# TYROSINE AMINOTRANSFERASE AS THE RATE-LIMITING STEP FOR TYROSINE CATABOLISM IN ISOLATED RAT LIVER CELLS

Alan J. DICKSON+, Fiona A. O. MARSTON* and Christopher I. POGSON+
*Biological Laboratory, University of Kent, Canterbury, Kent, CT2 9BT, England*

Received 9 March 1981

## 1. Introduction

In a number of species, hepatic tyrosine aminotransferase (TAT) activity increases rapidly in response to stress occassioned by nutritional or hormonal imbalance [1–5]. To a large extent the activity changes observed are attributable to direct hormonal effects on the rates of TAT synthesis or degradation in the liver [6–9]. TAT is the first enzyme in the catabolic pathway and is subject to numerous hormonal control mechanisms; it has therefore been proposed as the rate limiting enzyme for hepatic tyrosine catabolism [10]. There is, however, no direct evidence for this. From measurements of the rate of metabolism of specifically labelled tyrosine in liver cell suspensions, we have shown that TAT is indeed rate limiting for tyrosine breakdown within the intact cell. Comparison of enzyme activities in extracts with the rates of flux through TAT in whole cells under various conditions indicates that the enzyme is subject to regulation by several distinct factors in vivo.

## 2. Materials and methods

L-[*carboxyl*-$^{14}$C]Tyrosine (60 Ci/mol) and (hydroxy-[$^{14}$C]methyl)inulin (500 Ci/mol) were obtained from the Radiochemical Centre, Amersham, Bucks. Aminooxyacetate was obtained from Sigma, Poole, Dorset and all other chemicals and biochemicals were from the suppliers mentioned in [11–13].

Details of the animals used in this study and the methods of hepatocyte isolation and viability assessment have been given [11,13]. Hepatocytes (2–3 mg dry wt) were incubated for the times stated in 1.2 ml final vol. minimum essential medium (Eagles) containing 10% (v/v) dialysed, charcoal-treated foetal calf serum [13]. When present, hormones were added as 5 or 10 μl portions immediately before incubation [13]. Incubation was at 37°C with orbital shaking [11]. After the desired incubation time TAT was extracted from hepatocytes and assayed radiometrically [12]. A modification of this assay was also used to measure TAT flux (as $H_2O$ production) in the hepatocyte during incubation. L-[*side chain*-2,3-$^3$H]-Tyrosine was added to cells at the start of incubation (final spec. act. 2.06 μCi/μmol tyrosine); the reactions were terminated by inhibition of transaminase activity with aminooxyacetate (final conc. 1 mM) [14]. Preliminary experiments indicated that the presence of aminooxyacetate throughout 6 h incubations completely inhibited $^3H_2O$ production. Following centrifugation (12 000 × *g*, 4°C, 2 min), 50 μl portions of supernatants were added to 450 μl charcoal (Norit GSX) (20 mg/ml in 0.06% (w/v) bovine serum albumin). After mixing, the contents of the tubes were centrifuged (12 000 × *g*, 4°C, 2 min). Because tyrosine binds quantitatively to charcoal [12], counts remaining in the supernatant are solely $^3H_2O$. Portions (250 μl) of this supernatant were counted in 1 ml PCS.

The production of $^{14}CO_2$ from L-[*carboxyl*-$^{14}$C]-tyrosine was determined in cell incubations in glass vials (Kontes Glass Co., Wineland, NJ) fitted with centre wells; $^{14}CO_2$ was trapped in phenylethylamine: methanol (1:1, v/v) [11]. The final specific activity in incubations of L-[*carboxyl*-$^{14}$C]tyrosine was 0.045 μCi/μmol.

* Present Address: Department of Biochemistry, King's College, The Strand, London, WC2R 2LS, England

+ Present Address: Department of Biochemistry, University of Manchester, Manchester, M13 9PL, England



*Elsevier/North-Holland Biomedical Press*

Intracellular tyrosine concentrations and specific activities were determined after separation of hepatocytes from medium (20–30 mg cells dry wt in 10 ml medium) in special separation tubes [15]. Carrydown of medium with cells was estimated with (hydroxy-[$^{14}$C]methyl)inulin (final spec. act. 0.26 μCi/μmol). Intracellular contents were extracted with 4% (v/v) $HClO_4$ and 50 μl portions of extracts were counted before and after treatment with 450 μl Norit GSX suspension to measure the counts contributed by $^3H_2O$ plus L-[*side chain*-2,3-$^3$H]tyrosine and $^3H_2O$ only, respectively. The remaining volumes were neutralized and applied to columns of Amberlite CG 120 ($H^+$) (0.5 × 7 cm). After each column had been washed with 5 bed vol. water, bound tyrosine was eluted with 5 ml 5 M $NH_4OH$ and the eluants were freeze-dried. The residues were dissolved in 100 μl $H_2O$ and tyrosine contents were measured fluorimetrically [16].

Values are means ± SEM for the number of experiments indicated. In certain cases a representative experiment has been chosen and results are then presented as means ± SD for the triplicate samples at each point.

## 3. Results and discussion

Measurements of the intra- and extracellular concentrations of tyrosine and of the specific activity of the amino acid indicated complete equilibration of tyrosine between cellular water and medium during incubations of up to 2 h. We have therefore based flux measurements on the specific activity of the medium tyrosine. The rate of release of $^3H_2O$ from L-[*side chain*-2,3-$^3$H]tyrosine was dependent on the TAT activity and nutritional origin of the hepatocytes (fig.1). Significant $^3H_2O$ formation was observed after 5 min incubation. Initial rates of production of $^3H_2O$ were linear but some decrease was observed between 2–4 h incubation. During this time, the TAT activity of liver cell suspensions also decreases [13], but the decline in rate may be, in part, attributable to decreased extracellular tyrosine specific activity as a consequence of proteolysis [17].

Results obtained from $^3H_2O$ release by three preparations of fed rat hepatocytes indicated that tyrosine was metabolised at 0.103 ± 0.007 μmol . min$^{-1}$ . g dry cells$^{-1}$; fluorimetric analysis of the medium of these preparations showed the loss of 0.098 μmol tyrosine . min$^{-1}$ g dry cells$^{-1}$. This close correlation

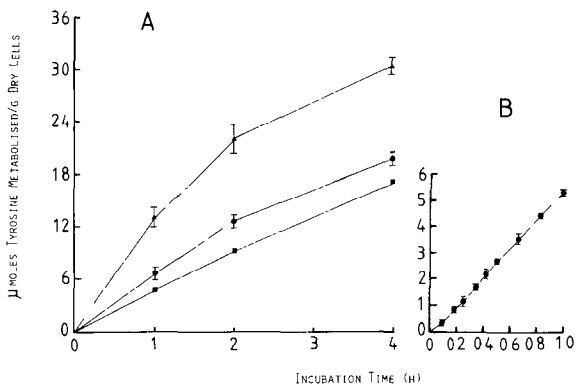


Fig.1. Rates of production of $^3H_2O$ from L-[*side chain*-2,3-$^3$H]tyrosine during hepatocyte incubations. Details of experimental conditions and isotope addition are given in the text. Initial medium tyrosine concentration was 0.18 mM.

(A) Values are means ± SEM for the conditions as follows:

| Symbol | Nutritional state of rat | No. expt. | Initial TAT activity (μmol tyrosine metabolized . min$^{-1}$ . g dry wt$^{-1}$) |
|---|---|---|---|
| (•) | Fed, intact | 4 | 1.51 ± 0.16 |
| (▪) | Fed, adrenalectomized | 2 | 0.98 ± 0.73 |
| (▲) | 48 h starved, intact | 3 | 2.12 ± 0.21 |

(B) Representative experiment with hepatocytes from fed rats. Values are means of triplicate determinations ± SD

supports the contention that $^3H_2O$ production is a good measure of tyrosine metabolism in cell suspensions. This observation also suggests that the isotope effect noted in assays of TAT in vitro with L-[*side chain*-2,3-$^3$H]tyrosine [12] is not found under cell incubation conditions. Similar discrepancies in respect of isotope effects resulting from differences in rate constants of partial reactions under various conditions have been noted previously [18,19].

The absence of a $^3$H-isotope effect was further confirmed in experiments in which the rates of metabolism as a function of tyrosine concentration were measured. The apparent $K_m$ for tyrosine is similar for the enzyme both isolated in cell homogenates and the whole cell incubations. In two independent experiments (with triplicate determinations in each), the maximal activity of TAT in cell extracts was apparently only 1.05 μmol . min$^{-1}$ . g dry wt$^{-1}$ as assayed by the $^3H_2O$ release method at 30°C [12]. When the isotope

Table 1
Comparison of the rates of $^{14}CO_2$ release from L-[*carboxyl*-$^{14}$C]tyrosine and $^{3}H_2O$ production from L-[*side chain*-2,3-$^{3}$H]tyrosine by hepatocytes from fed rats

| Time (h) | Tyrosine metabolised (nmol/mg dry wt) | |
|---|---|---|
| | Substrate: L-[*side chain*-2,3-$^{3}$H]-Tyrosine | L-[*carboxyl*-$^{14}$C]-Tyrosine |
| 1 | 7.16 ± 0.65 | 8.07 ± 0.90 |
| 2 | 15.87 ± 0.83 | 14.05 ± 1.05 |
| 3 | 18.12 ± 0.63 | 18.77 ± 1.40 |

Procedures for incubations were as given in the text. Initial tyrosine concentration was 0.18 mM. Values are means ± SEM from three independent observations

effect noted earlier [12] and the temperature are taken into account, this activity is corrected to 10.1 μmol . $min^{-1}$ . g dry $wt^{-1}$ at 37°C. This value is greater than that actually observed simultaneously in whole cell incubations with saturating concentrations of tyrosine (3.12 μmol . $min^{-1}$ . g dry $wt^{-1}$, again taking no account of potential isotope effects). The assay system in vitro, however, contains 100 mM 2-oxoglutarate. The reported $K_m$ value of TAT for 2-oxoglutarate is 2.1 mM [20]: in intact liver cells, the cytosolic concentration of 2-oxoglutarate is ~1 mM [21,22]. Taking this factor into account, the calculated activity of TAT in cell incubations is decreased from 10.1–3.3 μmol . $min^{-1}$ . g dry $wt^{-1}$, a value close to that found.

The rates of substrate flux through TAT and *p*-hydroxyphenylpyruvate oxidase using L-[*side chain*-2,3-$^{3}$H]tyrosine and L-[*carboxyl*-$^{14}$C]tyrosine, respectively, were found to be similar (table 1), and to be stimulated by glucagon ($2 \times 10^{-7}$ M) to the same extent (127.7 ± 4.6 and 132.2 ± 6.4%) of control, respectively; 3 independent obs. If TAT catalysed an 'equilibrium' reaction, then the rate of $^{3}H_2O$ formation should exceed the rate of $^{14}CO_2$ production because of rapid exchange of [$^{3}$H]tyrosine with intracellular water. Therefore one may conclude that TAT probably catalyses the rate-limiting reaction in tyrosine catabolism, despite its unpromising equilibrium constant.

Exposure of isolated cells to glucagon and triamcinolone lead to increased TAT activity (table 2). Glucagon produces an effect within 2 h which was decreased by 6 h; triamcinolone, however, is only seen to be effective after a lag phase [8]. Cell tyrosine metabolism was simulataneously increased in the presence of each hormone individually with triamcinolone having greater effects after 6 h than at shorter incubation periods. In neither case was the increase of tyrosine metabolism as marked as that of TAT activity. Both tyrosine metabolism and TAT activity maximally increased at similar concentrations of hormones (fig.2), so that the lack of correspondence between these parameters cannot be a result of differential sensitivities. Neither hormone increased the intra-cellular concentration or specific activity of tyrosine (not shown). We feel that the extent of stimulation of tyrosine metabolism by the intact cell probably reflects the internal concentration of 2-oxoglutarate. Although increasing the amount of potentially active enzyme, glucagon also decreases cellular 2-oxoglutarate concentrations [23,24] and thus produces much smaller effects on tyrosine flux than those expected from assay of TAT activity under the optimal conditions employed in vitro. No information is available on the effects of triamcinolone on cellular metabolites.

There is no simple correlation between the activity of TAT in extracts and the flux from tyrosine to *p*-hydroxyphenylpyruvate. Exposure of cells to both glucagon and triamcinolone together results in a much

Table 2
Hormonal stimulation of TAT activity and tyrosine metabolism of hepatocytes from fed rats

| Time (h) | Glucagon ($2.4 \times 10^{-7}$ M) | | Triamcinolone ($10^{-5}$ M) | |
|---|---|---|---|---|
| | Tyrosine metabolism | TAT activity | Tyrosine metabolism | TAT activity |
| 2 | 125.4 ± 1.7 | 166.6 ± 6.4 | 116.4 ± 1.3 | 150.1 ± 6.1 |
| 4 | 128.5 ± 2.7 | 169.7 ± 8.3 | 123.9 ± 3.1 | 240.3 ± 14.7 |
| 6 | 133.5 ± 2.3 | 144.9 ± 8.9 | 142.9 ± 3.7 | 208.0 ± 11.1 |

Procedures were as described in the text. Initial medium tyrosine was 0.18 mM. Values are expressed as % of the respective controls at each time point. Results are means from 4 independent obs. ± SEM

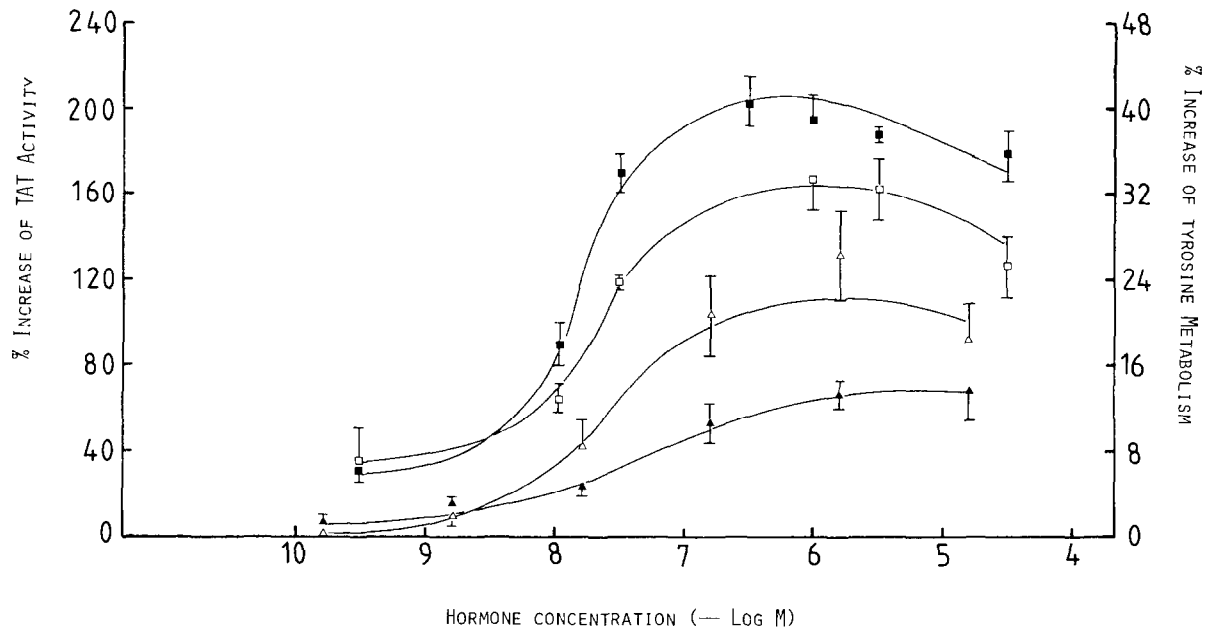


Fig.2. Effect of glucagon and triamcinolone concentration on TAT activity and the production of $^3H_2O$ from L-[*side chain*-2,3-$^3$H]tyrosine. Hepatocytes from fed rats were incubated for 5 h in the presence of 0.18 mM tyrosine. Values are expressed as % increases over control at 5 h, and are means ± SD of 3 experimental samples. Rates of cellular production of $^3H_2O$: (△) + glucagon; (□) + triamcinolone. Rates of TAT activity measured in vitro: (▲) + glucagon; (■) + triamcinolone

greater increase in assayable activity than in catabolism of tyrosine (table 3). Thus, enzyme protein concentration is not the sole determinant of activity in vivo.

Whereas the effects of the triamcinolone required >2 h incubation to become appreciable, some effect of glucagon was measurable 30 min after addition of the hormone (not shown). This would be consistent with a post-translational modification of the enzyme, as suggested [25] and experiments are in progress to investigate this possibility.

## Acknowledgements

We are grateful to the Medical Research Council for financial support and for a postgraduate studentship to F. A. O. M.

## References

[1] Lin, E. C. C. and Knox, W. E. (1957) Biochim. Biophys. Acta 26, 85–88.

Table 3
Additive effects of glucagon and triamcinolone on tyrosine metabolism and TAT activity

| Time (h) | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | Tyrosine metabolism | TAT activity | Tyrosine metabolism | TAT activity |
| 2 | 31.4 ± 2.4 (42.2) | 119.1 ± 11.3 (119.4) | 41.2 ± 8.4 (43.8) | 87.4 ± 17.3 (111.6) |
| 4 | 40.7 ± 8.2 (53.3) | 204.9 ± 17.8 (179.5) | 50.6 ± 7.4 (54.5) | 241.3 ± 17.3 (224.6) |
| 6 | 65.5 ± 7.3 (90.8) | 330.0 ± 28.2 (268.0) | 71.5 ± 10.2 (87.9) | 346.6 ± 12.3 (219.2) |

Hepatocytes isolated from fed rats were incubated with hormones singly or together. Initial medium tyrosine was 0.18 mM. Values from triplicate determinations are expressed as % increases over control value (i.e., in the absence of hormones) ± SD. Values calculated by addition of % effects observed in incubations with single hormones are given in parentheses

[2] Holten, D. and Kenney, F. T. (1967) J. Biol. Chem. 242, 4372–4377.
[3] Kido, R., Nishimo, M. and Tsuda, H. (1971) Am. J. Clin. Nutrit. 24, 766–769.
[4] Nemeth, S. (1978) Horm. Metabol. Res. 10, 144–147.
[5] Spencer, C. J., Heaton, J. H., Gelehrter, T. D., Richardson, K. I. and Garwin, J. L. (1978) J. Biol. Chem. 253, 7677–7682.
[6] Hager, C. B. and Kenney, F. T. (1968) J. Biol. Chem. 243, 3296–3300.
[7] Pariza, M. W., Kletzien, R. F., Butcher, F. R. and Potter, V. R. (1976) Adv. Enz. Regul. 14, 103–115.
[8] Ernest, M. J., Chen, C. L. and Feigelson, P. (1977) J. Biol. Chem. 252, 6783–6791.
[9] Gurr, J. A. and Potter, V. R. (1980) Exp. Cell Res. 126, 237–248.
[10] Coufalik, A. H. and Monder, C. (1980) Arch. Biochem. Biophys. 199, 67–75.
[11] Dickson, A. J. and Pogson, C. I. (1977) FEBS Lett. 83, 27–32.
[12] Marston, F. A. O. and Pogson, C. I. (1977) FEBS Lett. 83, 277–280.
[13] Marston, F. A. O., Dickson, A. J. and Pogson, C. I. (1981) Mol. Cell. Biochem. in press.
[14] Soling, H. D., Kleineke, J., Willms, B., Janson, G. and Kuhn, A. (1973) Eur. J. Biochem. 37, 233–243.
[15] Hems, R., Lund, P. and Krebs, H. A. (1975) Biochem. J. 150, 47–50.
[16] Waalkes, T. P. and Udenfriend, S. (1957) J. Lab. Clin. Med. 50, 733–736.
[17] Seglen, P. O. (1977) Biochim. Biophys. Acta 496, 182–191.
[18] Northrop, D. B. (1975) Biochemistry 14, 2644–2651.
[19] Bush, K., Shimer, V. J. and Mahler, H. R. (1973) Biochemistry 12, 4802–4805.
[20] Hayashi, S., Granner, D. L. and Tomkins, G. M. (1967) J. Biol. Chem. 242, 3998–4006.
[21] Siess, E. A., Brocks, D. G. and Wieland, O. H. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 785–798.
[22] Brocks, D. G., Siess, E. A. and Wieland, O. H. (1980) Eur. J. Biochem. 113, 39–43.
[23] Siess, E. A. and Wieland, O. H. (1978) FEBS Lett. 93, 301–306.
[24] Siess, E. A. and Wieland, O. H. (1980) Eur. J. Biochem. 110, 203–210.
[25] Wicks, W. D. and Su, J. L. (1978) J. Cyclic Nucl. Res. 4, 113–122.