The influence of starvation and tryptophan administration on the metabolism of phenylalanine, tyrosine and tryptophan in isolated rat liver cells

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1. Liver cells from fed Sprague–Dawley rats metabolized phenylalanine, tyrosine and tryptophan at rates consistent with the known kinetic properties of the first enzymes of each pathway. 2. Starvation of rats for 48 h did not increase the maximal activities of phenylalanine hydroxylase, tryptophan 2,3-dioxygenase and tyrosine aminotransferase in liver cell extracts, when results were expressed in terms of cellular DNA. Catabolic flux through the first two enzymes was unchanged; that through the aminotransferase was elevated relatively to enzyme activity. This is interpreted in terms of changes in the concentrations of 2-oxoglutarate and glutamate. 3. Cells from tryptophan-treated animals exhibited significant increases in the catabolism of tyrosine and tryptophan, but not of phenylalanine. The activities of tyrosine aminotransferase and tryptophan 2,3-dioxygenase were also increased, although the changes in flux and enzyme activity did not correspond exactly. 4. These results are discussed with reference to the control of aromatic amino acid catabolism in liver; the role of substrate concentration is emphasized.

In mammals, the aromatic amino acids phenylalanine and tryptophan are essential components of the diet; dietary tyrosine is supplemented by synthesis from phenylalanine. The concentrations of these amino acids in the blood are therefore a result of the balance between dietary intake, protein synthesis, protein degradation and irreversible disposal through hepatic pathways. The concentrations in the blood are particularly significant in view of the role of the amino acids as precursors of neuroactive amines in the central nervous system and elsewhere.

Protein intake and metabolism vary widely with nutritional, hormonal and other environmental stimuli; it is to be expected that the catabolism of all three amino acids is responsive to such changing circumstances and is regulated in a co-ordinated manner.

Both tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) and tryptophan 2,3-dioxygenase [L-tryptophan: oxygen 2,3-oxidoreductase (decyclizing), EC 1.13.11.11] have similar half-lives (Schimke *et al.*, 1965) and are induced by various factors, including glucocorticoid hormones, glucagon, insulin (Voigt *et al.*, 1978; Smith *et al.*, 1979; Nakamura *et al.*, 1980; Hill *et al.*, 1981; Granner & Hargrove, 1983), starvation (Brock *et al.*, 1971; Smith *et al.*, 1979;

Welch & Badawy, 1980) and tryptophan (Rosen & Mulholland, 1963; Schimke *et al.*, 1965; Yatvin & Pitot, 1970; Smith *et al.*, 1979). Nevertheless, despite the apparent similarities, there are reports that the two enzymes may not respond identically in all cases (Thompson *et al.*, 1966; Voigt & Sekeris, 1978; Smith *et al.*, 1979). In addition, both enzymes may be subject to acute control by other mechanisms, tyrosine aminotransferase by phosphorylation (Lee & Nickol, 1974; Wicks & Su, 1978) or cofactor availability (Black & Axelrod, 1969) and tryptophan 2,3-dioxygenase by interconversion of apo- and holo-enzymic forms (Badawy, 1979; Bender, 1983).

Phenylalanine hydroxylase [L-phenylalanine, tetrahydropteridine: oxygen oxidoreductase (4-hydroxylating), EC 1.14.16.1] has a longer half-life (Baker & Shiman, 1979; Goodwin, 1979), is induced by glucocorticoid hormones (Freedland, 1963; Greengard & Delvalle, 1976; Nambodiri & Ramasarma, 1978) and tryptophan (Freedland *et al.*, 1964). The activity is, however, reported to be decreased by starvation (Freedland *et al.*, 1964; Yuwiler *et al.*, 1969) and by phenylalanine administration (Freedland *et al.*, 1964). In the short term, phenylalanine hydroxylase activity is increased by phenylalanine itself (Shiman, 1980; Shiman & Gray, 1980) and by phosphorylation in-

volving the cyclic AMP-dependent protein kinase (Abita *et al.*, 1976; Donlon & Kaufman, 1978; Fisher & Pogson, 1984).

Other enzymes involved in the metabolism of the aromatic amino acids are also affected by nutritional factors. Hydroxyphenylpyruvate oxidase [4-hydroxyphenylpyruvate: oxygen oxidoreductase (hydroxylating, decarboxylating), EC 1.13.11.27] and homogentisate oxidase [homogentisate: oxygen 1,2-oxidoreductase (decyclizing), EC 1.13.11.5] activities are increased by diets of high protein content (Muramatsu *et al.*, 1975; Yamamoto *et al.*, 1979), and the latter is also steroid-sensitive (Lin & Knox, 1958). The activity of phenylalanine transaminase [L-phenylalanine (L-histidine): pyruvate aminotransferase, EC 2.6.1.58] is elevated by cortisol and in diabetes (Morris *et al.*, 1973). Glucagon (Brand & Harper, 1974a; Shih & Chan, 1979), also increases activity although this may not be reflected in increased flux *in vivo* (Brand & Harper, 1974b).

Although the factors influencing disposal of aromatic amino acids are relatively well known, the importance of each for the control of metabolic fluxes *in vivo* has been more difficult to assess. In this paper, we report on the effects of two conditions on aromatic amino acid catabolism in isolated liver cells, and relate these effects to the activities of the enzymes measured simultaneously.

Materials and methods

Animals

Male Sprague-Dawley rats (180–220 g) were used throughout. Unless otherwise stated, animals were given a standard laboratory diet (CRM diet; Labsure, Poole, Dorset, U.K.). L-Tryptophan (750 mg/kg body wt.) was administered by injection intraperitoneally (13 h and 5 h before each experiment) of a microcrystalline suspension of the amino acid (4 mg/ml) in 0.9% (w/v) NaCl containing 0.1% (w/v) Tween 80. Control animals were untreated, previous studies having shown that administration of vehicle alone was without effect.

Chemicals

All radiolabelled compounds were from Amersham International, Amersham, Bucks., U.K., except for L-[ring-2- ^{14}C]tryptophan, which was from CEA, Gif-sur-Yvette, France, through Fluorochem, Glossop, Derbyshire, U.K.

Ion-exchange resin Q15-S was obtained from Bio-Rad Laboratories (Watford, Herts., U.K.). The sources of other reagents were as given previously (Smith & Pogson, 1980; Smith *et al.*, 1980).

Purification of labelled compounds

Radiolabelled L-tryptophan was purified by affinity chromatography (Stewart & Doherty,

1973) as described in detail by Smith (1977). For phenylalanine and tyrosine, portions of freeze-dried labelled compounds (500 μCi and 50 μCi for ^3H and ^{14}C respectively) were dissolved in 0.5 ml of 0.1 M-HCl and applied to a bed (2.0 cm $\times$ 0.3 cm) of Q15-S previously equilibrated with 0.7 M-HCl in 40% (v/v) ethanol. The resin was then washed with 0.3 ml of 0.1 M-HCl and compounds were eluted with 0.7 M-HCl in 40% ethanol. Fractions (0.25 ml) were collected at a flow rate of 3 ml/h. All procedures were at room temperature. Fractions were pooled as appropriate, freeze-dried and made up in water to give solutions containing 40000 c.p.m. (^3H) or 6000 c.p.m. (^{14}C) per μl . These were freeze-dried again, reconstituted with water and stored at -20°C .

Preparation and incubation of cells

The procedures were as described previously (Smith & Pogson, 1980; Smith *et al.*, 1980) with the following amendments. (1) All experiments were started at 12:00–12:30 h each day. (2) Perfusion media contained 20 mM-D-glucose, except when animals had been previously starved. (3) Cells were washed in incubation medium (see below) without addition of albumin. (4) The incubation medium consisted of Krebs-Henseleit (1932) buffer containing 2.5 mM- Ca^{2+} and 2% (w/v) bovine serum albumin (defatted as described by Chen, 1967) and supplemented with 5 mM-D-glucose, 5 mM-L-glutamine, 4.5 mM-L-lactate and 0.5 mM-pyruvate (glucose was omitted for cells from starved animals). Glucose was used to maintain glycogen stores in cells from fed animals. Lactate and pyruvate were provided as a gluconeogenic substrate in their physiological ratio. Glutamine provided a nitrogen-containing substrate, with input into the citric acid cycle. The final cell concentration was approx. 3 mg dry wt./ml, final volume 2 ml. (5) Cells were preincubated for 25 min before addition of labelled amino acid substrate.

Flux through the tryptophan-catabolic pathway was assessed as described by Smith *et al.* (1980), that through phenylalanine hydroxylase as described by Fisher & Pogson (1984) and through tyrosine aminotransferase and hydroxyphenylpyruvate oxidase as described by Dickson *et al.* (1981), except that albumin was omitted from the medium added with charcoal. The principle of the flux determinations using ^3H - and ^{14}C -labelled substrates was to measure the respective release of $^3\text{H}_2\text{O}$, $^{14}\text{CO}_2$ and ^{14}C -labelled non-aromatic products during cell incubation. Charcoal was used to absorb the aromatic compounds in the deproteinized samples, after which $^3\text{H}_2\text{O}$ and ^{14}C -labelled non-aromatic products remained in their respective charcoal supernatants. $^{14}\text{CO}_2$ was collected as mentioned below. Flux through homogentisate

oxidase was calculated from measurements of the rates of $^{14}\text{CO}_2$ evolution and formation of non-aromatic products from L-[U- ^{14}C]tyrosine. Briefly, incubations were stopped by injection of 0.2 ml of 20% (v/v) HClO_4 into cell suspensions. $^{14}\text{CO}_2$ was collected in centre wells containing 250 μl of 2-phenethylamine/methanol (1:1, v/v, absorbed on pleated filter paper); total recovery was ensured by further incubation of flasks for 60 min at 75 oscillations/min at room temperature. Wells were then removed, their outer surfaces wiped dry, and they were added directly to 6 ml of scintillation fluid (Cocktail T; Hopkin and Williams, Chadwell Heath, Essex, U.K.) in mini-vials for counting. Rates for $^{14}\text{CO}_2$ evolution from reactions subsequent to homogentisate were obtained by subtraction of the rate of $^{14}\text{CO}_2$ production from L-[1- ^{14}C]tyrosine. Acidified suspensions were centrifuged at 1700 g for 10 min at 4°C to remove denatured protein. Portions of supernatants were neutralized with 0.5 M-triethanolamine/HCl containing 2 M-KOH. After storage on ice for 15 min, samples were centrifuged at 12000 g for 2 min (to remove KClO_4). Fractions (1 ml) were then treated with an equal volume of Norit GS-X in water (100 mg/ml, de-fined). Portions of the supernatants after centrifugation were counted for radioactivity as above. Neutralization of samples is important because acetoacetate and hydroxybutyrate are bound to charcoal in acid conditions.

A diagram of the pathways of the metabolism of radiolabelled tryptophan has been published (Smith *et al.*, 1980). Scheme 1 indicates the fate of radiolabelled phenylalanine and tyrosine in liver.

Assay of enzymes

Tryptophan 2,3-dioxygenase in cell incubations was assayed at 37°C generally as described by Metzler *et al.* (1982). Portions (1 ml) of cell suspension were centrifuged at 50 g for 2 min; the pellets were then resuspended in 0.6 ml of assay medium before sonication (three 5 s bursts, amplitude 10 μm ; MSE Soniprep 150).

Phenylalanine hydroxylase was extracted and assayed at 25°C as described by Shiman *et al.* (1979), except that cells were disrupted by three cycles of freezing (liquid N_2) and thawing (25°C).

Tyrosine aminotransferase was measured (Marston & Pogson, 1977) in cell extracts in 50 mM-potassium phosphate/2 mM-2-oxoglutarate/1 mM-EDTA/0.2 mM-pyridoxal phosphate, pH 7.6 at 30°C, after disruption as immediately above.

Cell pellets could be stored for at least a few days at -80°C without loss of enzymic activity.

Other assays

Cell viability was checked on each occasion by measurement of ATP content (Stanley & Williams,

Scheme 1. Pathways of metabolism of radiolabelled phenylalanine and tyrosine

The top line shows the sequence of metabolites in the major catabolic pathway. Numbers represent the names of the enzymes (or systems) catalysing individual reactions: (1) phenylalanine transaminase (EC 2.6.1.58); (2) phenylalanine hydroxylase (EC 1.14.16.1); (3) tyrosine aminotransferase (EC 2.6.1.5); (4) 4-hydroxyphenylpyruvate oxidase (EC 1.13.11.27); (5) homogentisate isomerase (EC 5.2.1.2); (6) maleylacetoacetate isomerase (EC 5.2.1.2); (7) fumarylacetoacetase (EC 3.7.1.2); (8) tricarboxylic acid cycle. Subsequent lines indicate the fate of specific radiolabelled forms.

1969); cell batches were discarded unless the ATP content was at least 8nmol/mg dry wt. DNA was measured as described by Burton (1956).

Results and discussion

Fed animals

The time-courses of the metabolism of all three aromatic amino acids by liver cells were followed in all experiments. With phenylalanine rates were linear for approx. 30 min only and then decreased gradually, as previously noted (Carr & Pogson, 1981). Rates of metabolism were linear with radiolabelled tryptophan, again as described previously (Smith *et al.*, 1980). Rates with radiolabelled tyrosine were also linear for at least 2 h. The time-courses remained similar for cells from starved and tryptophan-treated animals.

Rates of metabolism of the three separate amino acids are shown in Tables 1–3.

With cells from the fed (control) animals, the absolute rates of catabolism were quite similar for the three amino acids. The concentrations used in these experiments were in the physiological range, so that activities measured more closely reflect circumstances *in vivo* rather than maximal capacities. This was shown clearly in experiments with phenylalanine and tyrosine, where the fluxes through the hydroxylase and transaminase respectively were only 2–3% of maximal, as assessed from assays of the enzymes in cell-free extracts by standard procedures. With tryptophan, the rate was proportionately much higher.

The mechanism of phenylalanine hydroxylase is complex (Kaufman, 1971), so that calculation of the activity expected with 50 μ M-phenylalanine is difficult. Our previous studies (Carr & Pogson, 1981) showed, however, that this concentration of phenylalanine was associated with rates of hydroxylation approx. 10% of the maximum flux in cells. Kaufman (1971) recorded that the rate of the reaction *in vitro* with the natural cofactor is only 10% of that with the synthetic cofactor. From this information, and assuming a value for Q_{10} for the reaction of 2, we calculated that the ratio of flux to activity shown in Table 1 is very close to the expected value.

With the assumption that the mechanism of tyrosine aminotransferase is similar to that of other aminotransferases, a 'substituted enzyme' mechanism (Cornish-Bowden, 1979), it was possible to make some assessment of the expected tyrosine catabolic rate. Thus, by taking the K_m values for tyrosine and 2-oxoglutarate as 1.4 mM and 0.7 mM respectively (Hayashi *et al.*, 1967), the cytosolic concentrations of 2-oxoglutarate and glutamate as 0.7 mM and 7.8 mM respectively (Siess *et al.*, 1978), and assuming that the K_i value for glutamate is similar to its cytosolic concentration, that the concentration of 4-hydroxyphenylpyruvate is negli-

Table 1. Effect of 48 h starvation and tryptophan pre-treatment on the metabolism of L-phenylalanine by isolated rat liver cells

Conditions for the treatment of animals and for the preparation and incubation of liver cells were as described in the Materials and methods section. All incubations contained 5 mM-L-glutamine, 4.5 mM-L-lactate and 0.5 mM-pyruvate; 5 mM-D-glucose was added to incubations of cells from fed and tryptophan-treated animals. L-[4-³H]phenylalanine (final concn. 50 μ M; sp. radioactivity 1.5 Ci/mol) was added after a preincubation period of 25 min. Results are means $\pm$ S.E.M. from three independent observations; rates are expressed as (1) nmol/h per mg dry wt. of cells or (2) nmol/h per mg of DNA. Differences between means were assessed by Student's t test: P (versus fed controls) * < 0.05; ** < 0.01; other differences not significant.

Flux	Metabolic flux rates					
	Rats		Fed		Tryptophan-treated	
	(1)	(2)	(1)	(2)	(1)	(2)
Through phenylalanine hydroxylase	5.07 $\pm$ 0.27	330 $\pm$ 20	7.62 $\pm$ 0.29**	370 $\pm$ 10	4.60 $\pm$ 0.23	290 $\pm$ 20
Through hydroxyphenylpyruvate oxidase	1.14 $\pm$ 0.09	73.5 $\pm$ 5.9	1.04 $\pm$ 0.08	49.8 $\pm$ 3.9*	1.65 $\pm$ 0.12*	105 $\pm$ 9*
Through phenylalanine transaminase	0.033 $\pm$ 0.004	2.14 $\pm$ 0.24	0.028 $\pm$ 0.003	1.33 $\pm$ 0.12*	0.065 $\pm$ 0.006*	4.17 $\pm$ 0.40*
Activity of phenylalanine hydroxylase in cell extracts	232 $\pm$ 14	15101 $\pm$ 1007	357 $\pm$ 35*	17334 $\pm$ 2039	344 $\pm$ 44	21687 $\pm$ 4929
Flux through phenylalanine hydroxylase (% of enzyme maximal activity)	2.2 $\pm$ 0.1		2.1 $\pm$ 0.1		1.4 $\pm$ 0.2*	

gibly small ($<5\mu\text{M}$; Jones & Mason, 1978) and that the rate of reaction doubles for every 10°C rise in temperature, we found that the calculated rate with $100\mu\text{M}$ -tyrosine was approx. 3% of V_{max} , a value that agreed closely with the experimental value. This calculation takes into account that the presence of 5mM -glutamine in the incubation medium has no significant effect on flux through tyrosine aminotransferase (J. C. Stanley & C. I. Pogson, unpublished work). This coincidence was consistent with the view that tyrosine aminotransferase is controlled acutely by substrate availability, and suggested that modification by phosphorylation (Lee & Nickol, 1974) is not important in this respect (a conclusion supported directly by our own studies; J. C. Stanley & C. I. Pogson, unpublished work).

Similar calculations for tryptophan dioxygenase indicated that, when the K_m for tryptophan (0.17mM ; Cook, 1983) was taken into account with the fact that the concentration of free tryptophan in our incubations (with 2% albumin) was $40\text{--}50\mu\text{M}$ (Cook, 1983), the enzyme was operating in the cells as if limited by tryptophan alone. Although tryptophan dioxygenase is found in both holo- and apo-forms in liver (Badawy, 1979; Bender, 1983) and in extracts of liver cells (M. Salter, unpublished work), our results required that the total enzyme pool is fully active in its physiological environment.

The proportion of phenylalanine that is metabolized through 4-hydroxyphenylpyruvate oxidase is only 20–25% in cells from fed rats. In cells, in the absence of exogenous tyrosine and where the rate of intracellular proteolysis generates only small amounts of the amino acid (Seglen, 1975), the rate of flux through tyrosine aminotransferase (with its high K_m for tyrosine) will be low, and a large proportion of the phenylalanine metabolized will accumulate as tyrosine. Experiments in which radiolabelled phenylalanine was added to cells with various concentrations of unlabelled tyrosine gave results that were consistent with a complete equilibration of phenylalanine-derived tyrosine with exogenous tyrosine (L.-D. Luo & C. I. Pogson, unpublished work).

The proportion of phenylalanine transaminated to phenylpyruvate in these experiments was very low ($<1\%$ of that hydroxylated; Table 1), and, in particular, was lower than that found by Carr & Pogson (1981). This may have been a reflection not so much of different enzyme activities as of the fact that L-glutamine (5mM) was present in incubations in the present series of experiments. L-Glutamine is an alternative to phenylalanine as an amino donor for the transaminase (Cooper, 1977; Noguchi *et al.*, 1978) and would therefore compete effectively at the ratio of concentration used.

In contrast with phenylalanine (Carr & Pogson, 1981), tyrosine was very largely oxidized through the tricarboxylic acid cycle (Table 2). There was some loss of carbon between the amino acid and homogentisate, but nearly 80% was further catabolized.

Tryptophan was much less completely oxidized than tyrosine (Table 3). The rates observed in these experiments agreed well with previous data (Smith *et al.*, 1980).

Starved animals

Cells from the 48 h-starved rats all exhibited apparent increases in catabolic flux through the first enzymic step of each pathway (Tables 1–3). For phenylalanine hydroxylase and tryptophan dioxygenase, the assayable enzyme activities were also increased (cf. Yuwiler *et al.*, 1969, who reported a 50% decrease in the hydroxylase on starvation). When the results were corrected for the cell weight loss during starvation, however, i.e. were expressed in terms of DNA content, it was seen that the total aromatic amino acid catabolic potential in the liver was unchanged by starvation.

One effect of starvation was to decrease flux through phenylalanine aminotransferase (Table 1) to approx. 60% of the control value. This may perhaps be related to a parallel decrease in total phenylalanine aminotransferase protein, which is known to undergo fluctuations in concentration with changing nutritional and hormonal conditions (Morris *et al.*, 1973; Shih & Chan, 1979; Yamamoto *et al.*, 1982). Certainly, tyrosine aminotransferase activity in the same cells showed a small decrease (Table 2). Despite this, the flux through tyrosine aminotransferase as a function of total enzyme activity increased significantly. Siess *et al.* (1977) have shown that the cytosolic 2-oxoglutarate concentration increases in livers of animals starved for 48 h from 0.7 to 1.23mM , and that glutamate remains unchanged at 7.7mM . Such a change, interpreted with the assumptions made in the calculations above, is entirely consistent with the relative increases in the flux through the enzyme and therefore with the view that the 2-oxoglutarate concentration is a significant factor regulating tyrosine catabolism. There was a significant decrease in the proportion of tyrosine oxidized through homogentisate, again a possible reflection of changes in the cellular content of the oxygenase (Lin & Knox, 1958; Yamamoto *et al.*, 1979).

Effect of tryptophan administration

Tryptophan administration to rats resulted in significant increases in the activities of tyrosine aminotransferase and tryptophan dioxygenase (Tables 2 and 3; Smith *et al.*, 1979). Although there was no significant effect on phenylalanine

Table 2. Effect of 48 h starvation and tryptophan pre-treatment on the metabolism of L-tyrosine by isolated rat liver cells

Conditions for the treatment of animals and for the preparation and incubation of liver cells were as described in the Materials and methods section. All incubations contained 5 mM-L-glutamine, 4.5 mM-L-lactate and 0.5 mM-pyruvate; 5 mM-D-glucose was added to incubations of cells from fed and tryptophan-treated animals. L-Tyrosine [final concn. 100 µM; sp. radioactivity 0.75 Ci/mol (³H) or 0.15 Ci/mol (¹⁴C)] was added after a preincubation period of 25 min. Results are means ± S.E.M. from three independent observations; rates are expressed as (1) nmol/h per mg dry wt. of cells or (2) nmol/h per mg of DNA. Differences between means were assessed by Student's t test: P (versus fed controls) * < 0.05; ** < 0.01; *** < 0.001; other differences not significant.

Flux	Metabolic flux rates					
	Fed		48 h-starved		Tryptophan-treated	
	(1)	(2)	(1)	(2)	(1)	(2)
Through tyrosine aminotransferase	3.32 ± 0.20	214 ± 13	4.42 ± 0.10**	212 ± 6	5.00 ± 0.51*	320 ± 35*
Through hydroxyphenylpyruvate oxidase	2.88 ± 0.16	186 ± 10	3.85 ± 0.22*	185 ± 12	4.14 ± 0.22**	264 ± 17*
Through homogentisate oxidase	2.58 ± 0.23	167 ± 15	2.43 ± 0.17	117 ± 8*	3.64 ± 0.29*	232 ± 21
Activity of tyrosine aminotransferase in cell extracts	127 ± 6	8217 ± 386	127 ± 13	6087 ± 653*	387 ± 57**	24796 ± 3840*
Flux through tyrosine aminotransferase (% of enzyme maximal activity)	2.60 ± 0.06		3.55 ± 0.28*		1.31 ± 0.07***	
Flux through homogentisate oxidase (% of flux through tyrosine aminotransferase)	77.8 ± 3.6		54.8 ± 2.8**		73.1 ± 1.9	

Table 3. Effect of 48 h starvation and tryptophan pre-treatment on the metabolism of L-tryptophan by isolated rat liver cells

Conditions for the treatment of animals and for the preparation and incubation of liver cells were as described in the Materials and methods section. All incubations contained 5 mM-L-glutamine, 4.5 mM-L-lactate and 0.5 mM-pyruvate; 5 mM-D-glucose was added to incubations of cells from fed and tryptophan-treated animals. L-[¹⁴C]Tryptophan [final concn. 100 µM; sp. radioactivity 0.15 Ci/mol] was added after a preincubation period of 25 min. Results are means ± S.E.M. from three independent observations (five for cells from fed animals); rates are expressed as (1) nmol/h per mg dry wt. of cells or (2) nmol/h per mg of DNA. Differences between means were assessed by Student's t test: P (versus fed controls) ** < 0.01; *** < 0.001; other differences not significant.

Flux	Metabolic flux rates					
	Fed		48 h-starved		Tryptophan-treated	
	(1)	(2)	(1)	(2)	(1)	(2)
Through tryptophan 2,3-dioxygenase	2.58 ± 0.17	168 ± 10	4.33 ± 0.18**	170 ± 5	6.85 ± 0.05***	429 ± 15***
Through kynureninase	2.49 ± 0.14	162 ± 8	4.13 ± 0.19**	162 ± 5	6.58 ± 0.09***	412 ± 18***
To acetyl-CoA	0.38 ± 0.03	25 ± 2	0.62 ± 0.01**	24 ± 1	1.01 ± 0.07**	63 ± 6**
Activity of tryptophan 2,3-dioxygenase in cell extracts (2.5 mM-L-tryptophan)	13.7 ± 0.3	892 ± 16	24.9 ± 0.7***	979 ± 55	140 ± 7***	8792 ± 655***
Flux through tryptophan 2,3-dioxygenase (% of assayed dioxygenase activity)	18.8 ± 0.9		17.5 ± 1.2		4.9 ± 0.02***	
Flux to acetyl-CoA (% of flux through tryptophan 2,3-dioxygenase)	14.5 ± 0.4		14.2 ± 0.3		14.7 ± 0.9	

hydroxylase activity (Table 1; cf. Freedland *et al.*, 1964), the relative flux through the hydroxylase reaction was markedly decreased. The flux from phenylalanine through 4-hydroxyphenylpyruvate was greater under these conditions, an observation attributable to the elevated activity of tyrosine aminotransferase, which would thereby divert a larger proportion of the tyrosine formed towards oxidation. The conversion of phenylalanine into phenylpyruvate was also increased by tryptophan administration, again possibly as a reflection of increased transaminase activity.

The increase in tyrosine aminotransferase activity was reflected in increases in the flux through the whole catabolic pathway (Table 2). Nevertheless the relative flux through the transaminase was decreased. This may once more reflect changes in the cytosolic concentrations of 2-oxoglutarate and glutamate. Spydevold *et al.* (1974) calculated that quinolinate, which accumulates during tryptophan metabolism in the rat (McDaniel *et al.*, 1973; Smith *et al.*, 1980), causes a fall of two-thirds in cytosolic glutamate and a fall in 2-oxoglutarate to about 16% of its control value; such changes, if they persist in isolated liver cells, would be consistent with the present observations.

The increase in flux through tryptophan dioxygenase in cells from tryptophan-treated animals (Table 3) was paralleled by the increases in flux through the rest of the pathway. There was no significant change in the proportion of the carbon skeleton diverted to quinolinate and the nicotinamide nucleotides, although, in absolute terms, the flux through this part of the metabolic sequence was increased some 3-fold, consistent with observations *in vivo* (Bender, 1983). The expressed activity of tryptophan dioxygenase in the cells, although increased, was relatively much lower than the total enzyme activity. Although we cannot rule out the participation of an intracellular inhibitor such as reduced nicotinamide nucleotide (Cho-Chung & Pitot, 1967), we have shown that tryptophan uptake across the plasma membrane is relatively slow, and it is possible that tryptophan uptake plays a role in controlling flux at high dioxygenase concentrations (M. Salter & C. I. Pogson, unpublished work).

General comments

We have previously shown that tyrosine aminotransferase is the 'rate-limiting' enzyme in tyrosine catabolism (Dickson *et al.*, 1981), and it is generally assumed that phenylalanine hydroxylase and tryptophan dioxygenase play similar roles for their respective catabolic pathways. The present study indicates that the fluxes through the three enzymes are closely related to the concentrations of the enzymes themselves and that there is no intrinsic

co-ordination of the degradation of the aromatic amino acids in liver cells. Therefore, such co-ordination may rather be expressed through the concentrations of the amino acids themselves, factors which are primary regulators of the rates of catabolism.

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References

- Abita, J.-P., Milstien, S., Chang, N. & Kaufman, S. (1976) *J. Biol. Chem.* **251**, 5310–5314
- Badawy, A. A.-B. (1979) *Biochem. Soc. Trans.* **7**, 575–583
- Baker, R. E. & Shiman, R. (1979) *J. Biol. Chem.* **254**, 9633–9639
- Bender, D. A. (1983) *Mol. Aspects Med.* **6**, 101–197
- Black, I. B. & Axelrod, J. (1969) *J. Biol. Chem.* **244**, 6124–6129
- Brand, L. M. & Harper, A. E. (1974a) *Biochem. J.* **142**, 231–245
- Brand, L. M. & Harper, A. E. (1974b) *Proc. Soc. Exp. Biol. Med.* **147**, 211–215
- Brock, K. W., Krauss, E. & Fröhling, W. (1971) *Eur. J. Biochem.* **23**, 366–371
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Carr, F. P. A. & Pogson, C. I. (1981) *Biochem. J.* **198**, 655–660
- Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173–181
- Cho-Chung, Y. S. & Pitot, H. C. (1967) *J. Biol. Chem.* **242**, 1192–1198
- Cook, J. S. (1983) Ph.D. Thesis, University of Manchester
- Cooper, A. J. L. (1977) *J. Biol. Chem.* **252**, 2032–2038
- Cornish-Bowden, A. (1979) *Fundamentals of Enzyme Kinetics*, Butterworth, London
- Dickson, A. J., Marston, F. A. O. & Pogson, C. I. (1981) *FEBS Lett.* **127**, 28–32
- Donlon, J. & Kaufman, S. (1978) *J. Biol. Chem.* **253**, 6657–6659
- Fisher, M. J. & Pogson, C. I. (1984) *Biochem. J.* **219**, 79–85
- Freedland, R. A. (1963) *Biochim. Biophys. Acta* **73**, 71–75
- Freedland, R. A., Krakowski, M. C. & Waisman, H. A. (1964) *Am. J. Physiol.* **206**, 341–344
- Goodwin, B. L. (1979) in *Aromatic Aminoacid Hydroxylases and Mental Disease* (Youdim, M. B. H., ed.), pp. 5–76, Wiley, Chichester
- Granner, D. K. & Hargrove, J. L. (1983) *Mol. Cell. Biochem.* **53/54**, 113–128
- Greengard, O. & Delvalle, H. A. (1976) *Biochem. J.* **154**, 619–624
- Hayashi, S., Granner, D. K. & Tomkins, G. M. (1967) *J. Biol. Chem.* **242**, 3998–4006
- Hill, R. E., Lee, K.-L. & Kenney, F. T. (1981) *J. Biol. Chem.* **256**, 1510–1513
- Jones, D. P. & Mason, H. A. (1978) *Biochem. Biophys. Res. Commun.* **80**, 477–483
- Kaufman, S. (1971) *Adv. Enzymol. Relat. Areas Mol. Biol.* **35**, 245–319
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

- Lee, K. L. & Nickol, J. M. (1974) *J. Biol. Chem.* **249**, 6024–6026
- Lin, E. C. C. & Knox, W. E. (1958) *J. Biol. Chem.* **233**, 1186–1189
- Marston, F. A. O. & Pogson, C. I. (1977) *FEBS Lett.* **83**, 277–280
- McDaniel, H. G., Boshell, B. R. & Reddy, W. J. (1973) *Diabetes* **22**, 713–718
- Metzler, H., Gebhardt, R., Obarauch, W. & Mecke, D. (1982) *Anal. Biochem.* **121**, 10–16
- Morris, M. L., Lee, S.-C. & Harper, A. E. (1973) *J. Biol. Chem.* **248**, 1459–1468
- Muramatsu, K., Takei, M. & Nakamura, K. (1975) *J. Nutr.* **105**, 439–448
- Nakamura, T., Shinno, H. & Ichihara, A. (1980) *J. Biol. Chem.* **255**, 7533–7535
- Namoodiri, M. A. A. & Ramasarma, T. (1978) *Indian J. Biochem. Biophys.* **15**, 178–182
- Noguchi, T., Okuno, E., Takada, Y., Minatogawa, Y., Okai, K. & Kido, R. (1978) *Biochem. J.* **169**, 113–122
- Rosen, F. & Mulholland, R. J. (1963) *J. Biol. Chem.* **238**, 3730–3735
- Schimke, R. T., Sweeney, E. W. & Berlin, C. M. (1965) *J. Biol. Chem.* **240**, 322–331
- Seglen, P. O. (1975) *Biochem. Biophys. Res. Commun.* **66**, 44–52
- Shih, J. C. & Chan, Y.-L. (1979) *Arch. Biochem. Biophys.* **192**, 414–420
- Shiman, R. (1980) *J. Biol. Chem.* **255**, 10029–10032
- Shiman, R. & Gray, D. W. (1980) *J. Biol. Chem.* **255**, 4793–4800
- Shiman, R., Gray, D. W. & Pater, A. (1979) *J. Biol. Chem.* **254**, 11300–11306
- Siess, E. A., Brocks, D. G., Lattke, H. K. & Wieland, O. H. (1977) *Biochem. J.* **166**, 225–235
- Siess, E. A., Brocks, D. G. & Wieland, O. H. (1978) *Hoppe-Seyler's Z. Physiol. Chem.* **359**, 785–798
- Smith, S. A. (1977) Ph.D. Thesis, University of Kent
- Smith, S. A. & Pogson, C. I. (1980) *Biochem. J.* **186**, 977–986
- Smith, S. A., Marston, F. A. O., Dickson, A. J. & Pogson, C. I. (1979) *Biochem. Pharmacol.* **28**, 1645–1651
- Smith, S. A., Carr, F. P. A. & Pogson, C. I. (1980) *Biochem. J.* **192**, 673–686
- Spydevold, O. S., Zaheer-Baquer, N., McLean, P. & Greenbaum, A. L. (1974) *Arch. Biochem. Biophys.* **164**, 590–601
- Stanley, P. E. & Williams, S. G. (1969) *Anal. Biochem.* **29**, 381–392
- Stewart, K. K. & Doherty, R. F. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2850–2852
- Thompson, E. B., Tomkins, G. M. & Curran, J. F. (1966) *Proc. Natl. Acad. Sci. U.S.A.* **56**, 296–303
- Voigt, J. & Sekeris, C. E. (1978) *Am. J. Physiol.* **235**, E374–E380
- Voigt, J., Wieland, T. & Sekeris, C. E. (1978) *Arch. Biochem. Biophys.* **191**, 101–109
- Welch, A. N. & Badawy, A. A.-B. (1980) *Biochem. J.* **192**, 403–410
- Wicks, W. D. & Su, J. L. (1978) *J. Cyclic Nucleotide Res.* **4**, 113–122
- Yamamoto, Y., Toyoshima, R. & Muramatsu, K. (1979) *Agric. Biol. Chem.* **43**, 585–590
- Yamamoto, Y., Suzuki, T. & Muramatsu, K. (1982) *Agric. Biol. Chem.* **46**, 2491–2497
- Yatvin, M. B. & Pitot, H. C. (1970) *J. Biol. Chem.* **245**, 4673–4680
- Yuwiler, A., Geller, E. & Shapiro, S. (1969) *Can. J. Physiol. Pharmacol.* **47**, 317–328