REVISTA ESPAÑOLA DE FISIOLOGIA, 36, 21-26. 1980

Fasting-induced Changes of Tyrosine Transaminase Activity in Rat Tissues

A. Palou, X. Remesar,* Ll. Arola and M. Alemany

Bioquímica. Facultad de Ciencias
Universidad de Mallorca
Palma de Mallorca (Baleares). Spain

(Received on March 26, 1979)

A. PALOU, X. REMESAR, Ll. AROLA and M. ALEMANY. *Fasting Induced Changes of Tyrosine Transaminase Activity in Rat Tissues.* Rev. esp. Fisiol., 36, 21-26. 1980.

Tyrosine transaminase activity in liver, kidney, intestine, stomach, skin, adipose tissue, striated muscle and brain in fed and 24-hour fasted rats, has been studied. Maximal activity has been found in liver, with only fractional activity in the other tissues. 24 hour fasting induced significant decrease in liver and adipose tissue activity, while no changes have been detected in the other tissues. The possible implications of these facts are discussed.

Tyrosine transaminase (E. C. 2.6.1.5) regulates the initial step in the tyrosine catabolic pathway. This enzyme is mainly found in the liver (14, 33, 37), but its activity has also been detected in small amounts in other tissues as kidneys, heart, brain and stomach (20, 26, 27).

The effect of fasting upon rat tissues tyrosine transaminase has been sparsely studied. The effects upon the rat liver enzyme seem to be biphasic, as it has been described that 18-20 hours of fasting induced a significant decrease in its activity (26), but 48 hours of food deprivation provoked a significant increase (13). These changes are, however, strongly influenced by the alimentary habits of the animals (26, 51) and by other factors, because of the considerable inducibility of this enzyme by corticosteroids (27, 42, 53), adrenalin, insulin, glucagon and cyclic AMP (11, 15, 16, 18, 19, 25, 31, 38, 50) as well as growth hormone (21) and other factors. Tyrosine, the enzyme substrate, also induces the enzyme (31); glucose prevents this induction (5), and other amino acids can enhance it (6). Trauma or anesthesia provoke also a significant induction (35, 46, 47) of tyrosine transaminase activity in the liver.

It has been intended here to determine the effects of a 24-hour fast upon the activity of tirosine transaminase of several organs of the rat compared with the activity changes of liver.

* Fisiología General. Facultad de Biología. Universidad de Barcelona. Barcelona-7 (Spain).

Materials and Methods

Albino virgin Wistar female rats, weighing initially 193 ± 6 g were used. The animals were kept in a temperature (22 ± 1° C) and light controlled environment (12 hours on / 12 off). They were fed rat chow pellets *ad libitum;* the fasted group had all food removed 24 hours before sacrifice. Rats were killed, without anesthesia, by decapitation with guillotine at the beginning of the light cycle. Samples of their liver, kidneys, brain, stomach, skin, small intestine, lumbar adipose tissue and hind leg striated muscle were dissected, blotted, cleaned of its contents (stomach and intestine) and minced with scissors. Then the samples were weighed and homogenized in 10 volumes of chilled Krebs-Ringer bicarbonate buffer (8), containing 0.1 % dextran (Sigma, average molecular weight 200,000), 0.05 % bovine serum albumin (Sigma, fraction V), 5 mM 2-mercaptoethanol (Merck) and 0.1 % Triton-X-100 (Rohm and Haas) (2). The homogenization was carried on a glass-teflon motor driven Potter-Elvejhem type homogenizer. Homogenates were coarsely filtered through cheesecloth, diluted and used directly for enzyme determination. Tyrosine transaminase activity was measured with the method of DIAMOND-STONE (10) modified by GRANNER and TOMKINS (14), following the production of p-hydroxy-phenyl-pyruvate by its chromogenic reaction with KOH. Initial velocities were calculated and used for the determination of specific activities.

Aliquots of the homogenates were used for protein (30, 49) and DNA (41) estimation. Muscle mass was calculated using the method of AROLA *et al.* (1). The adipose tissue mass in the rat was assumed to be aproximately 20 % of their body weight — Wistar rats fed rat chow had a mean 66 % of fat in adipose tissue (44) and a mean of 13.6 % of body fat (44) —; thus, this factor was used for the estimative value given in the expression of results per unit of rat weight.

The use of four different expressions of enzyme activity for a better interpretation of results has been previously discussed (2); thus all data presented here are expressed per unit of animal, tissue protein and DNA weight.

Results

A 24-hour fast induced a mean 9.7 ± 0.3 % body weight loss in the fasted group studied.

In table I are shown the tyrosine transaminase activity observed in the different organs of adult fed and fasted rats. The data are expressed in μkat or nkat per 100 g of tissue weight, per gram of protein or DNA and per 100 g of animal weight (activity present in the whole organ referred to an uniform rat weight).

Tyrosine transaminase activity is maximal in the liver regardless of the expression mode selected. Per unit of tissue weight, the differences are somewhat smaller, with kidneys, adipose tissue, muscle, brain, stomach and the adipose tissue, intestine and skin, all of them in a close range between 1/12 and 1/60th of the activity of the liver. Per unit of protein weight, the differences are somewhat smaller, with kidneys, adipose tissue, muscle, stomach and brain having activities between 1/9 and 1/16 of that of liver, followed by skin and intestine, with even lower activities. The pattern is similar for the activity expressed per unit of DNA weight, being the differences even smaller, as now the intestine activity is about 1/38th of that of liver.

When expressed per unit of animal weight, the quantitative importance of liver is yet overwhelming, as is more than twice that of muscle, regardless of being the muscle mass about 10 times higher than that of liver. After the muscle activity we found those of adipose tissue, skin, kidneys, brain, intestine and stomach.

Table I. *Tyrosine transaminase activity in the organs of fed and 24-hour fasted rats.* All values are the mean ± s.e.m. of six to eight different animals. Statistical significance of the differences versus fed controls: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Organ	Feeding status	Activity in μkat/100 g of tissue	Activity in nkat/g of protein	Activity in μkat/g of DNA	Activity in nkat/100 g of animal
Liver	fed	4.91 ± 0.52	285 ± 30	7.60 ± 0.81	194.7 ± 17.1
	fasted	3.01 ± 0.22*	149 ± 10**	2.94 ± 0.20***	99.7 ± 6.9***
Kidney	fed	0.41 ± 0.05	31.2 ± 3.8	1.12 ± 0.12	3.27 ± 0.35
	fasted	0.41 ± 0.03	27.3 ± 1.9	0.89 ± 0.10	3.62 ± 0.25
Striated muscle	fed	0.22 ± 0.03	20.6 ± 0.3	1.52 ± 0.20	97.0 ± 13.2
	fasted	0.22 ± 0.02	18.0 ± 1.6	1.57 ± 0.15	97.0 ± 8.8
Brain	fed	0.19 ± 0.03	17.7 ± 2.7	1.08 ± 0.17	1.66 ± 0.29
	fasted	0.25 ± 0.01	18.9 ± 0.7	1.23 ± 0.05	2.34 ± 0.10
Stomach	fed	0.18 ± 0.03	17.9 ± 2.0	0.82 ± 0.11	0.87 ± 0.12
	fasted	0.15 ± 0.01	18.0 ± 1.5	0.88 ± 0.08	0.62 ± 0.07
Adipose tissue	fed	0.050 ± 0.007	21.3 ± 2.8	0.71 ± 0.12	10.0
	fasted	0.036 ± 0.008	5.1 ± 1.1**	0.37 ± 0.08*	7.2
Intestine	fed	0.048 ± 0.005	4.33 ± 0.40	0.20 ± 0.02	1.15 ± 0.14
	fasted	0.049 ± 0.011	3.42 ± 0.70	0.15 ± 0.03	1.54 ± 0.38
Skin	fed	0.034 ± 0.006	8.02 ± 1.29	0.20 ± 0.04	5.93 ± 1.22
	fasted	0.076 ± 0.017*	10.21 ± 2.04	0.32 ± 0.07	13.88 ± 3.21*

Fasting induces a significant decrease in the tyrosine transaminase activity of liver and adipose tissue, with a significant increase in that of skin (not observed in the expressions of activity per unit of protein and DNA weight). The combined effect of lower specific activity and smaller liver size results in a decrease of about 50 % in the liver activity expressed per unit of animal weight, thus equalling that of muscle, left unchanged by fasting as all other tissues studied.

Discussion

The degradation of tyrosine in the albino rat takes place mainly in the liver (45), being controlled by the activity of tyrosine transaminase (48), which activity is directly related to its concentration (22).

The high capacity of the liver enzyme to be induced by changes in tyrosine content of the diet (28) seems to be an effective homeostatic system for the maintenance of the tyrosine pool concentrations in the whole animal (24). This is highlighted by the higher tyrosine degrading ability of the livers from albino versus pigmented mice (34), because of the lack of activity of the melanine synthesizing pathway of the skin. The liver enzyme, as a considerably adaptative enzyme, follows closely circadian patterns with peaks reflecting the feeding schedules (26, 51, 52).

In all extrahepatic organs the activity of tyrosine transaminase per unit of tissue weight is one or two orders of magnitude lower than that of liver. The brain enzyme has been considerably studied (3, 12, 20, 32, 36). The activity in brain and intestine enzyme has been found mainly in the mitochondrial fraction (20), while liver contains only one sixth of its total activity in the mitochondria (33). This liver mitochondrial tyrosine transaminase enzyme has been found to be identical to aspartate transaminase (33); the same result has been obtained both with brain (36) and intestine enzyme (39). The lack of inducibility by stress of the brain, kidney and muscle enzyme in contrast with that of liver (35) also supports the general assumption that the extrahepatic activity found here could be the consequence of important mito-

chondrial aspartate transaminase activity in the tissues studied, as there is an important fraction of aspartate transaminase in muscle, brain and kidneys (4, 17). We have not found fasting induced changes in the tyrosine transaminase activity of these extrahepatic tissues.

The liver tyrosine transaminase activity found in this work is considerably higher than that found by GOSWAMI and CHATAGNER (13), lower than that of OHISALO (37) in the frog and much higher than that found in humans (9). The decrease observed after 24 hours of fasting in the liver enzyme is in agreement with the data found in the literature (26, 43), contrasting with the described increase of this enzyme activity after a 48 hour fast (13, 43). These changes seem to be more related to the availability of energy in the form of glucose than to the liver tyrosine pool size (13), as the circulating levels of tyrosine practically did not change during 48 hours of fasting (40). The liver and adipose tissue enzyme show significant changes with fasting, both in the expression per unit of protein and DNA weight; changes in enzyme activity that may follow rapidly the changes in liver available substrates because of the rapid turnover of the enzyme (29).

The splanchnic organs (liver, kidneys, stomach and intestine) studied showed a combined tyrosine transaminase activity in the fed state about twice as high as that of the peripheral organs (brain, muscle, skin and adipose tissue). With fasting, the peripheral organs activity remained practically unchanged, while the splanchnic bed organs activity was reduced to about one half. Thus, in the fasted state the tyrosine metabolizing activity of the combined peripheral organs is roughly similar to that of the splanchnic ones.

This lack of responsivennes in most extrahepatic tissues (except skin and adipose tissue) is in full accordance with the identification of their tyrosine transaminase activity with the aspartate transaminase. The situation in adipose tissue and skin could be tentatively explained as the consequence of an increased rate of tyrosine release from skin protein through its active proteolysis, together with the lack of capability of the albino rat skin to use tyrosine through melanine synthesis (23, 34). The decrease in tyrosine transaminase activity of adipose tissue homogenates is even more marked than that of liver; in contrast, the aspartate transaminase activity of adipose tissue increases with 24 hours of fasting (unpublished data). This seems to suggest that, probably, the skin, and specially the adipose tissue tyrosine transaminase could be an enzyme different from aspartate transaminase.

The considerable decrease of the liver and adipose tissue enzyme activity could be a consequence of the increased trend, during short term fasting, towards the conservation of alpha amino nitrogen (7), especially in the form of essential (or semiessential as in the case of tyrosine) amino acids.

Resumen

Se ha determinado la actividad tirosina transaminasa en hígado, riñón, intestino delgado, estómago, piel, tejido adiposo, músculo estriado y cerebro de ratas control y sometidas a un ayuno de 24 horas. Se ha encontrado la máxima actividad en el hígado con sólo una fracción de la misma en el resto de tejidos estudiados. El ayuno induce disminuciones estadísticamente significativas de la actividad en el hígado y tejido adiposo, sin que se observen cambios en los restantes tejidos. Se discuten las posibles implicaciones de estos fenómenos.

References

1. AROLA, LL., HERRERA, E. and ALEMANY, M.: *Rev. esp. Fisiol.*, 35, 215-218, 1979.
2. AROLA, LL., PALOU, A., REMESAR, X., HERRERA, E. and ALEMANY, M.: *Rev. esp. Fisiol.*, 34, 345-350, 1978.
3. AUNIS, D., MARK, J. and MANDEL, P.: *Life Sci.*, 10, 617-625, 1971.

4. BAUMBER, M. E. and DOONAN, S.: *Int. J. Biochem.*, **7**, 119-122, 1976.
5. BONKOWSKY, H. L., COLLINS, A., DOHERTY, J. M. and TSCHUDY D. P.: *Biochim. Biophys. Acta*, **320**, 561-576, 1973.
6. BOURDEL, G., GIRARD-GLOBA, A., FORESTIER, M. and GOUHOT-NUBEL, B.: *Biochim. Biophys. Acta*, **399**, 339-344, 1975.
7. CAHILL, G. F.: *New Eng. J. Med.*, **282**, 668-675, 1970.
8. DELUCA, H. F. and COHEN, P. P.: In «Manometric Techniques» (W. W. Umbreit, R. H. Burris and J. F. Stauffer, eds.). Burgess, Minneapolis, 1964, pp. 131-133.
9. DEL VALLE, J. A. and GREENGARD, O.: *Pediat. Res.*, **11**, 2-5, 1977.
10. DIAMONDSTONE, T. I.: *Anal. Biochem.*, **16**, 395-401, 1966.
11. EMANUEL, J. R. and GELEHRTER, T. D.: *Biochem. Biophys. Res. Commun.*, **63**, 825-831, 1975.
12. GIBB, J. W. and WEBB, J. C.: *Proc. Nat. Acad. Sci. U.S.*, **63**, 364-369, 1969.
13. GOSWAMI, M. N. D. and CHATAGNER, F.: *Experientia*, **22**, 370-371, 1966.
14. GRANNER, D. K. and TOMKINS, G. M.: In Methods in Enzymology (S. P. Colowick and N. O. Kaplan, eds.). Academic Press, New York, Vol. XVII, 1970. pp. 633-637.
15. GROSSMAN, A., BOCTOR, A. and MASUDA, Y.: *Eur. J. Biochem.*, **24**, 149-155, 1971.
16. HAGER, C. B. and KENNEY, F. T.: *J. Biol. Chem.*, **243**, 3296-3300, 1968.
17. HERZFELD, A. and GREENGARD, O.: *Biochim. Biophys. Acta*, **237**, 88-98, 1971.
18. HOLT, P. G. and OLIVER, T. T.: *FEBS Lett.*, **5**, 89-91, 1969.
19. HOLTEN, D. and KENNEY, F. T.: *J. Biol. Chem.*, **242**, 4372-4377, 1967.
20. IWASAKI, Y., LAMAR, C., DANENBERG, K. and PITOT, H. C.: *Eur. J. Biochem.*, **34**, 347-357, 1963.
21. JÄNNE, J. and RAINA, A.: *Biochim. Biophys. Acta*, **174**, 769-772, 1969.
22. KENNEY, F. T.: *J. Biol. Chem.*, **237**, 1611-1614, 1962.
23. KITANO, Y. and HU, F.: *J. Invest. Derm.*, **54**, 463-467, 1970.
24. KNOX, W. E. and GREENGARD, O.: *Adv. Enzyme. Regulat.*, **3**, 247-313, 1965.
25. LASKOWSKA-KLITA, T.: *Acta Biochim. Pol.*, **22**, 155-162, 1975.
26. LICHTENSTEIN, M. J., POTTER, VAN R. and HARPER, A. E.: *J. Nutr.* **104**, 1356-1364, 1974.
27. LINN, E. C. C. and KNOX, W. E.: *J. Biol. Chem.*, **233**, 1186-1189, 1958.
28. LITWACK, G., EBERT, P. S., YANKELOWITZ, S. and TAYLOR, N. W.: *Enzymologia*, **20**, 183-191, 1959.
29. LITWACK, G. and ROSENFIELD, S.: *Biochem. Biophys. Res. Commun.*, **52**, 181-188, 1973.
30. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J.: *J. Biol. Chem.*, **193**, 265-257, 1951.
31. MENDELSON, D., GROSSMAN, A. and BOCTOR, A.: *Eur. J. Biochem.*, **24**, 140-148, 1971.
32. MILLER, J. E. and LITWACK, G.: *Arch. Biochem. Biophys.*, **134**, 149-159, 1969.
33. MILLER, J. E. and LITWACK, G.: *J. Biol. Chem.*, **246**, 3234-3240, 1971.
34. MOJAMDAR, M. V., SHARMA, K. S. and SHAH, V. C.: *Indian J. Biochem. Biophys.*, **13**, 237-238, 1976.
35. NEMETH, S.: *Physiol. Bohemoslov.*, **26**, 557-563, 1977.
36. NOGUCHI, T., NAKATANI, M., MINATOGAWA, Y., OKUNO, E. and KIDO, R.: *J. Neurochem.*, **25**, 579-582, 1975.
37. OHISALO, J. J.: Thesis. University of Helsinki, 1977.
38. OHTSUKA, H.: *J. Biochem.*, **75**, 53-58, 1974.
39. OKUNO, E., MINATOGAWA, Y., NOGUCHI, T. and KIDO, R.: *Life Sci.*, **16**, 883-890, 1975.
40. PALOU, A., ALEMANY, M. and HERRERA, E.: *Horm. Metab. Res.*, **10**, 482-489, 1978.
41. PRASAD, A. S., DUMOUCHELLE, E., KONIUCH, D. and OBERLEAS, D.: *J. Lab. Clin. Med.*, **80**, 598-602, 1972.
42. SAMUELS, H. H. and TOMKINS, G. M.: *J. Mol. Biol.*, **52**, 57-74, 1970.
43. SAPICO, V., SHEAR, L. and LITWACK, G.: *J. Biol. Chem.*, **249**, 2122-2129, 1974.
44. SCHEMMEL, R.: *Amer. Zool.*, **16**, 661-670, 1976.

45. SCHEPARTZ, B.: In «Regulation of amino acid metabolism in mammals». Saunders, Philadelphia, 1973.
46. STEFAN, M., BOERESCU, J. and GHEORGHE, N.: *Rev. Roum. Physiol.*, **12**, 303-305, 1975.
47. STEFAN, M. and VLADESCU, C.: *Agressologie*, **16**, 273-277, 1975.
48. TANAKA, K. and ICHIHARA, A.: *Biochim. Biophys. Acta*, **399**, 302-312, 1975.
49. WANG, C. S. and SMITH, R. L.: *Anal. Biochem.*, **63**, 414-417, 1975.
50. WICKS, W. D.: *Science*, **160**, 997-998, 1968.
51. WURTMAN, R. J.: *Life Sci.*, **15**, 827-847, 1974.
52. WURTMAN, R. J. and AXELROD, J.: *Proc. Natl. Acad. Sci. U.S.*, **57**, 1594-1596, 1967.
53. YAMAMOTO, K. R. and ALBERTS, B. M.: *Ann. Rev. Biochem.*, **45**, 721-746, 1976.