Biochemical Pharmacology, Vol. 28, pp. 2145–2148.
Pergamon Press Ltd. 1979. Printed in Great Britain.

INHIBITION OF HEPATIC GLUCONEOGENESIS BY TRYPTOPHAN METABOLITES IN RATS AND GUINEA PIGS

STEPHEN A. SMITH, KEITH R. F. ELLIOTT* and CHRISTOPHER I. POGSON
Biological Laboratory, University of Kent, Canterbury, CT2 7NJ, Kent, U.K.

(*Received* 23 *December* 1978; *accepted* 28 *February* 1979)

Abstract—Gluconeogenesis from lactate in rat liver cells is inhibited by 0.5 mM kynurenine, picolinate, 5-hydroxytryptamine, indole 3-acetate and tryptamine. In contrast, in guinea pig liver cells only the last three of these are effective; kynurenine and 3-hydroxyanthranilate are activators. Fifty per cent inhibition is achieved by 0.6 mM- and 0.1 mM-indole 3-acetate in the rat and guinea pig respectively, and by 0.12 mM-tryptamine in both species. *p*-Chlorophenylalanine, MK-486, pargyline and disulfiram do not affect the inhibition of rat hepatocyte gluconeogenesis by tryptophan. The results are discussed in relation to the induction of hypoglycaemia in the rat *in vivo* by tryptophan.

Tryptophan inhibits gluconeogenesis *in vivo* [1–3], in the perfused rat liver [4, 5] and in the isolated rat hepatocyte [6, 7]. Studies with a range of substrates and direct measurement of metabolites have pinpointed the locus of inhibition at the reaction catalysed by phosphenolpyruvate carboxykinase (GTP:oxaloacetate carboxylase (transphosphorylating) EC 4.1.1.32) [2, 3, 7].

The time-course of the onset of inhibition by tryptophan is consistent with the idea that the actual inhibitor is a metabolite rather than the aminoacid itself [2, 7]. Lardy's group [1, 2, 4] have suggested that quinolinate may be the effector concerned, although other compounds have also been proposed [8].

In guinea pig liver cells, in contrast, tryptophan does not inhibit gluconeogenesis even at relatively high concentrations [6, 7]. Quinolinate is still an inhibitor in this species, but the sensitivity is lower [9, 10]; this may be, at least in part, attributable to a slow rate of penetration across the plasma membrane [9].

We now report on the effects of a larger number of tryptophan metabolites on glucose production in both rat and guinea pig liver cells, and on the related actions of appropriate inhibitors of tryptophan metabolism. The possibility that metabolites other than quinolinate may be responsible for the inhibitory effects of tryptophan in the rat is discussed.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley CSE/ASH strain rats (200–250 g) and Dunkin–Hartley guinea pigs (300–400 g) were used throughout. All animals were deprived of food for 48 hr before cell preparation.

Chemicals and biochemicals. 3-Hydroxyanthranilic acid and nicotinic acid were from Koch-Light Laboratories, Colnbrook, Bucks, U.K. and Fisons Ltd. Loughborough, U.K. respectively. All other tryptophan metabolites, *p*-chlorophenylalanine, allopurinol (4-hydroxy-pyrazole[3,4-*d*]pyrimidine), disulfiram (tetraethylthiurium disulphide), L-lactate and inulin were from the Sigma (London) Chemical Co., Poole, Dorset, U.K. Radiochemicals were from the Radiochemical Centre, Amersham, Bucks, U.K. MK-486 (Carbidopa; L-α-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid monohydrate) and pargyline (n-benzyl-*N*-methylprop-2-ynylamine) were gifts from Merck, Sharp and Dohme Labs, Rahway, NJ, U.S.A. and Abbott Laboratories, Queenborough, Kent, respectively. Other materials were of the purest grade available from standard suppliers.

Cell preparation and incubation. Cells were prepared from rats and guinea pigs by procedures detailed elsewhere [11, 12], with the exception that, in some experiments, guinea pig livers were perfused in the physiological direction from the hepatic portal vein to the inferior vena cava. Incubations followed a standard procedure [7]; specific details are given in the legends to tables. Cell 'viability' was established in each experiment from the rates of glucose production from lactate under standard conditions [3].

The uptake of [2-^{14}C]indole 3-acetate was followed as previously described [9], with 'Hems' tubes [13] being used to effect separation of cells from medium. [^{3}H]Inulin was added immediately before centrifugation [9] to act as a marker for cell pellet contamination with extracellular fluid.

Since many tryptophan metabolites interfere very strongly with the peroxidase-based glucose assay ([14]; S.A.S., unpublished work), glucose was in all cases measured in neutralised extracts with hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) and glucose 6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP^{+} 1-oxidoreductase, EC 1.1.1.49) [15].

RESULTS AND DISCUSSION

We have investigated whether the differences in sensitivity of hepatic gluconeogenesis to tryptophan [7] and quinolinate [9, 10, 16] in the rat and the guinea pig might be reflected in similar differential responses to metabolites of both the "kynurenine" and "indole" pathways. The results of this study are given in Table 1. The only compounds significantly effective at 0.5 mM

* Present address: Department of Biochemistry, University of Manchester, Manchester M13 9PL.

Table 1. Effect of tryptophan metabolites on glucose production from lactate in rat and guinea pig liver cells

Metabolite	Glucose formation (as per cent of rate with lactate alone)	
	Rat	Guinea pig
None	100	100
L-Kynurenine	46 ± 5* (3)	147 ± 18* (5)
Nicotinate	81 ± 6 (4)	95 ± 3 (3)
Picolinate	62 ± 8* (4)	101 ± 6 (3)
3-Hydroxyanthranilate	75 ± 4 (3)	115 ± 1* (3)
5-Hydroxytryptophan	80 ± 3 (5)	81 ± 12 (3)
5-Hydroxytryptamine	28 ± 6† (3)	56 ± 16* (3)
5-Hydroxyindole 3-acetate	100 ± 5 (3)	85 ± 5 (3)
Tryptamine	4 ± 2‡ (4)	14 ± 5‡ (3)
Indole 3-acetate	62 ± 6* (3)	13 ± 3‡ (3)

Hepatocytes were incubated with tryptophan metabolites (0.5 mM final concentration in medium) for 40 min before addition of lactate (10 mM final). Rates of glucose production were determined between 30 and 90 min after addition of lactate. Rates with lactate alone were 231 ± 32(4), (rat) and 192 ± 13(3), (guinea pig) nmoles glucose/hr/mg dry wt cells. All values are means ± S.E.M. with the number of independent observations in parentheses. P (vs lactate alone; Student's *t* test) * < 0.05; † < 0.01; ‡ < 0.005; other differences are not significant.

in the rat cell incubations were kynurenine, picolinate, 5-hydroxytryptamine, indole 3-acetate and, especially, tryptamine. In the guinea pig, 3-hydroxyanthranilate and, particularly, kynurenine stimulated gluconeogenesis, while indole 3-acetate was as effective as tryptamine.

The response of glucose synthesis to various concentrations of tryptamine is shown in Table 2. This metabolite is equally effective in both rat and guinea pig, producing 50 per cent inhibition at approx. 0.12 mM.

Dose–response curves for indole 3-acetate (Fig. 1) show that half-maximal effects are found at 0.6 mM in the rat and at 0.1 mM in the guinea pig. It was possible that this species difference, like that for quinolinate [9],

Table 2. Sensitivity of lactate gluconeogenesis to tryptamine in rat and guinea pig hepatocytes

[Tryptamine] (mM, final)	Glucose formation (as per cent of uninhibited rates)	
	Rat	Guinea pig
0	100	100
0.05	99 ± 3	76 ± 12
0.10	74 ± 6	66 ± 7
0.25	29 ± 10	33 ± 13
0.50	3 ± 3	14 ± 5

Hepatocytes were incubated with various concentrations of tryptamine for 40 min before addition of lactate (10 mM, final). Rates of glucose production were determined between 30 and 90 min after addition of lactate. Rates with lactate alone were 271 ± 46 (rat) and 192 ± 13 (guinea pig) nmoles glucose/mg dry wt cells/hr. All values are means ± S.E.M. from 3 independent experiments.

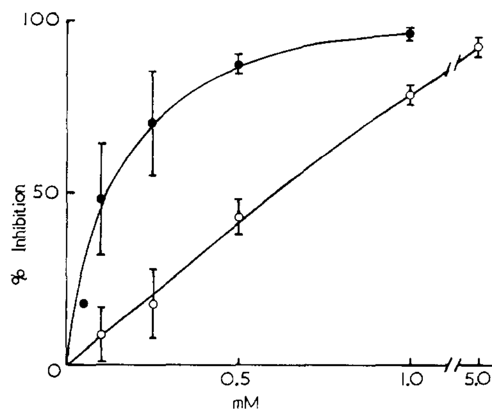

Fig. 1. Inhibition of lactate gluconeogenesis in rat and guinea pig hepatocytes by indole 3-acetate. Indole 3-acetate was added to incubations 40 min before L-lactate (final 10 mM); rates of gluconeogenesis were measured between 30 and 90 min after addition of substrate. Results are means ± S.E.M. for three independent observations or (0.05 mM-indole 3-acetate) means of two experiments. ○ Rat; ● guinea pig.

might be attributable to unequal rates of uptake of indole 3-acetate across the liver cell plasma membrane. This possibility was investigated by measuring the rate of uptake of 0.5 mM [2-^{14}C]indole 3-acetate according to the method described previously [9]. At 37°, uptake of indole 3-acetate by isolated rat liver cells obeyed simple kinetics and was completely equilibrated between cell cytoplasm and medium in 2 min. Using this procedure, however, the minimum time after addition of indole 3-acetate in which cells can be separated from incubation medium is approx. 15 sec. During this time, a quantity of indole 3-acetate, equal to that finally taken into the cell, becomes associated with the plasma membrane.

It is therefore probable that the species differences in the response to indole 3-acetate reside in intracellular events rather than rates of uptake. This is further supported by an earlier finding that gluconeogenesis in rat kidney cortex, a tissue more permeable generally than liver, is relatively insensitive towards exogenous indole 3-acetate [17].

Somewhat surprisingly, mouse liver cells resemble those of the guinea pig rather than the rat in sensitivity to indole, 3-acetate. This compound inhibited lactate gluconeogenesis by 38, 69 and 95 per cent at 0.1, 0.5 and 1 mM respectively (D. M. Crisp and C. I. Pogson, unpublished work).

A number of metabolites have been proposed as being involved in the mechanism of tryptophan-induced hypoglycaemia. These include indole 3-acetate [18, 19] and, more recently, 5-hydroxytryptamine [2]. We have investigated the possibility of the direct involvement of these metabolites with various inhibitors of tryptophan metabolism in liver. Table 3 details the dose–response of gluconeogenesis to tryptophan in hepatocytes from 48 hr-starved *p*-chlorophenylalanine-treated rats. Comparison with cells from control animals shows that *p*-chlorophenylalanine has no effect on the potency of tryptophan. Since *p*-chlorophenylalan-

Table 3. Effect of *p*-chlorophenylalanine pretreatment on the sensitivity of gluconeogenesis to tryptophan in rat hepatocytes

[Tryptophan] (mM, final)	Glucose formation (as per cent of rate with lactate alone)	
	Control cells	"Pretreated" cells
0	100	100
0.05	96 ± 3 (3)	92 ± 6 (3)
0.10	64 ± 5 (4)	63 ± 2 (3)
0.15	—	36 (2)
0.25	23 ± 7 (6)	20 ± 9 (3)
0.50	11 ± 3 (3)	6 ± 4 (3)
1.0	6 ± 2 (3)	5 ± 3 (3)

Control cells were from 48 hr-fasted rats; "pretreated" cells are those from 48 hr-fasted rats, injected 24 hr before use with *p*-chlorophenylalanine (313 mg/kg body weight, i.p.). Cells were incubated with tryptophan at various concentrations for 40 min before addition of lactate (final 10 mM). Rates of glucose production were determined between 30 and 90 min after addition of lactate. Rates with lactate alone were 217 ± 16(11), (control cells) and 275 ± 32(3), ("pretreated" cells) nmoles glucose/mg dry wt cells/hr. All values are means, ± S.E.M. where applicable, with the numbers of independent observations in parentheses. Results for control cells are recalculated from [7].

ine pre-treatment blocks the formation of 5-hydroxytryptophan [21], this metabolite and its products cannot be the responsible agents. Furthermore, measurements of tryptophan hydroxylation (S. A. Smith, unpublished work) and 5-hydroxytryptamine concentrations [22] in liver indicate that the flow through this pathway is very limited.

We have similarly found no reversal of the tryptophan effect with 0.05 mM MK-486 which blocks aromatic aminoacid decarboxylase (aromatic L-aminoacid carboxylase, EC 4.1.1.28) [23], with 0.1 mM pargyline, which inhibits monoamine oxidase (amine:oxygen oxidoreductase [deaminating] [flavin-containing], EC 1.4.3.4) [24], or with 0.1 mM disulfiram, which blocks aldehyde dehydrogenase (aldehyde:NAD^+ oxidoreductase, EC 1.2.1.3) [25], all compounds which influence the "indole" metabolism of tryptophan. These drugs are similarly ineffective when tryptophan is replaced by either tryptamine or 5-hydroxytryptamine. The failure of pargyline to alter the pattern of inhibition when these latter metabolites are present is consistent with the view that inhibition is attributable to the amines themselves rather than to the formation of further metabolites within the cell.

The difference between rats and guinea pigs in the effect of quinolinate on gluconeogenesis has been associated with the pattern of distribution of phosphoenolpyruvate carboxykinase activities within the cell [16]. If this were so, then all metabolites in the pathway from tryptophan to quinolinate in the guinea pig should show weak effects comparable to those of quinolinate itself. Kynurenine in this species, however, is an activator of lactate-dependent gluconeogenesis. Furthermore, insensitivity to tryptophan and weak effects of quinolinate, similar to those in the guinea pig, are also found with liver cells from the gerbil, whose hepatic phosphoenolpyruvate carboxykinase distribution resembles that of the rat (R. A. Muñoz-Clares and C. I. Pogson, unpublished work).

We have previously shown [20] that tryptophan-induced hypoglycaemia in rats is relieved by pre-treatment with *p*-chlorophenylalanine, and that the effect itself is attributable to formation of 5-hydroxytryptamine outside the blood–brain barrier. From the experiments reported here, it is again clear that there is no direct association between the hypoglycaemia *in vivo* and the short-term impairment of gluconeogenesis *in vitro*.

Quantitatively, the major pathway of tryptophan metabolism in both rats and guinea pigs is by the "kynurenine" pathway [26, 27]. Hence it is unlikely that any of the "indole" metabolites of tryptophan influence gluconeogenesis directly *in vivo* [20], unless present at artificially-induced pharmacological concentrations.

Acknowledgements—We are grateful to Merck, Sharp & Dohme Laboratories and Abbott Laboratories for their gifts of drugs and to the British Diabetic Association and the Medical Research Council for financial support.

REFERENCES

1. D. O. Foster, P. D. Ray and H. A. Lardy, *Biochemistry* **5**, 563 (1966).
2. P. D. Ray, D. O. Foster and H. A. Lardy, *J. biol. Chem.* **241**, 3904 (1966).
3. H. G. McDaniel, B. R. Boshell and W. J. Reddy, *Diabetes* **22**, 713 (1973).
4. C. M. Veneziale, P. Walter, N. Kneer and H. A. Lardy, *Biochemistry* **6**, 2129 (1967).
5. F. L. Alvarez and P. D. Ray, *J. biol. Chem.* **249**, 2058 (1974).
6. K. R. F. Elliott, C. I. Pogson and S. A. Smith, *Biochem. Soc. Trans.* **4**, 1048 (1976).
7. S. A. Smith, K. R. F. Elliott and C. I. Pogson, *Biochem. J.* **176**, 817 (1978).
8. M. Oravec and T. L. Sourkes, *Biochemistry* **6**, 2788 (1967).
9. K. R. F. Elliott, C. I. Pogson and S. A. Smith, *Biochem. J.* **164**, 283 (1977).
10. H-D. Söling and J. Kleineke, in *Gluconeogenesis: Its Regulation in Mammalian Species* (Eds. R. W. Hanson and M. A. Mehlman) pp. 369–462. John Wiley and Sons, New York (1976).
11. K. R. F. Elliott, R. Ash, D. M. Crisp, C. I. Pogson and S. A. Smith, in *Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies* (Eds. J. M. Tager, H.-D. Söling and J. R. Williamson) pp. 139–143. North Holland, Amsterdam (1976).
12. K. R. F. Elliott and C. I. Pogson, *Molec. Cell Biochem.* **16**, 23 (1977).
13. R. Hems, P. Lund and H. A. Krebs, *Biochem. J.* **150**, 47 (1975).
14. D. R. Nelson and A. K. Huggins, *Analyt. Biochem.* **59**, 46 (1974).
15. H.-U. Bergmeyer, E. Bernt, F. Schmidt and H. Stork, in *Methods of Enzymatic Analysis* (Ed. H.-U. Bergmeyer) 2nd edn., pp. 1196–1201. Academic Press, New York (1974).
16. H.-D. Söling, B. Willms, J. Kleineke and M. Gehlhoff, *Eur. J. Biochem.* **16**, 289 (1970).
17. C. I. Pogson, D. M. Crisp and S. A. Smith, *Acta vitam. Enzym.* **29**, 232 (1975).
18. I. A. Mirsky, G. Perisutti and D. Diengott, *Endocrinology* **59**, 369 (1956).

19. I. A. Mirsky, G. Perisutti and R. Jinks, *Endocrinology* **60**, 318 (1957).
20. S. A. Smith and C. I. Pogson, *Biochem. J.* **168**, 495 (1977).
21. B. K. Koe, *Fedn Proc.* **30**, 886 (1971).
22. C. A. Fischer and M. H. Aprison, *Analyt. Biochem.* **46**, 67 (1972).
23. J. J. Warsh and H. C. Stancer, *J. Pharmac. exp. Ther.* **197**, 545 (1976).
24. M. E. Jarvik, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. Goodman and A. Gilman) 4th edn., pp. 151–203. Macmillan, New York (1970).
25. W. D. Graham, *J. Pharm. Pharmac.* **3**, 160 (1951).
26. O. Hayaishi, H. Ijichi and A. Ichiyama, *Adv. Enzyme Reg.* **5**, 9 (1967).
27. J. K. Yeh and R. R. Brown, *J. Nutr.* **107**, 261 (1977).