The results are consistent with an inhibition of gluconeogenesis at the level of phosphoenolpyruvate carboxykinase in the rat liver cells. The time-course of the large increases in intracellular malate and aspartate, and decrease in phosphoenolpyruvate, is similar to that of the decrease in glucose output by the cells. The lack of inhibition of glucose production from glycerol or D-fructose is also consistent with an inhibition of the pathway before triose phosphate formation.

The absence of inhibition of gluconeogenesis in guinea-pig hepatocytes by 0.5 mM-L-tryptophan is yet another example of a difference between the rat and the guinea pig. There are many possible explanations for the different sensitivities to L-tryptophan. For example: (a) there may be differences in the metabolism of L-tryptophan between the two species; (b) compartmentation of metabolites may be important, in view of the differing distributions of phosphoenolpyruvate carboxykinase; and (c) the enzyme phosphoenolpyruvate carboxykinase may itself differ in its sensitivity to L-tryptophan in the two species.

This work was supported by grants from the British Diabetic Association and the Medical Research Council.


The Hypoglycaemic Action of Tryptophan in the Rat

STEPHEN A. SMITH and CHRISTOPHER I. POGSON

Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ, U.K.

The hypoglycaemic effect of tryptophan in the starved rat was initially demonstrated by Gullino et al. (1955). Mirsky et al. (1957) confirmed and extended this finding and found that the hypoglycaemic response could be reproduced by several tryptophan metabolites, namely indol-3-ylacetic acid, 5-hydroxytryptophan and nicotinic acid. Ray et al. (1966) showed that tryptophan administration caused a change in the distribution of certain hepatic metabolites which was consistent with an inhibition of gluconeogenesis at the locus of phosphoenolpyruvate carboxykinase. Lardy’s group found that tryptophan caused an inhibition of gluconeogenesis from alanine in the isolated perfused rat liver, an effect which could be duplicated by its metabolite, quinolinate (Veneziale et al., 1967).

Thus the hypoglycaemic effect of tryptophan has been variously attributed to the intracellular formation of indol-3-ylacetic acid, quinolinate, 5-hydroxytryptophan or nicotinic acid.

The present report describes a reinvestigation of this phenomenon with a view to identifying the metabolite(s) of tryptophan responsible and determining the mechanisms involved.

Tryptophan (750 mg/kg body wt.) was administered, as a suspension in 0.9% (w/v) NaCl, containing 0.1% (w/v) Tween 80, to rats intraperitoneally. All other compounds were administered intraperitoneally either as a solution in 0.9% NaCl or as a neutralized aqueous solution. Control solutions consisted of the appropriate solvent. Blood samples were taken from the tail vein, just before, and at intervals of 1 h after, injection. Plasma glucose was assayed by the glucose oxidase method (Krebs et al., 1963).

However, in those experiments involving tryptamine, 5-hydroxytryptophan and 5-hydroxytryptamine, blood samples were taken from the heart, owing to severe peripheral vasoconstriction preventing adequate blood flow to the tail. Concentrations of hepatic metabolites were determined by using standard techniques in neutralized HClO₄ extracts of liver samples obtained by the freeze-clamp technique (Wollenberger et al., 1960).

Vol. 4
Administration of L-tryptophan to rats intraperitoneally caused an initial hyperglycaemia after 1 h, a return to normal blood glucose concentrations after 3 h, followed by a period of severe hypoglycaemia; the blood glucose then returned slowly to the initial value. The effect is maximal at 5–6 h, when an 80% fall is observed. Tryptophan elicited this effect both in normal, fed rats and in those that had been starved for 36 h. The hypoglycaemic effect of tryptophan may be blocked by the pretreatment of the animal with either MK486 or p-chlorophenylalanine, inhibitors of tryptophan decarboxylase and tryptophan 5-hydroxylase respectively. This response is potentiated in rats which have been pretreated with the monoamine oxidase inhibitor, pargyline. Administration of quinolinate or indol-3-ylacetic acid in equivalent amounts to that of tryptophan produced no hypoglycaemia; indeed, indol-3-ylacetic acid caused a marked hyperglycaemia. Tryptamine when injected into pargyline-pretreated rats produced the expected central nervous-system effects, but was ineffective in altering blood glucose concentrations. Exogenous 5-hydroxytryptamine caused hyperglycaemia in rats previously treated with pargyline. The effects of 5-hydroxytryptophan in pargyline-pretreated rats resembled those of tryptophan, but occurred both earlier and at lower doses. Further, the hypoglycaemic response to tryptophan may be prevented by the specific 5-hydroxytryptamine antagonist methysergide. Adrenalectomy renders the animal more sensitive to this tryptophan treatment. Plasma insulin concentrations are also increased and correlate with the changes in glucose concentration. Experimental diabetes, induced by either alloxan or streptozotocin, abolishes the hypoglycaemic response. Livers from 36-h-starved animals, pretreated for various times with tryptophan, were freeze-clamped and assayed for metabolite changes. The effects were rapid and did not correlate in any way with the alterations in blood glucose concentration. There are, within 1 h, large increases in malate, aspartate, lactate and 3-hydroxybutyrate; the concentration of acetoacetate falls and there is no change in adenine nucleotide content. Similar changes in liver metabolite contents occur in tryptophan-treated diabetic rats.

It appears that the hypoglycaemic effect of tryptophan is not direct, but is mediated via one or more of its metabolites. The abolition of the hypoglycaemic response in animals pretreated with inhibitors of tryptophan 5-hydroxylase or tryptophan decarboxylase indicates the involvement of 5-hydroxytryptamine (or a derivative thereof). This is supported by the finding that pargyline (which decreases the rate of oxidation of 5-hydroxytryptamine) potentiates the hypoglycaemic effect of tryptophan, whereas methysergide prevents the response. The mediation of the tryptophan-induced hypoglycaemia is dependent on the presence of functional pancreatic \( \beta \) cells, suggesting that insulin is involved.

The time-course of changes in liver metabolite concentrations agrees closely with those found by Ray et al. (1966) in the intact animal, and those found by Veneziale et al. (1967) in the isolated perfused liver, but do not correlate in any way with the fluctuations in blood glucose concentrations. In the fed animal gluconeogenesis is not a major contributor to blood glucose; tryptophan, however, is as effective in fed rats as in starved rats. Similar changes in liver metabolites occur in tryptophan-treated diabetic rats. These two findings support the view that the changes in liver metabolite concentrations and blood glucose concentrations are unrelated processes. Thus, although there may be some inhibition of hepatic gluconeogenesis, the major contribution to the fall in blood glucose concentration is provided by an increased concentration of circulating insulin.

This work was supported by a grant from the British Diabetic Association.


1976