Biochemical Pharmacology, Vol. 31, No. 22, pp. 3563–3569, 1982.
Printed in Great Britain.

0006-2952/82/223563-07 $03.00/0

FACTORS AFFECTING TRYPTOPHAN-INDUCED HYPOGLYCAEMIA IN RATS

PETER LLOYD,* STEPHEN A. SMITH,† DONALD STRIBLING‡ and CHRISTOPHER I. POGSON§

Biological Laboratory, University of Kent, Canterbury CT2 7NJ, U.K.; and Imperial Chemical Industries plc, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.

(*Received* 25 *January* 1982; *accepted* 5 *May* 1982)

Abstract—The mechanisms whereby tryptophan administration leads to hypoglycaemia in some groups of rats but not others have been investigated. Animals insensitive to tryptophan are rendered responsive by adrenalectomy. This effect is reversed by steroid replacement. Turnover studies with [2-^{3}H]glucose show that hypoglycaemia in sensitive animals is associated with a decrease in glucose synthesis. Tryptophan administration causes a marked and sustained increase in plasma glucagon concentrations in all animals. The locus of the inhibition of gluconeogenesis in tryptophan-sensitive animals is the reaction catalysed by phosphoenolpyruvate carboxykinase. The sensitivities to tryptophan of gluconeogenesis in isolated hepatocytes from normal and adrenalectomized animals were similar. Cells from chronically streptoxotocin-diabetic animals required higher concentrations of the amino acid for the same effect. These results are discussed in relation to previous discrepancies in the literature, and a unifying hypothesis for tryptophan-induced hypoglycaemia is proposed.

The interaction between tryptophan and plasma glucose concentrations has been the subject of many publications over a period of more than 30 years. Howard and Modlinger [1] and Mann *et al.* [2] found no effect of the amino acid, but subsequent reports have claimed that, in rats, at least, substantial hypoglycaemia can result from administration of pharmacological doses of tryptophan [3–9]. Even when the effect is pronounced, however, results differ in the time-courses of the changes. Gullino *et al.* [3] and Smith and Pogson [9] found a transient initial rise in blood glucose concentration but others have seen only a rapid hypoglycaemia [5, 6]; these latter reports differ, however, in the dose effectiveness of the tryptophan administered.

Studies with perfused rat liver [10, 11] and isolated liver cells [12, 13] have shown that quinolinate may be responsible for the hypoglycaemic effect of tryptophan. *In vivo*, however, 5-hydroxytryptophan (which is not a precursor of quinolinate) is a more potent hypoglycaemic agent than is tryptophan itself [14–16]. Experiments with specific inhibitors of biogenic amine metabolism suggest that 5-hydroxytryptamine may be a causative agent in these instances and also with tryptophan itself [9, 14, 15].

Finally, controversy also exists as to whether tryptophan is hypoglycaemic in other species, and particularly in man. Again, both positive [17, 18] and negative [19, 20] results have been obtained.

In this paper, we report investigations into the variability of the response to tryptophan in rats and provide evidence for the molecular mechanisms involved in the induction of hypoglycaemia by the amino acid.

MATERIALS AND METHODS

Animals. At the University of Kent, male rats (200–250 g) of the Sprague–Dawley CD/ASH strain (Charles River U.K., Manston, U.K.) were inbred in the laboratory. They were, unless otherwise specified, allowed food (86S diet, Grain Harvesters Ltd, Wingham, U.K.) and water *ad lib.* At ICI, Pharmaceuticals Division (Alderley Park, U.K.), rats (male 180–220 g) of the Alderley Park strain, were supplied by the Animal Breeding Unit, Alderley Park, U.K. They were, unless otherwise specified, allowed food (standard diet, BP Nutritional) and water *ad lib.*

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 by intravenous injection of alloxan or streptozotocin (60 mg/kg body weight). Animals were used after 48 hr ('acute') or 14 days ('chronic'). Diabetes was confirmed by glucosuria [Clinistix (Ames Co., Stoke Poges, U.K.)] and subsequent plasma glucose determination. Rats with plasma glucose concentrations below 15 mM were not used.

Tryptophan was administered intraperitoneally as a microcrystalline suspension [37.5 mg/ml in 0.9% (w/v) NaCl, 0.1% (w/v) Tween 80]. Control animals received a similar volume of vehicle.

Metyrapone was administered intraperitoneally as a solution in 0.9% (w/v) NaCl, 0.5% (w/v) Tween

Present addresses: * Nuffield Department of Clinical Biochemistry, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K. (to whom correspondence should be addressed); † Beecham Pharmaceuticals, Biosciences Research Centre, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ, U.K.; ‡ Imperial Chemical Industries plc, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.; and § Department of Biochemistry, University of Manchester, Oxford Road, Manchester M13 9PL, U.K.

80. Treated animals were given 1% (w/v) NaCl to drink.

Other drugs and dosing regimes were as described in Refs 9 and 21.

Preparation of samples. Routinely, blood samples were removed from a tail vein, just before, and at intervals after, appropriate injection. Chilled, heparinized, blood samples were centrifuged at 12,000 *g* for 1 min; plasma was stored at −20° for subsequent glucose assay. The validity of the sampling technique was checked in all conditions by comparison with plasma derived from cardiac blood at the final time point. In experiments with pargyline (where there was pronounced vasoconstriction), cardiac samples only were used.

Samples for determination of immunoreactive insulin and glucagon were obtained either by cardiac puncture or from a carotid arterial catheter. 0.5 ml of blood was mixed with 0.04 ml of EDTA (20 mg/ml), Trasylol (10^4 kI.U./ml); after chilling, samples were centrifuged at 12,000 *g* as before. Portions of the plasma supernatant were stored at −20° before assay (glass tubes for glucagon, plastic tubes for insulin).

Glucose turnover in vivo. Rats were anaesthetized with 2.0–2.5% Halothane in oxygen at a flow rate of 2 l/min; during infusion this was decreased to 1.0–1.5%. Cannulation of one carotid artery and jugular vein were performed as described in Ref. 22. After an initial 'priming' dose of [2-^{3}H]glucose (10 Ci/mmole; 0.5 ml of 8 μCi/ml), infusion of radiolabel (10 μCi/mmole; 6 μCi/ml) was continued at a rate of approximately 0.8 ml/hr—similar to the rate of removal of blood for assay. Data were analysed as discussed in Ref. 23.

Liver cell incubations. Liver cells were prepared [24] and incubated as previously described [12].

For estimation of the production of [^{14}C]glucose from [U-^{14}C]lactate, portions of neutralised cell extract were added to mixed-bed ion-exchange columns [0.5 ml Amberlite CG120, Na^+ form; above 0.5 ml Amberlite CG400, acetate form; 200-mesh (BDH, Poole, U.K.)]. Uncharged labelled compounds (essentially all [^{14}C]glucose) were eluted from columns with 2 ml of water.

Biochemical assays. Metabolites in freeze-clamped liver samples were assayed as described in Ref. 9.

Glucose in plasma samples was routinely measured with glucose oxidase and peroxidase [25]. Since many compounds, including tryptophan metabolites, inhibit colour development in this assay [26], concentrations were checked by a second method using hexokinase and glucose 6-phosphate dehydrogenase [27]. No differences were noted in any case. Glucose formed in cell incubations was measured by the latter method. Insulin was assayed by the single-antibody method [28], glucagon by a modification [29] of the method described in Ref. 30. The glucagon antibody showed no cross-reactivity with gut glucagon-like immunoreactive substances.

Sources of materials. With the exception of [^{125}I]glucagon [from New England Nuclear Corp. (South Wanston, U.K.)], radiochemicals were from Amersham International Ltd (Amersham, U.K.).

Pargyline (*N*-benzyl-*N*-methylprop-2-ynylamine) was from Abbot Laboratories (Queenborough, U.K.), compound MK-486 [L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid monohydrate] from Merck, Sharp & Dohme Research Laboratories (Rahway, NJ), Halothane from ICI, Pharmaceuticals Division, triamcinolone {9α-fluoro-11β,21-dihydroxy-16α,17[1-methylethylidene bis(oxy)] pregna-1,4-diene-3,20-dione}, as the acetonide (Adcortyl) from E.R. Squibb & Sons (Twickenham, U.K.), and metyrapone [2-methyl-1,2-bis(3-pyridyl)-1-propanone] from Ciba-Geigy (Horsham, U.K.).

Other chemicals, of the purest grade available, were from standard suppliers.

RESULTS AND DISCUSSION

Tryptophan injection and glucose response in vivo

Previous studies performed in the Biological Laboratory at the University of Kent (from 1973 to 1975) showed that tryptophan administration to rats [group IA(TS); TS = tryptophan-sensitive] resulted in profound hypoglycaemia with a characteristic time-course. These effects were highly reproducible between individuals and groups of rats in the same population. Experiments with agents affecting tryptophan metabolism strongly suggested that the effect was mediated through 5-hydroxytryptamine [9].

Experiments carried out in a continuation of this project, after a gap of more than 1 year (1976–1979), gave equally consistent but contrasting results in that no hypoglycaemic response whatsoever could be elicited at any dose of the amino acid [group IB(TR); TR = tryptophan-resistant; see Fig. 1(a) and (b)].

Simultaneously, parallel studies were made at the laboratories of ICI, Pharmaceuticals Division, during the same two time periods. In the earlier period [i.e. contemporaneously with work with group IA(TS)], no hypoglycaemic response was observed [group IIA(TR)], while in the later period [cf. group IB(TR)], a sharp decrease in blood glucose concentration could be seen, but with a time-course distinct from that obtained with group IA(TS) [group IIB(TS); see Fig. 2]. The same batch of tryptophan was used for groups IA, IB and IIB.

A systematic survey of the effects and importance of timing, changes of experiments, routes and methods of tryptophan administration, rat strain, age, weight, sex and diet did not reveal any factor(s) responsible for the variation in responsiveness (results not shown). Rats in group IIB(TS) did, however, lose their sensitivity to tryptophan on transfer to the University of Kent, but again no simple explanation for this phenomenon could be adduced. We were unable to perform experiments with group IB(TR) rats transferred to ICI.

Further investigation revealed that group IB(TR) animals responded to tryptophan after adrenalectomy, although the dose required was significantly greater than for group IA(TS) animals similarly treated [Fig. 1(c) and (d)]. That these observations may be of more general relevance is suggested by the literature (see Introduction). As a particular case, Pitot's group [31] found that tryptophan at doses of above 400 mg/kg killed adrenalectomized rats rapidly, while Schimke *et al.* [32], in a similar

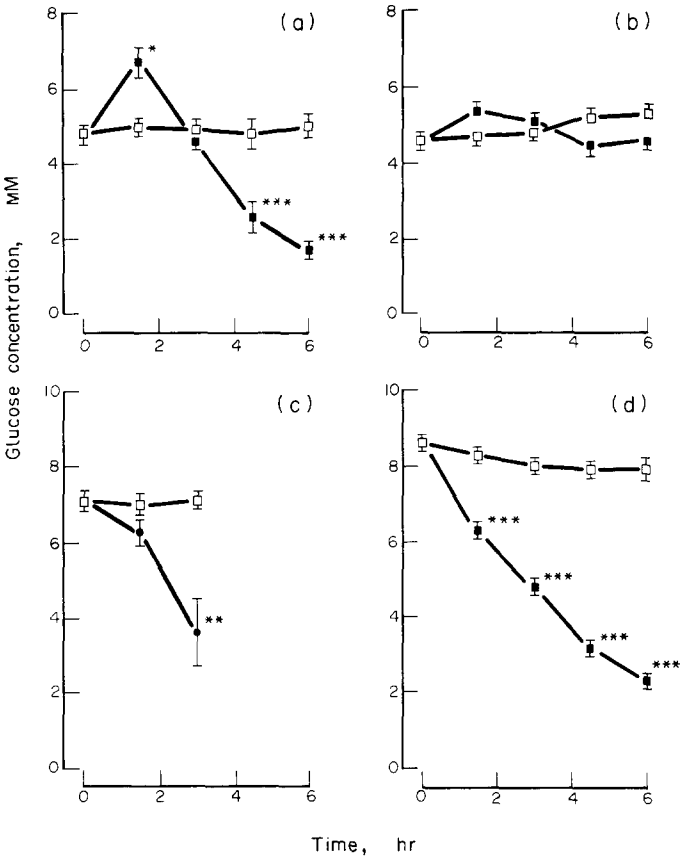

Fig. 1. Effect of tryptophan on plasma glucose concentrations in rats: (a) 48-hr starved, group IA(TS); (b) 48-hr starved, group IB(TR); (c) adrenalectomised, group IA(TS); (d) adrenalectomised, group IB(TR). Results are means ± S.E.M. of at least four determinations. P (vs corresponding controls): * <0.05; ** <0.01; *** <0.001. □, controls; ■, tryptophan (750 mg/kg i.p.); ●, tryptophan (200 mg/kg i.p.). In (d) results with tryptophan at 200 mg/kg did not differ significantly from controls. In (c) all treated animals died after 4 hr. Data in (a) and (c) are from Smith and Pogson [9].

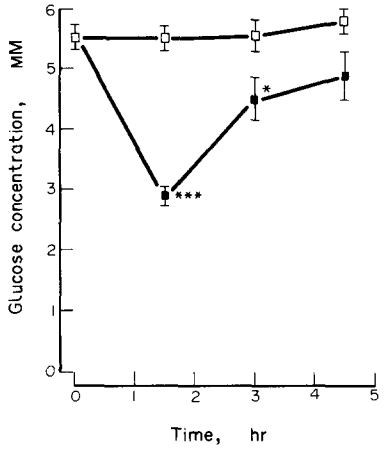

Fig. 2. Effect of tryptophan (750 mg/kg body weight) on plasma glucose concentrations in 48-hr starved rats of group IIB(TS). Results are means ± S.E.M. from six observations. P (vs control): * <0.05, *** <0.001. Symbols as in Fig. 1.

study, were able to administer four successive doses of 1 g/kg at 4-hr intervals!

The results of tryptophan administration on plasma glucose concentrations in diabetic rats are more difficult to assess because of the high initial values. Smith and Pogson [9] found that there was no significant change in plasma glucose in acutely alloxan-diabetic group IA(TS) animals, although concentrations appeared to decline over the whole 6-hr time-course. With group IB(TR), results were broadly similar with comparable falls in the absolute glucose concentrations; these became significantly different from controls at 6-hr ($P < 0.05$; results not shown).

Group IB(TR) animals, rendered diabetic with streptozotocin, exhibited apparently paradoxical results in that tryptophan-induced hypoglycaemia was found 48 hr after the diabetogen but not after 10 days. This may be attributable to the known short-term effects of streptozotocin on NAD, and hence tryptophan, metabolism [33], and to the slower induction of picolinate carboxylase in chronically diabetic animals [34, 35].

Table 1. Effect of MK-486 on tryptophan-induced hypoglycaemia in adrenalectomized group IB(TR) rats

Time (hr)	Control (I)	Tryptophan† (II)	MK-486‡ (III)	MK-486 + tryptophan (IV)
0	7.6 ± 0.3		6.3 ± 0.3	
1.5	6.8 ± 0.2	6.0 ± 0.3	5.9 ± 0.4	4.5 ± 0.4*
3	6.9 ± 0.2	5.2 ± 0.4**	4.5 ± 0.7**	1.2 ± 0.5**
4.5	6.7 ± 0.3	3.8 ± 0.5**	4.4 ± 0.7**	0.7 ± 0.1**
6	6.6 ± 0.4	3.6 ± 0.4***	4.7 ± 0.5**	

Results are means ± S.E.M.; five animals per group. P (II vs I, III vs I, IV vs III): * <0.05, ** <0.01, *** <0.001.
† 750 mg/kg body weight.
‡ 68 mg/kg body weight, 30 min before tryptophan.

Pargyline, a general monoamine oxidase inhibitor [36], amplifies the effect of tryptophan in acute streptozotocin-diabetic group IB(TR) rats, and induces some sensitivity to the amino acid in 48-hr starved rats of the same group. 5-Hydroxytryptophan is an equally effective hypoglycaemic agent in all groups of rats tested, and its effects are potentiated by pargyline (in agreement with Refs 14 and 15).

The aromatic amino acid decarboxylase inhibitor, MK-486, suppressed the hypoglycaemia induced by tryptophan in group IA(TS) rats [9]. In group IB(TR), however, similar treatment of adrenalectomized animals revealed a *greater* responsiveness (Table 1). *p*-Chlorophenylalanine, a chronic inhibitor of tryptophan hydroxylase [37], also increased the tryptophan hypoglycaemic effect in these animals, again in contrast with earlier observations with group IA(TS). Thus, although a role for monoamines as mediators of tryptophan-induced hypoglycaemia is clear for animals such as those in group IA(TS), the situation may be more confused for other groups, with other factors involved. The permissive effect of adrenalectomy in group IB(TR) rats is mimicked in animals chronically treated with metyrapone, an 11β-hydroxylase inhibitor [38] and this is accentuated by starvation (Fig. 3). Conversely, treatment of adrenalectomized animals with triamcinolone returned those in group IB(TR) to their normal tryptophan-insensitive state.

Measurement of blood tryptophan concentrations after injection [into rats of group IB(TR)] showed that the constant fall to basal values, seen in normal animals, 1.5–6 hr after a tryptophan load, was arrested by adrenalectomy and restored by triamcinolone (Fig. 4); metyrapone did not affect the normal pattern of tryptophan clearance. At the higher tryptophan concentrations, the proportion of the amino acid bound to plasma protein is very small (<5%, [35]).

Measurements of the rate of tryptophan oxidation by hepatocytes from adrenalectomized group IB(TR) rats showed that this was not significantly different from that in untreated animals [35]. This is reflected in the fact that the early changes in blood tryptophan concentrations after administration of the amino acid are similar in both adrenalectomised and control animals. The reason for the subsequent decrease in tryptophan clearance in the former group is not known, but may possibly reflect the low level of tryptophan dioxygenase activity or involve limitation of amino acid uptake from the peritoneal cavity.

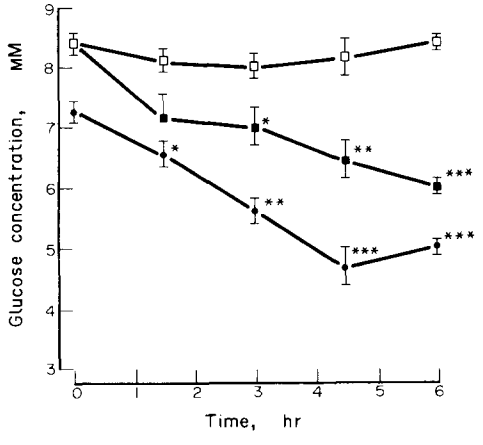

Fig. 3. Effect of tryptophan (750 mg/kg body weight) on plasma glucose concentrations in metyrapone-treated group IB(TR) rats, either fed (■) or starved 18 hr before (●) tryptophan administration. Animals were given metyrapone (100 mg/kg body weight) once per day for 6 days. Results are means ± S.E.M. from five observations. Values for 18-hr starved control animals showed a time-course similar to that for fed controls (□) and are not shown. P (vs respective controls): * <0.05, ** <0.01, *** <0.001.

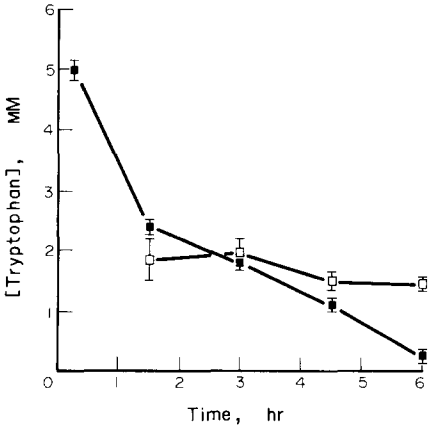

Fig. 4. Effect of tryptophan loading (750 mg/kg body weight) on plasma tryptophan concentrations. Group IB(TR): (□) adrenalectomized; (■) triamcinolone-treated adrenalectomized.

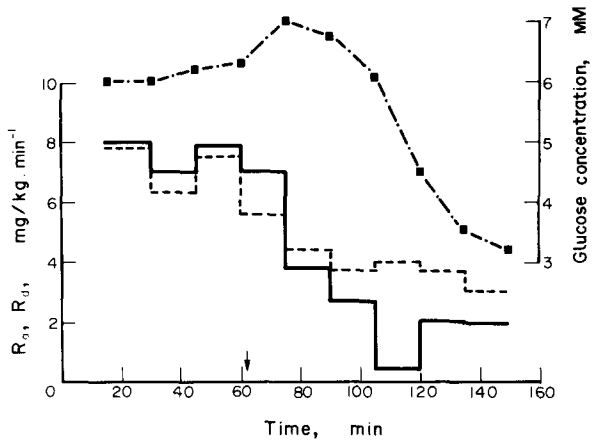

Fig. 5. Glucose turnover in 48-hr starved group IIB(TS) rats given tryptophan (750 mg/kg body weight). [2-^{3}H]Glucose was infused as described in Materials and Methods; tryptophan was injected i.p. where indicated (↓). The controls, without tryptophan, yielded time-courses essentially parallel to the ordinate axis. This experiment is typical of three performed. (———) Rate of appearance of glucose, R_a; (---) rate of disappearance of glucose, R_d; (-■-■-) plasma glucose concentration.

Tryptophan, glucose turnover and plasma hormone concentrations

Infusion of [2-^{3}H]glucose into 48-hr starved rats of group IIB(TS) showed that the hypoglycaemia was very largely attributable to an inhibition of gluconeogenesis, changes in glucose disposal being relatively minor (Fig. 5). The theoretical basis for these conclusions is discussed fully in Ref. 39.

The time-course of plasma glucose changes in these experiments differed somewhat from that shown in Fig. 2, an observation attributable to the decreased demand for glucose in the anaesthetized state. Similar experiments with group IB(TR) animals revealed an immediate transient hyperglycaemia followed by a period in which there were no substantial changes in either glucose synthesis or removal. The hyperglycaemia correlated closely with a 3–4-fold rise in plasma immunoreactive glucagon (now shown). Tryptophan also caused appreciable changes in plasma glucagon concentrations in starved group IB(TR) rats over a longer time period (Fig. 6; see also Ref. 20), but very much smaller and more variable changes in insulin concentrations. In adrenalectomized group IB(TR) animals, the hormone profiles were very similar, despite the pronounced hypoglycaemia in this group.

Direct evidence for the inhibition of gluconeogenesis by tryptophan *in vivo* was obtained from measurements of the conversion of [U-^{14}C]alanine into [U-^{14}C]glucose (Table 2).

Table 2. Effect of tryptophan (750 mg/kg) on conversion of [U-^{14}C]alanine into [^{14}C]glucose in 48-hr starved group IIB(TS) rats

Treatment	Number of observations	% incorporation into [^{14}C]glucose
Control	6	8.17 ± 0.99
Tryptophan	6	2.99 ± 0.12 ($P < 0.01$)

0.5 ml of 1.0 mole/l [U-^{14}C]alanine was injected i.p. 1 hr after tryptophan; 20 min later, a cardiac blood sample was taken for [^{14}C]glucose determination. Rates were linear over this 20-min period.

Tryptophan and liver metabolites

Measurement of metabolites in freeze-clamped livers from adrenalectomized group IB(TR) rats (Table 3) revealed a pattern similar to that observed for group IA(TS) [9], and consistent with a block at the level of phosphoenolpyruvate carboxykinase with quinolinate as the putative inhibitor [13, 35].

Studies with isolated hepatocytes

Tryptophan added *in vitro* to incubations of isolated hepatocytes produces a marked inhibition of gluconeogenesis from many substrates. This inhibition requires a lag phase during which quinolinate accumulates [12, 13]. We have found no differences in responsiveness of hepatocytes from groups IA and IB. The dose–response curves for hepatocytes from fed and adrenalectomized group IB(TR) animals are similar (Fig. 7), consistent with observations on rates of tryptophan oxidation [35]. Cells from chronically

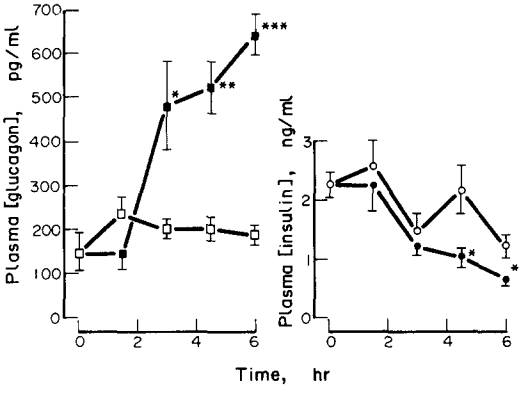

Fig. 6. Effect of trypotophan (750 mg/kg body weight) on plasma insulin (●) and glucagon (■) concentrations in 48-hr starved rats from group IB(TR). Plasma insulin (○) and glucagon (□) concentrations were also measured in control animals given saline vehicle. Results are means ±S.E.M. from eight observations. P (vs control): *<0.05, ** <0.01, *** <0.001.

Table 3. Effect of tryptophan (750 mg/kg body weight) on liver metabolites in fed adrenalectomised group IB(TR) rats

	Treatment	Time (hr) 0	1.5	3	4.5	6
Glycogen (glucose units)	Control	113 ± 15		157 ± 48		49 ± 18
	Tryptophan		139 ± 30	96 ± 14	17 ± 10	5 ± 3*
Aspartate	Control	0.69 ± 0.07		0.63 ± 0.06		0.59 ± 0.02
	Tryptophan		1.40 ± 0.05	1.66 ± 0.22**	1.65 ± 0.37	2.01 ± 0.22**
Malate	Control	0.30 ± 0.04		0.16 ± 0.03		0.24 ± 0.03
	Tryptophan		0.13 ± 0.04	0.65 ± 0.05**	1.49 ± 0.08	2.13 ± 0.37**
2-Oxoglutarate	Control	0.06 ± 0.01		0.06 ± 0.01		0.07 ± 0.01
	Tryptophan		0.13 ± 0.01	0.18 ± 0.01***	0.18 ± 0.01	0.21 ± 0.01***
Phosphoenolpyruvate	Control	0.09 ± 0.01		0.08 ± 0.02		0.08 ± 0.01
	Tryptophan		0.17 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.06 ± 0.03

Results are means ± S.E.M. for four animals per group. Values are μmoles/g wet weight of liver. P (vs control): * <0.05, ** <0.01, *** <0.001.

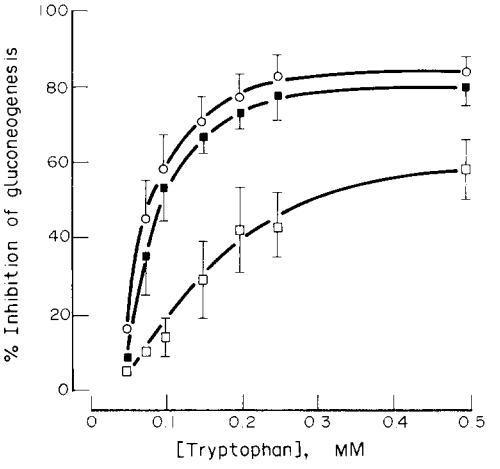

Fig. 7. Inhibition of the conversion of [U-^{14}C]lactate into [^{14}C]glucose in hepatocytes by tryptophan. Hepatocytes were prepared from fed (○), adrenalectomised (■) and chronic streptozotocin-diabetic (□) rats of group IB(TR). Methods of incubation and analysis are described in Materials and Methods. Results are means ± S.E.M. from three independent experiments in each case.

streptozotocin-diabetic animals are less sensitive, because of the diversion of tryptophan carbon from quinolinate to other oxidation products [34, 35].

GENERAL DISCUSSION

It is clear that the mechanism of tryptophan-induced hypoglycaemia is complex, and probably involves interactions brought about by two products derived from the amino acid, namely 5-hydroxytryptamine and quinolinate.

The literature clearly reveals that apparently similar rats show a wide spectrum of response to tryptophan. Although we have not been able to define the factors which predispose any group to be sensitive or not, it is possible nevertheless to clarify the biochemical nature of the differences.

First, quinolinate accumulates in rat liver cells *in vivo* and *in vitro* after exposure to tryptophan [8, 13]; this is accompanied by an apparent inhibition, as revealed by metabolite measurements, of phosphoenolpyruvate carboxykinase. There is no evidence to suggest that TS and TR populations differ in this respect [13, 40], nor do they differ in their responsiveness to 5-hydroxytryptophan ± pargyline. In our view the variation between TS and TR rats cannot be explained by differences in tryptophan metabolism but is a result of other factors which influence the severity of tryptophan action.

Second, tryptophan administration to both control and adrenalectomized TR rats is associated with a prolonged rise in plasma glucagon concentrations which may be a response to the incipient hypoglycaemia [41]. In controls, there was, however, no significant change in plasma glucose concentrations. One likely explanation for this is that, although phosphoenolpyruvate carboxykinase is indeed inhibited, flux through the enzyme (and hence gluconeogenesis) remains constant because of the increased provision of precursors elicited by glucagon. In starved animals, glycogen is, of course, not a significant source of plasma glucose.

In the adrenalectomized animals, glucagon will be unable to stimulate gluconeogenesis because of the lack of the 'permissive' effect of glucocorticoids [42]; such animals will therefore be TS.

We have no data on glucagon concentrations in tryptophan-treated TS rats. Nevertheless, the increased hormone levels in adrenalectomized and endotoxin-treated [43] TR animals suggest that a similar response may occur. If this is so, then the difference between TR and TS animals may reside in differential sensitivity to glucagon; a possible mechanism for this is suggested by the recent report describing competition between tryptophan methyl ester and dexamethasone for the glucocorticoid receptor in liver-derived cells [44].

Acknowledgements—P.L. was the holder of a Science Research Council CASE studentship. We are indebted to Dr D. C. N. Earl, Merck, Sharp & Dohme, Ciba-Geigy and E. R. Squibb & Sons for gifts of compounds.

REFERENCES

1. F. Howard and R. Modlinger, *Am. J. Physiol.* **153**, 425 (1948).
2. G. V. Mann, S. S. Kahn and F. J. Stare, *Am. J. Physiol.* **158**, 38 (1949).
3. P. Gullino, M. Winitz, S. M. Birnbaum, J. Cornfield, M. C. Otey and J. P. Greenstein, *Archs Biochem. Biophys.* **58**, 252 (1955).
4. I. A. Mirsky, G. Perisutti and D. Diengott, *Endocrinology* **359**, 369 (1956).
5. I. A. Mirsky, G. Perisutti and R. Jinks, *Endocrinology* **60**, 318 (1957).
6. J. J. Gagliardino, L. H. Ziehre, F. C. Iturriza, R. E. Hernandez and R. R. Rodriguez, *Hormone Metab. Res.* **3**, 145 (1971).
7. K. H. Bässler and H. Brinkrolf, *Z. ges. exp. Med.* **156**, 52 (1971).
8. H. G. McDaniel, B. R. Boshell and W. J. Reddy, *Diabetes* **22**, 713 (1973).
9. S. A. Smith and C. I. Pogson, *Biochem. J.* **168**, 495 (1977).
10. P. D. Ray, D. O. Foster and H. A. Lardy, *J. biol. Chem.* **241**, 3904 (1966).
11. C. M. Veneziale, P. Walter, N. Kneer and H. A. Lardy, *Biochemistry* **6**, 2129 (1967).
12. S. A. Smith, K. R. F. Elliott and C. I. Pogson, *Biochem. J.* **176**, 817 (1978).
13. S. A. Smith, F. P. A. Carr and C. I. Pogson, *Biochem. J.* **192**, 673 (1980).
14. I. Lundquist, R. Ekholm and L. E. Ericson, *Diabetologia* **7**, 414 (1971).
15. B. L. Furman, *Br. J. Pharmac.* **50**, 574 (1974).
16. B. L. Furman and G. A. Wilson, *Diabetologia* **19**, 386 (1980).
17. A. B. Ajdukiewicz, P. Keane, J. Pearson, A. E. Read and P. R. Salmon, *Scand. J. Gastroenter.* **3**, 622 (1968).
18. P. Furst, G. Guarnieri and E. Hultman, *Scand J. clin. Lab. Invest.* **27**, 183 (1971).
19. J. H. MacIndoe and R. W. Turkington, *J. clin. Invest.* **52**, 1972 (1973).
20. J. A. Hedo, M. L. Villanueva and J. Marco, *Metabolism* **26**, 1131 (1977).
21. I. D. Longshaw and C. I. Pogson, *J. clin. Invest.* **51**, 2277 (1972).
22. V. Popovic and P. Popovic, *J. appl. Physiol.* **15**, 727 (1959).
23. L. Sacca, G. Perez, F. Rengo and M. Condorelli, *Diabetes* **23**, 532 (1974).
24. K. R. F. Elliott, R. Ash, C. I. Pogson, S. A. Smith and D. M. Crisp, in *Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies* (Eds. J. M. Tager, H-D. Söling and J. R. Williamson), p. 139. North-Holland, Amsterdam (1976).
25. H. A. Krebs, D. A. H. Bennett, P. de Gasquet, T. Gascoyne and T. Yoshida, *Biochem. J.* **86**, 22 (1963).
26. D. R. Nelson and A. K. Huggins, *Analyt. Biochem.* **59**, 46 (1974).
27. H-U. Bergmeyer, E. Bernt, F. Schmidt and H. Stork, in *Methods of Enzymatic Analysis* (Ed. H-U. Bergmeyer), 2nd edition p. 1196. Academic Press, New York (1974).
28. J. D. Albano, R. P. Ekins, G. Mantz and R. C. Turner, *Acta endocr, Copenh.* **70**, 487 (1972).
29. J. P. Ashby and R. N. Speake, *Biochem. J.* **150**, 89 (1975).
30. R. H. Unger, E. Aguilar-Parada, W. A. Muller and A. M. Eisentraut, *J. clin. Invest.* **49**, 837 (1970).
31. A. Cihak, C. Lamar and H. C. Pitot, *Archs Biochem. Biophys.* **156**, 188 (1973).
32. R. T. Schimke, E. W. Sweeney and C. M. Berlin, *J. biol. Chem.* **240**, 322 (1965).
33. P. S. Schein, K. G. M. M. Alberti and D. H. Williamson, *Endocrinology* **89**, 827 (1971).
34. M. Ikeda, H. Tsuji, S. Nakamura, A. Ichiyama, Y. Nishizuka and O. Hayaishi, *J. biol. Chem.* **240**, 1395 (1965).
35. S. A. Smith and C. I. Pogson, *Biochem. J.* **200**, 605 (1981).
36. N. H. Neff and H-Y. T. Yang, *Life Sci.* **14**, 2061 (1974).
37. B. K. Koe and A. Weissman, *J. Pharmac. exp. Ther.* **154**, 499 (1966).
38. A. F. de Nicola and V. Dahl, *Endocrinology* **89**, 1236 (1971).
39. J. Katz, in *Techniques in the Life Sciences: Biochemistry* (Eds. H. L. Kornberg *et al.*). Elsevier/North-Holland, Amsterdam (1979).
40. P. Lloyd, Ph.D. thesis, University of Kent at Canterbury (1979).
41. R. H. Unger and L. Orci, *New Engl. J. Med.* **304**, 1575 (1981).
42. J. H. Exton, *Metabolism* **21**, 945 (1972).
43. P. Lloyd, D. Stribling and C. I. Pogson, *Biochem. Pharmac.* **31**, 3571 (1982).
44. M. E. Baker, D. A. Vaughn and D. D. Farrestil, *J. Ster. Biochem.* **13**, 993)(1980).