sharp and Dohme Research Laboratories, Rahway, NJ, U.S.A., and Sandoz Products, Feltham, Middx, U.K., respectively. Streptozotocin was a gift from Dr. D. C. N. Earl, ICI Ltd., Alderley Park, Cheshire, U.K.

All other enzymes and biochemicals were from Boehringer Corp. (London), Lewes, Sussex, U.K. Other basic chemicals were of the purest grade available from standard suppliers.

Preparation of samples for plasma glucose assay

Routinely, blood samples (approx. 0.1 ml) were taken into heparinized Pasteur pipettes from the tail vein just before, and at intervals after, appropriate injections. For sampling, animals were lightly anaesthetized with diethyl ether and the tails warmed in water to ensure suitable blood flow. The validity of this method was checked in all conditions by comparison of glucose concentrations in tail-vein and heart samples at the final time point. When severe vasoconstriction was a side effect, as in pargyline-treated rats, samples were removed from the heart immediately after cervical dislocation.

Chilled samples were centrifuged at 12000g for 2 min in a Beckman microfuge. Plasma was removed and stored at -20°C for assay.

Preparation of blood samples for metabolite assays

Rats were killed by decapitation and blood was collected in heparinized plastic beakers. Samples (0.5 ml) of whole blood were immediately deproteinized by thorough mixing with 1.5 ml of ice-cold 2% (w/v) HClO₄. After centrifugation at 2000g for 5 min at 4°C, 1.2 ml portions of the supernatant were neutralized with 0.5 M-triethanolamine hydrochloride pH 7.4, containing 2 M-KOH. After further centrifugation as before at 4°C, the supernatants were decanted and assayed immediately for pyruvate, lactate, ketone bodies and glycerol.

Further portions of whole blood were centrifuged at 2000g for 5 min at 4°C. The resulting plasma fractions were stored at -20°C before assay.

Preparation of liver extracts

Rats were killed by cervical dislocation, and samples of liver were freeze-clamped as rapidly as possible (Wollenberger *et al.*, 1960). The frozen tissue was powdered, weighed and extracted in 5 ml of ice-cold 2% (w/v) HClO₄ with an Ultra-Turrax homogenizer (The Scientific Instrument Centre, London WC1X 9JA, U.K.). Precipitated protein was removed by centrifugation at 2000g for 5 min at 4°C; 4 ml portions of the supernatants were neutralized as described

above. Samples were assayed immediately for pyruvate, phosphoenolpyruvate and acetoacetate. The remainder of the extracts were stored at -20°C for other metabolite determinations.

Assay procedures

The following compounds were measured by standard procedures: lactate (Gutmann & Wahlefeld, 1974a), pyruvate and phosphoenolpyruvate (Czok & Lamprecht, 1974), hydroxybutyrate (Williamson & Mellanby, 1974), acetoacetate (Mellanby & Williamson, 1974), ATP (Jaworek *et al.*, 1974a), ADP and AMP (Jaworek *et al.*, 1974b), malate (Gutmann & Wahlefeld, 1974b), aspartate (Bergmeyer *et al.*, 1974b), glycerol (Garland & Randle, 1962), non-esterified fatty acid (Duncombe, 1964), triacylglycerol (Eggstein & Kreutz, 1966), urea (Fawcett & Scott, 1960).

Tryptophan was determined fluorimetrically by using a modification (Bloxam & Warren, 1974) of the method of Denckla & Dewey (1967). 'Bound' and 'free' tryptophan were separated (Lipsett *et al.*, 1973) by dialysis of plasma samples against Krebs-Henseleit bicarbonate (Krebs & Henseleit, 1932) gassed with O_2/CO_2 (19:1). Insulin was measured by radioimmunoassay by using Norit GSX as adsorbent (Albano *et al.*, 1972). Antibody-bound ^{125}I -labelled insulin was determined in supernatants after separation with charcoal, by β -liquid-scintillation counting in PCS solubilizer (^3H channel, Packard model 3375). The overall counting efficiency was better than 60%.

Glucose was routinely measured with glucose oxidase and peroxidase (Krebs *et al.*, 1963). Since many compounds, including 5-hydroxytryptamine, are potent inhibitors of colour development in this assay (Nelson & Huggins, 1974), plasma glucose concentrations were checked by a second method by using hexokinase and glucose 6-phosphate dehydrogenase (Bergmeyer *et al.*, 1974a). No difference was noted in any case between results derived by these two methods.

Results and Discussion

Effects of L-tryptophan and its metabolites on plasma glucose concentrations in the intact rat

The time course of changes in plasma glucose concentrations alter intraperitoneal injection of L-tryptophan is shown in Fig. 1. Fed animals differed from 48h-starved animals only in the degree of responsiveness. In both states, the time course was characterized by an initial hyperglycaemia, a return to control values and a sustained period of severe hypoglycaemia. Minimum plasma glucose concentra-

Fig. 1. Effect of tryptophan on plasma glucose concentrations in fed and 48 h-starved rats

Tryptophan (750 mg/kg body wt.) was administered intraperitoneally at 0h. Experimental procedures were as described in the Materials and Methods section. Results are means $\pm$ S.E.M. for four determinations. $\circ$, Fed control; $\bullet$, fed, plus tryptophan; $\square$, starved control; $\blacksquare$, starved, plus tryptophan. *P* (versus corresponding controls): * <0.05 ; ** <0.01 ; *** <0.001 .

tions were seen approx. 6 h after the administration of tryptophan; after this time the concentration returned slowly to control values.

There has been disagreement in the literature as to whether tryptophan is hypoglycaemic *in vivo* or not. Howard & Modlinger (1948) and Mann *et al.* (1949) were unable to detect any effect, but more recent studies record marked changes in blood glucose concentrations (Gullino *et al.*, 1955; Mirsky *et al.*, 1956, 1957; Gagliardino *et al.*, 1971; Bässler & Brinkrolf, 1971; McDaniel *et al.*, 1973). Even where changes have been observed, however, there have been marked variations, in terms of both time course and the extent of hypoglycaemia, even though similar experimental conditions have been established. Mirsky *et al.* (1957) and Gagliardino *et al.* (1971) differ as to the dose effectiveness of tryptophan, but agree that the onset of the hypoglycaemic response is more rapid than in our experiments. Gullino *et al.* (1955), however, have noted a transient hyperglycaemic phase.

The explanation for these wide differences remains elusive. We have very recently (P. Lloyd & C. I. Pogson, unpublished work) found that fresh batches of rats of the same strain are much less sensitive to tryptophan than are those used earlier (in the studies reported in the present paper). We have checked a number of parameters, including age (cf. Mirsky *et al.*, 1956), weight, diet and housing conditions without finding

Table 1. *Effects of p-chlorophenylalanine, compound MK-486 and methysergide on tryptophan-induced hypoglycaemia in 48 h-starved rats*

p-Chlorophenylalanine (313 mg/kg body wt.), compound MK-486 (68 mg/kg body wt.) and methysergide (0.25 mg/kg body wt.) were given intraperitoneally 36h, 30min and 90min respectively before administration of tryptophan (750 mg/kg body wt; zero time). Controls received similar volumes of vehicle throughout. Details of experimental procedures were as described in the Materials and Methods section. Results are means $\pm$ S.E.M. (four animals). NS, Not significant, $P > 0.05$, by Student's *t* test.

Plasma glucose concentration (mM)					<i>P</i>			
	Time (h)	Control (I)	Inhibitor alone (II)	Tryptophan (III)	Tryptophan plus inhibitor (IV)	I versus II	I versus III	II versus IV
<i>p</i> -Chlorophenylalanine	0	6.7 $\pm$ 0.4	7.1 $\pm$ 0.1	6.6 $\pm$ 0.5	7.3 $\pm$ 0.3	NS	NS	NS
	1.5	6.2 $\pm$ 0.7	7.6 $\pm$ 0.2	7.9 $\pm$ 0.4	7.1 $\pm$ 0.4	NS	NS	NS
	3	5.9 $\pm$ 0.4	7.7 $\pm$ 0.7	6.4 $\pm$ 0.7	5.8 $\pm$ 0.2	NS	NS	<0.05
	4.5	6.6 $\pm$ 0.8	7.8 $\pm$ 0.4	3.0 $\pm$ 0.3	5.9 $\pm$ 0.7	NS	<0.01	NS
	6	7.0 $\pm$ 0.5	7.1 $\pm$ 0.6	1.6 $\pm$ 0.6	6.0 $\pm$ 0.5	NS	<0.001	NS
	8	6.7 $\pm$ 0.6	6.8 $\pm$ 0.9	1.7 $\pm$ 0.3	6.3 $\pm$ 0.3	NS	<0.001	NS
MK-486	0	5.9 $\pm$ 0.5	6.4 $\pm$ 0.3	5.7 $\pm$ 0.4	6.7 $\pm$ 0.3	NS	NS	NS
	1.5	5.6 $\pm$ 0.6	4.5 $\pm$ 0.4	6.1 $\pm$ 0.4	6.3 $\pm$ 0.3	NS	NS	<0.01
	3	5.4 $\pm$ 0.6	4.8 $\pm$ 0.5	4.6 $\pm$ 0.7	6.8 $\pm$ 0.4	NS	NS	<0.05
	4.5	5.8 $\pm$ 0.7	6.7 $\pm$ 0.6	2.8 $\pm$ 0.3	5.9 $\pm$ 0.9	NS	<0.01	NS
	6	6.4 $\pm$ 0.5	6.4 $\pm$ 0.3	1.6 $\pm$ 0.4	4.1 $\pm$ 0.5	NS	<0.001	<0.001
	7.5	6.3 $\pm$ 0.7	7.4 $\pm$ 0.2	1.1 $\pm$ 0.6	4.4 $\pm$ 0.4	NS	<0.001	<0.001
Methysergide	0	6.3 $\pm$ 0.3	6.6 $\pm$ 0.2	6.0 $\pm$ 0.3	6.7 $\pm$ 0.2	NS	NS	NS
	2	5.8 $\pm$ 0.4	6.9 $\pm$ 0.4	7.4 $\pm$ 0.5	6.1 $\pm$ 0.2	NS	<0.05	NS
	4	6.7 $\pm$ 0.4	7.6 $\pm$ 0.6	3.9 $\pm$ 0.4	5.3 $\pm$ 0.5	NS	<0.01	<0.05
	6	6.3 $\pm$ 0.4	7.6 $\pm$ 0.9	1.5 $\pm$ 0.4	5.1 $\pm$ 0.4	NS	<0.001	<0.05

any factor responsible for this diminished responsiveness. We would emphasize, however, that when such animals are made more sensitive to tryptophan by adrenalectomy or treatment with appropriate drugs (see below), the responses observed are qualitatively similar to those recorded earlier (P. Lloyd & C. I. Pogson, unpublished work).

Pretreatment of animals with *p*-chlorophenylalanine, a long-term non-competitive specific inhibitor of tryptophan and phenylalanine hydroxylases (Koe & Weissman, 1966; Koe, 1971; Scheme 1), abolished the hypoglycaemic response to tryptophan (Table 1). The inhibitor alone had no effect on this parameter. Inhibition of the 'adjacent' enzyme, aromatic L-amino acid decarboxylase, by compound MK-486 (Scheme 1) had similar results, consistent with the interpretation that the hypoglycaemic response is dependent, in part at least, on conversion into 5-hydroxytryptamine or its further metabolites. That 5-hydroxytryptamine itself is probably the agent responsible *in vivo* is indicated by experiments with methysergide, a blocker of 5-hydroxytryptamine-receptor sites (Franchamps *et al.*, 1960; Table 1). Attempts to obtain a complete inhibition of the effect with higher doses of the drug were unsuccessful, since methysergide, in common with other 5-hydroxytryptamine antagonists, itself produces a stimulatory effect at inappropriate concentrations (Gyermek, 1966).

Table 2. *Effect of pargyline on tryptophan-induced hypoglycaemia in fed and 48 h-starved rats*

Pargyline (75 mg/kg body wt.) was given intraperitoneally 45 min before administration of tryptophan (750 mg/kg body wt; zero time). Controls received similar volumes of vehicle throughout. The procedure was as described in the Materials and Methods section; the initial blood samples were from the tail vein and those at 3.5 h were from the heart. Results are means $\pm$ S.E.M. (four animals). *P* (versus corresponding controls): * <0.05 , ** <0.01 , by Student's *t* test.

		Plasma glucose concentration (mM)	
Animals	Time (h)	Pargyline alone	Tryptophan $\pm$ pargyline
Fed	0	12.6 $\pm$ 1.3	13.7 $\pm$ 2.4
	3.5	13.2 $\pm$ 2.7	5.2 $\pm$ 0.7*
48 h-starved	0	6.6 $\pm$ 0.6	8.0 $\pm$ 0.3
	3.5	7.8 $\pm$ 0.7	1.0 $\pm$ 0.1**

The conclusion is strengthened by the results of experiments with pargyline, a non-hydrazine-based monoamine oxidase inhibitor (Jarvik, 1970; Scheme 1). By itself, pargyline is somewhat hyperglycaemic, an effect possibly attributable to an inhibition of insulin release (Aleyassine & Lee, 1971). The effect of tryptophan is, however, potentiated by pargyline, with most treated animals dying within 4 h (Table 2).

Table 3. *Effects of quinolinate, indol-3-ylacetate and tryptamine on plasma glucose concentrations in 48 h-starved rats* Quinolinate, indol-3-ylacetate (both 750 mg/kg body wt.) and tryptamine (50 mg/kg body wt.) were administered intraperitoneally. Experimental conditions were as described in the Materials and Methods section. Results are means $\pm$ S.E.M. (four animals). *P* (versus corresponding controls): ** <0.01 , *** <0.001 , by Student's *t* test.

Time (h)	Plasma glucose concentration (mm)					
	Control	Quinolinate	Control	Indol-3-ylacetate	Control	Tryptamine
0	5.1 $\pm$ 0.3	4.8 $\pm$ 0.2	4.5 $\pm$ 0.4	5.9 $\pm$ 0.2	6.1 $\pm$ 0.6	5.9 $\pm$ 0.4
1.5	4.9 $\pm$ 0.2	4.8 $\pm$ 0.2	5.2 $\pm$ 0.2	5.9 $\pm$ 0.6	5.2 $\pm$ 0.6	6.1 $\pm$ 0.2
3	5.1 $\pm$ 0.2	5.6 $\pm$ 0.4	5.0 $\pm$ 0.3	7.7 $\pm$ 0.6**	5.2 $\pm$ 0.6	6.1 $\pm$ 0.4
4.5	5.4 $\pm$ 0.3	6.1 $\pm$ 0.4	5.4 $\pm$ 0.4	8.2 $\pm$ 0.3**	5.7 $\pm$ 0.5	6.1 $\pm$ 0.2
6	6.4 $\pm$ 0.4	6.8 $\pm$ 0.3	5.9 $\pm$ 0.3	8.4 $\pm$ 0.2***	6.7 $\pm$ 0.7	7.4 $\pm$ 0.5
7.5	6.7 $\pm$ 0.3	7.6 $\pm$ 0.1	6.1 $\pm$ 0.3	7.8 $\pm$ 0.2**	6.3 $\pm$ 0.6	7.5 $\pm$ 0.5

Table 4. *Effect of 5-hydroxy-L-tryptophan on plasma glucose concentrations in pargyline-pretreated 48 h-starved rats*

Pargyline (75 mg/kg body wt.) was given intraperitoneally 45 min before administration of 5-hydroxytryptophan (20 mg/kg body wt.). Controls received similar volumes of vehicle throughout. Experimental conditions were as described in the Materials and Methods section; blood was taken from the heart after cervical dislocation in each case. Results are means $\pm$ S.E.M. (four animals). *P* (versus corresponding controls): ** <0.01 , by Student's *t* test.

Time (h)	Plasma glucose concentration (mm)	
	Pargyline alone	5-Hydroxytryptophan + pargyline
0	6.6 $\pm$ 0.6	—
1	7.8 $\pm$ 0.7	7.6 $\pm$ 0.3
2	7.3 $\pm$ 0.6	2.7 $\pm$ 0.6**

The accompanying behavioural disturbances and severe vasoconstriction are indications of the accumulation of 5-hydroxytryptamine (Douglas, 1970).

A number of tryptophan metabolites were tested for their ability to induce hypoglycaemia (Table 3). Quinolinate, an inhibitor of gluconeogenesis (Williamson *et al.*, 1971; Alvarez & Ray, 1974), had no effect, in agreement with the results of other workers (Mirsky *et al.*, 1957; Blackshear *et al.*, 1975). Nevertheless, inhibition of gluconeogenesis must not of necessity cause hypoglycaemia, if peripheral utilization of glucose is equally and simultaneously decreased. The effects of quinolinate on glucose uptake have not, however, been studied.

Indol-3-ylacetate, previously reported to be hypoglycaemic (Mirsky *et al.*, 1957), elicited in our hands a marked hyperglycaemia. Tryptamine, at low doses, induced very apparent behavioural changes, but did not influence plasma glucose concentrations,

even in the presence of pargyline (results not shown); higher doses of tryptamine were rapidly fatal.

Low doses (5 mg/kg body wt.) of 5-hydroxytryptamine itself, administered to pargyline-pretreated 48 h-starved rats, were strikingly hyperglycaemic; the plasma glucose concentration rose from 7.85 $\pm$ 0.35 mm at zero time to 12.1 $\pm$ 0.2 mm after 30 min, and to 13.7 $\pm$ 0.6 mm (means $\pm$ S.E.M. for four observations in each case) after 1½ h. The value remained above 12 mm for 4 h. This result was not surprising in view of the ability of exogenous 5-hydroxytryptamine to stimulate release of catecholamines (Douglas *et al.*, 1967) and block release of insulin (Porte, 1969; Woods & Porte, 1974).

We found that 5-hydroxytryptophan was an effective hypoglycaemic agent. The effects were manifest both earlier and at much lower doses (Table 4), and generally confirm previous observations (Lundquist *et al.*, 1971; Furman, 1974).

Effects of hormonal insufficiency on tryptophan-mediated hypoglycaemia

Bilateral adrenalectomy renders both the fed and 48 h-starved rat more sensitive to the actions of tryptophan (Table 5). Animals given either 100 mg or 200 mg of L-tryptophan/kg body wt. died within 5 h; the minimum effective dose is 20 mg/kg body wt. In contrast, intact 48 h-starved or fed rats do not even become hypoglycaemic when given 200 mg/kg body wt. The sensitivity of the adrenalectomized animal to tryptophan may be a consequence of the toxicity of the amino acid itself, or may result from derangement of tryptophan metabolism. The activity of tryptophan 2,3-dioxygenase, which catalyses the initial step of the 'kynurenine' pathway, is induced by glucocorticoid hormones, and is correspondingly decreased in adrenalectomized animals (Rosen & Milholland, 1963; S. A. Smith, unpublished work). Since this pathway constitutes a major route of

Table 5. *Effect of tryptophan on plasma glucose concentrations in fed and 18 h-starved adrenalectomized rats*
Experimental procedures were as described in the Materials and Methods section. Results are means $\pm$ S.E.M. (four animals). *P* (versus corresponding controls): ** <0.01 , *** <0.001 , by Student's *t* test.

Animal	Time (h)	Plasma glucose concentration (mm)		
		Control	Tryptophan (100mg/kg body wt.)	Tryptophan (200mg/kg body wt.)
Fed	0	7.2 $\pm$ 0.4	7.8 $\pm$ 0.3	6.9 $\pm$ 0.2
	1.5	7.0 $\pm$ 0.3	6.4 $\pm$ 0.2	6.3 $\pm$ 0.4
	3	7.1 $\pm$ 0.2	4.6 $\pm$ 0.6**	3.6 $\pm$ 0.9**
18 h-starved	0	4.2 $\pm$ 0.6	5.5 $\pm$ 0.3	4.6 $\pm$ 0.6
	1.5	4.0 $\pm$ 0.3	5.1 $\pm$ 0.6	3.1 $\pm$ 0.2
	3	3.9 $\pm$ 0.4	1.9 $\pm$ 0.2***	0.8 $\pm$ 0.2***

Table 6. *Effect of tryptophan on plasma glucose concentrations in fed and 18 h-starved alloxan-diabetic rats*
Fed animals received tryptophan (750mg/kg body wt.) at 0h and 6h; starved animals received the same dose at 0h only. Experimental conditions were as described in the Materials and Methods section. Results are means $\pm$ S.E.M. (four animals).

Time (h)	Plasma glucose concentration (mm)			
	Fed animals		18 h-starved animals	
	Control	+Tryptophan	Control	+Tryptophan
0	43.3 $\pm$ 2.4	49.1 $\pm$ 2.5	41.4 $\pm$ 3.2	49.3 $\pm$ 1.8
2	—	—	44.9 $\pm$ 4.1	47.6 $\pm$ 2.8
3	39.2 $\pm$ 2.6	47.2 $\pm$ 4.5	—	—
4	—	—	43.8 $\pm$ 5.1	43.9 $\pm$ 3.2
6	35.9 $\pm$ 2.0	45.4 $\pm$ 4.6	41.7 $\pm$ 2.6	40.9 $\pm$ 3.5
7.5	—	—	43.8 $\pm$ 3.8	40.6 $\pm$ 3.2
9	37.3 $\pm$ 2.5	37.3 $\pm$ 4.9	—	—
12	44.1 $\pm$ 1.3	36.8 $\pm$ 5.0	—	—

tryptophan metabolism (Young & Sourkes, 1976; Green *et al.*, 1976; S. A. Smith & C. I. Pogson, unpublished work), such inhibition may lead to increased flux through other pathways, including that of 5-hydroxytryptamine synthesis. An additional factor in these animals is the absence of any ability to counter hypoglycaemia by release of catecholamines (Goldfien *et al.*, 1958).

Acutely alloxan-diabetic rats were apparently insensitive to tryptophan (Table 6). There was no significant decrease in plasma glucose concentration even after 12h in fed diabetic animals. Animals rendered diabetic with streptozotocin exhibited similar insensitivity to tryptophan (results not shown). These findings confirm and extend those of Mirsky *et al.* (1957) and McDaniel *et al.* (1973), although it is clearly difficult to dismiss the possibility that tryptophan may elicit the same absolute changes in both normal and diabetic animals, and that these changes are masked by variations in the prevailing high plasma glucose values in the diabetic. Nevertheless, neither tryptophan plus pargyline nor 5-hydroxytryptophan plus pargyline, the two most effective treatments in

the intact animals, had any noticeable effect on plasma glucose values in alloxan-diabetic rats.

The observation that 5-hydroxytryptophan is unable to elicit hypoglycaemia in these experiments is at variance with two earlier studies (Lundquist *et al.*, 1971; Furman, 1974). Since these investigators used mice rather than rats, we have repeated this experiment with alloxan-diabetic mice (Table 7). Although control (non-diabetic) animals were clearly responsive, no effect could be demonstrated in diabetic mice. Although no simple explanation can be offered to reconcile these differences, it should be noted that small doses of 5-hydroxytryptophan (10mg/kg body wt.), sufficient to induce hypoglycaemia in the intact mouse, are ineffective in the diabetic animal (Lundquist *et al.*, 1971).

Changes in hepatic and blood metabolites after tryptophan administration

The time course of changes in several key hepatic metabolites in 48h-starved animals treated with

Table 7. *Effect of 5-hydroxytryptophan on plasma glucose concentrations in pargyline-pretreated normal and alloxan-diabetic mice*

All animals received pargyline (50 mg/kg body wt.) intraperitoneally 60 min before 5-hydroxytryptophan (55 mg/kg body wt.; zero time). Experimental details were as described in the Materials and Methods section. Results are means $\pm$ S.E.M. (four animals). *P* (versus corresponding control): † <0.02 , by Student's *t* test.

Time (h)	Plasma glucose concentration (mM)			
	Normal fed		Alloxan-diabetic	
	Pargyline alone	5-Hydroxytryptophan + pargyline	Pargyline alone	5-Hydroxytryptophan + pargyline
0	11.3 $\pm$ 0.6	11.7 $\pm$ 0.3	26.9 $\pm$ 1.1	26.4 $\pm$ 0.4
1	10.5 $\pm$ 0.6	5.9 $\pm$ 1.2†	25.9 $\pm$ 3.3	23.8 $\pm$ 7.1

Table 8. *Effect of tryptophan on the concentrations of metabolites in livers of 48 h-starved rats*

Tryptophan (750 mg/kg body wt.) was administered intraperitoneally at 0 h. Procedures for extraction of livers and assay of metabolites were as described in the Materials and Methods section. Results are expressed as μ mol/g fresh wt., or as ratios, and are means $\pm$ S.E.M. (four determinations). *P* (versus corresponding controls): * <0.05 , ** <0.01 , *** <0.001 , † <0.02 , by Student's *t* test.

Time after tryptophan administration (h) . . .	1		3		5.5	
Metabolite	Control	+Tryptophan	Control	+Tryptophan	Control	+Tryptophan
Pyruvate	0.04 $\pm$ 0.009	0.05 $\pm$ 0.01	0.03 $\pm$ 0.006	0.10 $\pm$ 0.03	0.03 $\pm$ 0.007	0.07 $\pm$ 0.01†
Lactate	0.29 $\pm$ 0.07	0.90 $\pm$ 0.28	0.29 $\pm$ 0.09	1.79 $\pm$ 0.28*	0.30 $\pm$ 0.06	1.39 $\pm$ 0.04***
Lactate/pyruvate	6.8 $\pm$ 1.3	19.4 $\pm$ 4.1*	10.6 $\pm$ 4.8	13.0 $\pm$ 1.2	9.5 $\pm$ 0.7	21.4 $\pm$ 3.8*
Phosphoenolpyruvate	0.05 $\pm$ 0.01	0.05 $\pm$ 0.02	0.07 $\pm$ 0.004	0.03 $\pm$ 0.01**	0.04 $\pm$ 0.007	0.05 $\pm$ 0.005
Malate	0.23 $\pm$ 0.04	4.14 $\pm$ 1.00**	0.23 $\pm$ 0.05	6.24 $\pm$ 0.32***	0.29 $\pm$ 0.07	6.30 $\pm$ 0.37***
Aspartate	0.42 $\pm$ 0.04	2.14 $\pm$ 0.45**	0.35 $\pm$ 0.04	2.20 $\pm$ 0.16***	0.35 $\pm$ 0.03	3.57 $\pm$ 0.52***
Acetoacetate	0.25 $\pm$ 0.04	0.10 $\pm$ 0.02†	0.28 $\pm$ 0.02	0.10 $\pm$ 0.01***	0.30 $\pm$ 0.04	0.10 $\pm$ 0.02**
β -Hydroxybutyrate	0.32 $\pm$ 0.06	0.71 $\pm$ 0.15	0.16 $\pm$ 0.03	0.33 $\pm$ 0.06*	0.19 $\pm$ 0.06	0.48 $\pm$ 0.06†
β -Hydroxybutyrate/ acetoacetate	1.3 $\pm$ 0.1	7.8 $\pm$ 2.0†	0.6 $\pm$ 0.1	3.3 $\pm$ 0.7**	0.7 $\pm$ 0.2	5.2 $\pm$ 0.9**
ATP	2.58 $\pm$ 0.08	2.84 $\pm$ 0.20	2.75 $\pm$ 0.17	2.35 $\pm$ 0.15	2.68 $\pm$ 0.25	2.14 $\pm$ 0.13
ADP	1.17 $\pm$ 0.05	0.87 $\pm$ 0.12	1.03 $\pm$ 0.07	0.86 $\pm$ 0.07	0.93 $\pm$ 0.05	0.87 $\pm$ 0.13
AMP	0.48 $\pm$ 0.04	0.29 $\pm$ 0.05†	0.37 $\pm$ 0.04	0.32 $\pm$ 0.03	0.28 $\pm$ 0.05	0.33 $\pm$ 0.07

tryptophan is shown in Table 8. There were large increases in lactate, malate, aspartate and β -hydroxybutyrate and a fall in acetoacetate concentrations within 1 h of injection. These changes were somewhat greater at 3 h, and persisted at 5½ h. There was no consistent change in phosphoenolpyruvate concentration and no significant change in adenine nucleotide concentrations throughout. The increased [lactate]/[pyruvate] and [β -hydroxybutyrate]/[acetoacetate] ratios are consistent with a shift towards a more reduced state in both cytoplasmic and mitochondrial compartments (Krebs & Veech, 1969). These changes confirm and extend other observations (Ray *et al.*, 1966; McDaniel *et al.*, 1973). The changes do not, however, correlate with the tryptophan-induced alterations in plasma glucose concentrations (see Fig. 1). At 1 h after injection the pattern of metabolites is consistent with an inhibition of gluconeogenesis at the level of phosphoenolpyruvate

carboxykinase (EC 4.1.1.32), but this coincides with the period of transient hyperglycaemia.

McDaniel *et al.* (1973) and Alvarez & Ray (1974) failed to find any tryptophan-dependent changes in metabolites in livers from diabetic rats, and correlated the lack of a hypoglycaemic response with this apparent lack of inhibition of gluconeogenesis. We have reinvestigated this claim, and found that the pattern of metabolite changes after tryptophan injection is broadly similar in both diabetic and intact rats (Table 9). The only difference of note is that pyruvate accumulates in the tryptophan-treated diabetic liver; the [lactate]/[pyruvate] ratio is unchanged overall. The pattern is thus again consistent with an inhibition of gluconeogenesis. The role of quinolinate in such inhibition is unclear, since this metabolite does not accumulate in livers of diabetic animals (McDaniel *et al.*, 1973); this has been ascribed to an increased flux through the alternative pathway

Table 9. *Effect of tryptophan on the concentrations of metabolites in livers of alloxan-diabetic rats*

Tryptophan (750 mg/kg body wt.) was administered intraperitoneally at 0 h. Procedures for extraction of livers and assay of metabolites were as described in the Materials and Methods section. Results are expressed as $\mu\text{mol/g}$ fresh wt., $\mu\text{mol/ml}$ of plasma or as ratios, and are means $\pm$ S.E.M. (four determinations). *P* (versus corresponding controls): * <0.05 , ** <0.01 , *** <0.001 , $\dagger <0.02$, by Student's *t* test.

Time after tryptophan administration (h) . . .	1		3		6	
Metabolite	Control	+Tryptophan	Control	+Tryptophan	Control	+Tryptophan
Pyruvate	0.03 ± 0.006	$0.07 \pm 0.009^{**}$	0.03 ± 0.002	$0.07 \pm 0.007^{**}$	0.03 ± 0.003	0.14 ± 0.05
Lactate	1.17 ± 0.26	1.81 ± 0.60	0.84 ± 0.21	3.02 ± 0.89	0.58 ± 0.04	3.23 ± 1.11
Lactate/pyruvate	43 ± 1	28 ± 5	31 ± 9	42 ± 9	19 ± 2	22 ± 2
Phosphoenolpyruvate	0.11 ± 0.02	$0.04 \pm 0.007\dagger$	0.09 ± 0.02	0.06 ± 0.02	0.07 ± 0.008	0.08 ± 0.03
Malate	0.35 ± 0.05	$5.13 \pm 0.42^{***}$	0.40 ± 0.06	$5.23 \pm 1.63^*$	0.28 ± 0.02	3.57 ± 1.41
Aspartate	0.54 ± 0.06	$3.82 \pm 0.28^{***}$	0.54 ± 0.03	$1.94 \pm 0.39\dagger$	0.57 ± 0.01	$1.69 \pm 0.32\dagger$
Acetoacetate	0.35 ± 0.05	$0.14 \pm 0.01^{**}$	0.47 ± 0.19	0.18 ± 0.04	0.36 ± 0.10	0.19 ± 0.003
β -Hydroxybutyrate	0.29 ± 0.07	0.78 ± 0.08	0.24 ± 0.10	$0.75 \pm 0.17^*$	0.33 ± 0.12	$0.83 \pm 0.04^{**}$
β -Hydroxybutyrate/ acetoacetate	0.79 ± 0.10	$5.7 \pm 0.30^{***}$	0.52 ± 0.07	$4.2 \pm 0.50^{***}$	0.87 ± 0.10	$4.4 \pm 0.20^{***}$
ATP	3.62 ± 0.21	3.90 ± 0.18	3.91 ± 0.59	3.75 ± 0.58	4.08 ± 0.55	3.20 ± 0.24
ADP	1.56 ± 0.15	$0.87 \pm 0.18^*$	1.10 ± 0.12	0.99 ± 0.08	1.13 ± 0.09	1.07 ± 0.23
AMP	0.27 ± 0.04	0.25 ± 0.02	0.43 ± 0.14	0.28 ± 0.06	0.31 ± 0.04	0.19 ± 0.04
Plasma glucose	28.0 ± 2.9	28.3 ± 1.4	26.2 ± 1.3	33.6 ± 7.5	28.5 ± 1.1	35.3 ± 3.2

Table 10. *Effect of tryptophan on the concentrations of lactate, pyruvate and ketone bodies in the blood of 48 h-starved rats*

Tryptophan (750 mg/kg body wt.) was administered intraperitoneally at 0 h. Procedures for preparation and assays of extracts were as described in the Materials and Methods section. Results are expressed as $\mu\text{mol/ml}$ of blood or as ratios, and are means $\pm$ S.E.M. (four determinations). *P* (versus corresponding controls): * <0.05 , ** <0.01 , *** <0.001 , $\dagger <0.02$, by Student's *t* test.

Metabolite	Time after treatment (h) . . .	0	1.5	3	4.5	6
Lactate	Control	1.00 ± 0.23	0.46 ± 0.080	1.05 ± 0.33	0.62 ± 0.17	0.41 ± 0.03
	Tryptophan	—	$1.11 \pm 0.01^{**}$	0.85 ± 0.20	$1.88 \pm 0.38\dagger$	$1.73 \pm 0.11^{***}$
Pyruvate	Control	0.08 ± 0.01	0.05 ± 0.01	0.11 ± 0.04	0.09 ± 0.01	0.05 ± 0.01
	Tryptophan	—	$0.10 \pm 0.01\dagger$	0.10 ± 0.01	$0.18 \pm 0.03\dagger$	$0.14 \pm 0.01^{***}$
Lactate/pyruvate	Control	12.1 ± 1.2	12.5 ± 3.2	9.4 ± 1.8	7.3 ± 1.6	8.2 ± 0.8
	Tryptophan	—	12.2 ± 1.1	8.3 ± 0.8	10.5 ± 1.0	$11.6 \pm 0.6^{**}$
Acetoacetate	Control	0.51 ± 0.02	0.51 ± 0.03	0.46 ± 0.05	0.45 ± 0.04	0.43 ± 0.05
	Tryptophan	—	$0.21 \pm 0.03^{***}$	$0.25 \pm 0.03\dagger$	$0.19 \pm 0.03^{**}$	$0.15 \pm 0.02^{**}$
β -Hydroxybutyrate	Control	1.13 ± 0.01	1.17 ± 0.05	1.16 ± 0.20	1.17 ± 0.03	1.20 ± 0.15
	Tryptophan	—	0.96 ± 0.20	1.35 ± 0.10	1.26 ± 0.08	0.91 ± 0.08
Total ketone bodies	Control	1.63 ± 0.10	1.62 ± 0.06	1.62 ± 0.22	1.62 ± 0.32	1.63 ± 0.20
	Tryptophan	—	1.17 ± 0.20	1.60 ± 0.15	1.45 ± 0.08	$1.06 \pm 0.08^{**}$
β -Hydroxybutyrate/ acetoacetate	Control	2.2 ± 0.2	2.2 ± 0.1	2.6 ± 0.5	2.5 ± 0.4	2.8 ± 0.2
	Tryptophan	—	4.6 ± 1.0	$5.4 \pm 0.4^{**}$	$7.2 \pm 1.6^*$	$6.4 \pm 0.1\dagger$

involving picolinate carboxylase (aminocarboxy-muconate semialdehyde decarboxylase, EC 4.1.1.45) in diabetic animals (Ikeda *et al.*, 1965).

Measurements of blood metabolites in tryptophan-treated animals (Table 10) reveal increased concentrations of circulating lactate and pyruvate, and a marked decrease in that of acetoacetate. This is responsible for the significantly increased [β -hydroxybutyrate]/[acetoacetate] ratio, which reflects similar changes in the liver.

In passing, it should be noted that the measurements of hepatic and blood ketone-body concentrations (Tables 8 and 10) were made in samples taken from entirely different groups of animals and, further, the two experiments were performed several months apart. The discrepancy between these values may be attributable to this temporal separation; no direct comparison of these two sets of data is justified.

Bässler & Brinkrolf (1971) reported a rapid inhibition of ketogenesis by tryptophan in the starved rat.

Table 11. *Time course of changes in the plasma concentrations of glucose, insulin and tryptophan after administration of tryptophan to 48 h-starved rats*

Tryptophan (750 mg/kg body wt.) was administered intraperitoneally at 0 h. The procedures for preparation and assay of plasma samples were as described in the Materials and Methods section. Results are expressed as means $\pm$ s.e.m. (four determinations). One unit of insulin is equivalent to 40.8 μ g. *P* (versus corresponding controls): **<0.01, ***<0.001, by Student's *t* test.

Time (h)	Glucose (μ mol/ml)		Insulin (μ units/ml)		Tryptophan (μ mol/ml)	
	Control	+Tryptophan	Control	+Tryptophan	Control	+Tryptophan
0	4.8 $\pm$ 0.2	—	4.4 $\pm$ 1.2	—	0.07 $\pm$ 0.01	—
1.5	5.0 $\pm$ 0.1	6.7 $\pm$ 0.4**	2.4 $\pm$ 1.1	3.7 $\pm$ 0.7	0.07 $\pm$ 0.009	2.2 $\pm$ 0.2***
3	4.9 $\pm$ 0.3	4.7 $\pm$ 0.3	2.5 $\pm$ 0.7	6.3 $\pm$ 0.6**	0.05 $\pm$ 0.007	1.6 $\pm$ 0.09***
4.5	4.8 $\pm$ 0.4	2.6 $\pm$ 0.4**	4.3 $\pm$ 1.0	4.1 $\pm$ 0.9	0.05 $\pm$ 0.006	1.0 $\pm$ 0.12**
6	5.0 $\pm$ 0.3	1.7 $\pm$ 0.2***	4.6 $\pm$ 1.2	2.6 $\pm$ 0.8	0.06 $\pm$ 0.003	0.25 $\pm$ 0.05***

We saw no evidence of this effect, although there was a significant fall in the total ketone-body concentration 6 h after tryptophan administration.

There were no significant changes in the plasma concentrations of urea, triacylglycerol, non-esterified fatty acid or glycerol in tryptophan-treated animals over a 6 h period (results not shown).

Tryptophan itself was, not unexpectedly, present at significantly higher concentrations in the plasma of tryptophan-treated than in that of control animals (Table 11). Even 6 h after injection the concentration was some 4 times that in controls. Measurement of 'bound' and 'free' tryptophan (Knott & Curzon, 1972; Curzon *et al.*, 1973) showed that, although most (approx. 75%) was bound to protein in control plasma, the excess tryptophan in plasma of experimental animals remained largely in the free state (less than 30% bound).

Plasma insulin concentrations were increased some 2.5-fold 3 h after tryptophan administration (Table 11), but were not significantly different from control values at other times. This observation agrees with that of Gagliardino *et al.* (1971).

General discussion

The pattern of hypoglycaemic responsiveness with tryptophan itself, its metabolites and various inhibitors is consistent with the hypothesis that 5-hydroxytryptamine is the effective agent. The failure of 5-hydroxytryptamine itself to elicit a similar response is unsurprising, since this metabolite is rapidly compartmented into platelets and other tissues (Hagen & Cohen, 1966), and, at the concentrations used, is associated with a complex interaction of pharmacological events (Douglas, 1970).

Tryptophan may be hydroxylated by at least two distinct enzyme systems. In brain, a specific tryptophan hydroxylase with low activity is present (Guroff, 1975), whereas in liver and kidney tryptophan hydroxylation depends on phenylalanine hydroxylase, with an unphysiologically high K_m for tryptophan

(Renson *et al.*, 1961, 1962). In other tissues that synthesize 5-hydroxytryptamine, the nature of the enzymes involved is less clear. Both tryptophan hydroxylase and phenylalanine hydroxylase catalyse rate-limiting steps in the metabolism of their respective substrates (Guroff, 1975). The regulation of both enzymes is complex and may involve both direct control by metabolites (Fisher & Kaufman, 1973; Lovenberg & Victor, 1974) and changes in total enzyme protein (Zivkovic *et al.*, 1973; McClure *et al.*, 1975; Diez *et al.*, 1976; Sze *et al.*, 1976). In addition, it has been shown that phenylalanine hydroxylase may be modified by phosphorylation through a cyclic AMP-dependent mechanism (Abita *et al.*, 1976). The relative effectiveness of 5-hydroxytryptophan as a hypoglycaemic agent may thus be explained in terms of a 'by-pass' of the rate-limiting step of 5-hydroxytryptamine synthesis, and by its rapid transport into, and decarboxylation within, a number of tissues (Udenfriend & Weissbach, 1958).

There are several possible mechanisms whereby 5-hydroxytryptamine accumulation might produce hypoglycaemia. The evidence that biogenic amines, including 5-hydroxytryptamine, play a role in the regulation of peptide-hormone secretion (Kamberi *et al.*, 1970, 1971; Bivens *et al.*, 1973; Marco *et al.*, 1976; Smith & Porte, 1976) suggests that an alteration of endocrine function may be involved. The changes in plasma insulin content in our experiments are, however, insufficient on their own to account for the dramatic hypoglycaemia. Moreover, the function of 5-hydroxytryptamine in the pancreatic β -cell is inhibition rather than stimulation of insulin release (Lernmark, 1971; Wilson *et al.*, 1974).

Compound MK-486 is an inhibitor of peripheral aromatic L-amino acid decarboxylase (Porter *et al.*, 1962; Bartholini *et al.*, 1971; Warsh & Stancer, 1976). The inhibition of tryptophan-dependent hypoglycaemia by this compound suggests that 5-hydroxytryptamine synthesis from injected tryptophan is outside the central nervous system. Further evidence is required before one may speculate fruitfully on the

site of this effect. It is reasonable, however, to discount the theory that the anti-gluconeogenic action of tryptophan explains the hypoglycaemic response. The appearance of hypoglycaemia in the fed animal, and the lack of correlation between liver metabolites and plasma glucose concentrations, indicates that these two events are little, if at all, related.

The discrepancy between our results and those of other workers with regard to the changes in liver metabolites in diabetic rats is not easily explained. It may, however, perhaps be attributed to the different animal models used: in our experiments, acute (48 h), but non-ketotic, alloxan-diabetes; in earlier studies, chronic (14 day) alloxan-diabetes. The longer period of less-profound diabetes may permit slow adaptations of enzyme activities with consequent alteration of metabolic flux patterns.

This work was supported by a grant from the British Diabetic Association. We are indebted to Merck, Sharp and Dohme Research Laboratories, Sandoz Products and Dr. D. C. N. Earl for their gifts of chemicals.

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