The metabolism of L-tryptophan by liver cells prepared from adrenalectomized and streptozotocin-diabetic rats

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(Received 14 July 1981/Accepted 16 September 1981)

1. The metabolism of L-tryptophan by liver cells prepared from fed normal, adrenalectomized and streptozotocin-diabetic rats was studied. 2. At physiological concentrations (0.1 mM), the rate of oxidation of tryptophan by tryptophan 2,3-dioxygenase was 3-fold greater in liver cells from diabetic rats than in those from fed rats. In liver cells from diabetic rats, oxidation of tryptophan to CO₂ and metabolites of the glutarate pathway was increased 7-fold. Quinolate synthesis was decreased by 50%. These findings are consistent with an increase in picolinate carboxylase activity. 3. Rates of metabolism of 0.1 mM-tryptophan by hepatocytes from fed and adrenalectomized rats were similar. 4. In all three types of cell preparation, fluxes through tryptophan 2,3-dioxygenase with 2.5 mM-tryptophan were 7-fold greater than those obtained with 0.1 mM-tryptophan. Tryptophan 2,3-dioxygenase and kynureninase fluxes in hepatocytes from fed and adrenalectomized rats were comparable, whereas those in liver cells from diabetic rats were increased 2.5-fold and 3.3-fold respectively. Picolinate carboxylase activities of liver cells from diabetic rats were 15-fold greater than those of cells from fed rats, but rates of quinolate synthesis were unchanged. 5. It is concluded that: (i) adrenal corticosteroids are not required for the maintenance of basal activities of the kynurenine pathway, whereas (ii) chronic insulin deficiency produces changes in both the rate of oxidation and metabolic fate of tryptophan carbon.

The oxidative metabolism of L-tryptophan by the kynurenine pathway of the liver is known to be influenced by the hormonal status of the animal. The hepatic activity of tryptophan 2,3-dioxygenase [L-tryptophan: O₂ 2,3-oxidoreductase (decyclizing), EC 1.13.11.11], the first and rate-limiting enzyme of the kynurenine pathway, is increased in diabetic rats (Mehler *et al.*, 1958; Ikeda *et al.*, 1965) and by the administration of adrenal corticosteroids to intact or adrenalectomized rats (Knox & Auerbach, 1955). Similarly, the activity of picolinate carboxylase [aminocarboxymuconate-semialdehyde carboxylase, EC 4.1.1.45], an enzyme that determines the balance of tryptophan metabolism between nicotinamide nucleotide synthesis and acetyl-CoA production, is increased markedly in the livers of pancreatectomized and diabetic rats (Mehler *et al.*, 1958).

That these hormonally induced changes in enzyme activity measured *in vitro* result in both qualitative and quantitative changes in flux of tryptophan through the various sections of the kynurenine pathway in the whole animal is suggested from measurements of urinary tryptophan metabolites. For example, in diabetic rats and in diabetic man, the urinary excretion of xanthurenic acid and 3-hydroxy-L-kynurenine is increased (Akarte & Shastri, 1974; Rosen *et al.*, 1955), whereas the excretion of *N*-methylnicotinamide has been shown to be decreased in diabetic rats (Mehler *et al.*, 1958).

Such studies, however, do not allow precise determinations of hormonal influences on kynurenine-pathway flux rates of the liver to be made, because other tissues, notably the kidney, possess the necessary enzymic complement (except tryptophan 2,3-dioxygenase) for the oxidation of tryptophan (Rose, 1972).

In the experiments described in the present paper we have examined directly the influences of adrenal corticosteroid and chronic (10 day) insulin deficiency on flux rates of tryptophan carbon through trypto-

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phan 2,3-dioxygenase and kynureninase [L-kynurenine hydrolase, EC 3.7.1.3] and to acetyl-CoA using hepatocytes isolated from adrenalectomized and streptozotocin-diabetic rats.

Some of these data have been presented in preliminary form elsewhere (Smith & Pogson, 1980a).

Materials and methods

Chemicals

Streptozotocin was kindly given by Dr. D. C. N. Earl (I.C.I. Pharmaceuticals, Alderley Park, Cheshire, U.K.). The sources of all other materials have been detailed earlier (Smith & Pogson, 1980b; Smith *et al.*, 1980).

Animals and liver cell preparation

Male Sprague-Dawley CSE/ASH-strain rats (Charles River U.K. Ltd., Manston, Kent, U.K.; inbred in this laboratory), weighing 200–250 g, were used throughout. Animals were fed *ad libitum* (no. 1 maintenance diet; Cooper Nutritional Products, Witham, Essex, U.K.). Bilateral adrenalectomy was performed as described by Smith & Pogson (1977). Diabetes was induced by intravenous injection of streptozotocin (60 mg/kg body wt. in 10 mM-sodium citrate, pH 4.5). Animals with blood glucose concentrations of less than 25 mM, 10 days after administration of streptozotocin, were discarded.

Isolated liver cells were prepared from normal fed rats, from rats 5 days after adrenalectomy and from rats 10 days after streptozotocin injection by the procedure detailed elsewhere (Elliott *et al.*, 1976). Metabolic integrity of cell preparations was assessed on the basis of cellular ATP content (Dickson & Pogson, 1977).

Cell incubation conditions and measurement of rates of tryptophan metabolism

The incubation conditions for determining fluxes through tryptophan 2,3-dioxygenase and

kynureninase and to non-aromatic products of the glutarate pathway in isolated liver cell preparations by using L-[ring-2-¹⁴C]-, L-[carboxyl-¹⁴C]- and L-[benzene-ring-U-¹⁴C]-tryptophan respectively were identical with those described previously (Smith & Pogson, 1980b; Smith *et al.*, 1980).

Expression of results

Rates of hepatocyte tryptophan metabolism, determined from radioisotope-release measurements (Smith *et al.*, 1980), are expressed as nmol of tryptophan/h per mg dry wt. of cells. There was no significant difference in the DNA content (expressed on a dry-cell-weight basis) of cells prepared from normal fed, adrenalectomized or streptozotocin-diabetic rats. The rates of metabolism of radiolabelled 0.1 mM- and 2.5 mM-tryptophan were determined simultaneously with each cell batch; these rates were linear throughout. The data for rates of tryptophan metabolism by liver cells from fed rats are taken from Smith *et al.* (1980) and have been included for comparative purposes.

Results and discussion

Metabolism of 0.1 mM-L-tryptophan by isolated liver cells

Flux rates of tryptophan carbon through tryptophan 2,3-dioxygenase and kynureninase and through the glutarate section of the kynurenine pathway in hepatocytes prepared from fed normal, adrenalectomized and streptozotocin-diabetic rats incubated with a low (0.1 mM) physiological concentration of L-tryptophan are shown in Table 1.

In liver cells from diabetic rats the flux through tryptophan 2,3-dioxygenase (flux I) was increased 3-fold compared with that of similar preparations from fed rats. Such an observation is consistent with the increased activity of tryptophan 2,3-dioxygenase when measured in homogenates of livers from diabetic rats (Mehler *et al.*, 1958; Ikeda *et al.*,

Table 1. Metabolism of 0.1 mM-L-tryptophan by liver cells isolated from fed normal, adrenalectomized and streptozotocin-diabetic rats

Liver cells were preincubated for 10 min before addition of pyruvate (10 mM). Radiolabelled L-tryptophan was added after a further 20 min. Rates of radioisotope release were determined between 30 and 90 min after addition of tryptophan (the sp. radioactivity of ¹⁴C-labelled tryptophan radioisomers was 0.25 μ Ci/ μ mol). Results are expressed as means $\pm$ S.E.M. (numbers of experiments given in parentheses). **P* < 0.05; ***P* < 0.001 versus corresponding values for fed rats (Student's *t* test).

Metabolic flux	Flux (nmol/h per mg) in:		
	Fed rats (<i>n</i> = 4)	Diabetic rats (<i>n</i> = 5)	Adrenalectomized rats (<i>n</i> = 3)
I. Through tryptophan 2,3-dioxygenase	2.71 $\pm$ 0.36	6.82 $\pm$ 0.85**	2.27 $\pm$ 0.30
II. Through kynureninase	1.60 $\pm$ 0.13	5.27 $\pm$ 0.73**	2.23 $\pm$ 0.23
III. To acetyl-CoA	0.70 $\pm$ 0.09	4.84 $\pm$ 0.26**	0.70 $\pm$ 0.21
Quinolate synthesis (flux II – flux III)	0.90 $\pm$ 0.11	0.43 $\pm$ 0.13*	1.43 $\pm$ 0.25

1965). Similarly the metabolism by kynureninase (flux II) of 3-hydroxy-L-kynurenine was increased 3-fold, although this change is probably merely a reflection of increased substrate supply, since the total tissue content of kynureninase is reported to be unchanged in diabetic-rat liver (Ikeda *et al.*, 1965). The rate of formation of by-products from kynurenine and 3-hydroxy-L-kynurenine (flux I—flux II) was similar to that of control liver cell preparations and is in agreement with studies *in vivo* in which no changes in basal rates of urinary excretion of xanthurenic acid and kynurenic acid occur in diabetic rats (Akarte & Shastri, 1974).

The rates of oxidation by diabetic-rat liver cells of tryptophan carbon to CO₂ and metabolites of the glutarate pathway (flux III) were 7-fold greater than those of cells from fed rats. The relative proportions of tryptophan carbon that enter the glutarate pathway or that are directed towards NAD biosynthesis are determined by the activity of picolinate carboxylase, because the formation of quinolinate from 2-amino-3-carboxymuconate 6-semialdehyde, the substrate of picolinate carboxylase, is non-enzymic (Bokman & Schweigert, 1951). The activity of this enzyme is increased by about 10-fold in the livers of pancreatectomized or chemically induced diabetic rats (Mehler *et al.*, 1958). Administration of insulin decreases the activity of picolinate carboxylase to normal values (Ikeda *et al.*, 1965). Hence an increased activity of picolinate carboxylase will shift the balance of tryptophan metabolism in favour of oxidation by the glutarate pathway (flux III), with a consequent decrease in quinolinate formation and NAD biosynthesis. We have previously shown that the maximal rate of quinolinate synthesis can be reliably determined from the difference between kynureninase activities (flux II) and total oxidation rates (flux III) (Smith *et al.*, 1980). In liver cells from diabetic rats, quinolinate synthesis (flux II—flux III) was decreased by 50% (Table 1). Studies *in vivo* show that diabetic rats excrete decreased amounts of

urinary *N*-methylnicotinamide (Mehler *et al.*, 1958), have a decreased hepatic content of quinolinate (McDaniel *et al.*, 1973) and have impaired conversion of tryptophan into hepatic NAD (Ikeda *et al.*, 1965).

Liver cells prepared from diabetic rats display a greatly decreased sensitivity to inhibition by tryptophan of glucose synthesis from added lactate (Smith *et al.*, 1978). The decreased rate of quinolinate accumulation observed under similar incubation conditions may be responsible for this phenomenon.

Although the activity of tryptophan 2,3-dioxygenase measured *in vitro* is lower in adrenalectomized rats than in control animals (Smith *et al.*, 1979), the rates of metabolism of 0.1 mM-L-tryptophan through the three segments of the kynurenine pathway by liver cells prepared from adrenalectomized rats were not significantly different from those determined for cells from fed rats (Table 1). These findings are in agreement with the radiotracer studies *in vivo* by Madras & Sourkes (1968), who found that adrenalectomy had no effect on the rate of oxidation of a number of tryptophan radioisomers.

Metabolism of 2.5 mM-L-tryptophan by isolated liver cells

In liver cell preparations from fed, adrenalectomized and streptozotocin-diabetic rats, increasing the concentration of tryptophan in the incubation medium from 0.1 mM to 2.5 mM produced 7-fold increases in tryptophan 2,3-dioxygenase flux (Table 2). Since the K_m for tryptophan of tryptophan 2,3-dioxygenase (from measurements *in vitro*) is about 0.1 mM (Feigelson & Greengard, 1962), such increases in flux are attributable to simple substrate-saturation of the enzyme (Smith *et al.*, 1980). Kynureninase activities of all three types of cell preparation were increased 6-fold.

In liver cells from diabetic rats, tryptophan 2,3-dioxygenase activity was 2.5-fold greater and

Table 2. *Metabolism of 2.5 mM-L-tryptophan by liver cells isolated from fed normal, adrenalectomized and streptozotocin-diabetic rats*

Liver cells were preincubated for 10 min before addition of pyruvate (10 mM). Radiolabelled L-tryptophan was added after a further 20 min. Rates of radioisotope release were determined between 30 and 90 min after addition of tryptophan (the specific radioactivity of ¹⁴C-labelled tryptophan radioisomers was 0.025 μ Ci/ μ mol). Results are expressed as means $\pm$ S.E.M. (numbers of separate experiments given in parentheses). * $P < 0.001$ versus corresponding value for fed rats (Student's *t* test).

Metabolic flux	Flux (nmol/h per mg) in:		
	Fed rats (<i>n</i> = 4)	Diabetic rats (<i>n</i> = 5)	Adrenalectomized rats (<i>n</i> = 3)
I. Through tryptophan 2,3-dioxygenase	19.00 $\pm$ 3.00	49.84 $\pm$ 5.88*	15.78 $\pm$ 1.56
II. Through kynureninase	9.20 $\pm$ 1.60	29.86 $\pm$ 4.89*	14.82 $\pm$ 1.64
III. To acetyl-CoA	1.41 $\pm$ 0.60	20.54 $\pm$ 2.30*	1.91 $\pm$ 0.34
Quinolinate synthesis (flux II—flux III)	7.79 $\pm$ 1.24	9.32 $\pm$ 4.08	12.34 $\pm$ 2.16

kynureninase activity was 3.3-fold greater than in liver cell preparations from fed rats. By virtue of these increased fluxes, the rate of formation of by-products from kynurenine and 3-hydroxy-L-kynurenine (flux I—flux II) was increased (20 nmol/h per mg for diabetic cells versus 10 nmol/h per mg for fed cells). The urinary excretion of kynurenine, xanthurenic acid and kynurenic acid is known to be increased after administration of a tryptophan load to diabetic rats (Tenconi, 1964; Akarte & Shastri, 1974) or to diabetic man (Rosen *et al.*, 1955).

In contrast, tryptophan 2,3-dioxygenase and kynureninase activities of hepatocytes from adrenalectomized rats were essentially equal; hence by-product formation from intermediates of this section of the kynurenine pathway must be minimal. Porter *et al.* (1951) and Brown & Berg (1956) found that tryptophan-loaded rats excrete less kynurenic acid but unchanged amounts of kynurenine or xanthurenic acid.

Picolinate carboxylase activities (flux III) of liver cells from diabetic rats incubated with 2.5 mM-tryptophan were 15-fold greater than those of 'fed' liver cells. Again this is indicative of a shift in the balance of tryptophan oxidation away from quinolinate formation. However, because of the greater overall flux through the kynurenine pathway in the diabetic state, rates of quinolinate formation by both 'fed' and 'diabetic' liver cells were similar (Table 2). Clearly, at low extracellular tryptophan concentrations the increased activity of picolinate carboxylase in diabetic-rat liver cells results in a decrease of quinolinate synthesis, whereas the large increases in flux that occur with a high tryptophan concentration exceed the capacity of picolinate carboxylase resulting in normal rates of quinolinate synthesis. It is noteworthy that in liver cell preparations from diabetic rats tryptophan only inhibits gluconeogenesis at high extracellular concentrations (greater than 0.5 mM) (Smith *et al.*, 1978).

Rates of quinolinate formation and total oxidation of tryptophan by liver cells from adrenalectomized rats were not significantly different from those of control cell preparations.

General discussion

It is clear from the data presented in this paper that the kynurenine pathway of tryptophan metabolism in liver cells from adrenalectomized rats displays only minor quantitative differences from that observed in cells from normal fed rats. Thus the intrinsic capacity of the enzymic system of hepatocytes for total oxidation of tryptophan does not appear to be dependent on the presence of adrenal corticosteroid hormones in the whole animal, despite the observation that the activity of tryptophan 2,3-dioxygenase, measured *in vitro*, is

depressed in the livers of adrenalectomized animals. Nevertheless, adrenal corticosteroids are important in regulating rates of tryptophan oxidation after administration of loading doses of the amino acid to whole animals. In addition to substrate-mediated enhancement, the activity of tryptophan 2,3-dioxygenase is known to be increased by adrenal corticosteroids (Knox & Auerbach, 1955).

The rate at which adrenalectomized rats metabolize a tryptophan load is much lower than in intact rats (P. Lloyd, personal communication). For example, 6 h after intraperitoneal administration to normal rats of tryptophan (750 mg/kg body wt.) the plasma tryptophan concentration is decreased to basal (about 0.05–0.1 mM) values, whereas, in adrenalectomized rats, the plasma tryptophan concentration 6 h after tryptophan administration remains elevated at about 1.5 mM. The removal of plasma tryptophan is restored to normal in adrenalectomized rats pretreated with triamcinolone.

Tryptophan is considerably more toxic in adrenalectomized than in normal rats (Smith & Pogson, 1977). This may be a consequence of the toxicity of the amino acid itself or may result from the decreased metabolism of tryptophan by the kynurenine pathway, thereby allowing increased flux through other pathways such as those of 5-hydroxytryptamine or tryptamine synthesis.

The pattern of tryptophan metabolism in liver cells from streptozotocin-diabetic rats showed a number of differences from that of the cells from fed rats. The activity of tryptophan 2,3-dioxygenase is increased markedly, suggesting that the rate of entry of tryptophan into the kynurenine pathway in the liver of the diabetic animals is increased. This contention is supported by the finding that the plasma concentration of tryptophan is decreased in diabetic rats (MacKenzie & Trulson, 1978). The high concentrations of plasma unesterified fatty acids that are associated with diabetes will also tend to increase the rate of tryptophan oxidation by increasing tryptophan availability as a consequence of decreased binding of tryptophan to plasma albumin (Smith & Pogson, 1980b). In addition, it might be predicted that inappropriately high rates of hepatic tryptophan oxidation in diabetic animals could severely restrict brain uptake of tryptophan and hence 5-hydroxytryptamine biosynthesis. Although diabetic rats do indeed have decreased brain tryptophan concentrations, brain concentrations of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetate are maintained at normal values, probably as a result of a compensatory increase in the activity of tryptophan hydroxylase (Trulson & MacKenzie, 1980).

Flux rates through picolinate carboxylase in liver cells from diabetic rats were increased 7–15-fold, depending on the extracellular tryptophan con-

centration (see Tables 1 and 2). However, quinolin-ate formation, and hence the potential for NAD biosynthesis, is impaired only at physiological tryptophan concentrations. The possibility that hepatic NAD synthesis from dietary tryptophan may be defective in diabetic man [a species in which the basal activity of picolinate carboxylase is twice that of the rat (Ikeda *et al.*, 1965)] cannot therefore be discounted.

We gratefully acknowledge financial support from the British Diabetic Association and the Medical Research Council.

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