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VARIATION OF TYROSINE AMINOTRANSFERASE EXPRESSION DURING THE DAY IN RATS OF DIFFERENT AGES

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The activity of the enzyme tyrosine aminotransferase and the synthesis of its specific mRNA were evaluated at different hours of the day in the liver of 3-, 12- and 24-month old BN rats. The enzyme activity has a circadian rhythm with a peak at midnight in 3- and 12-month old, which shifts to 03.00 hrs in 24-month old animals, in agreement with previous results. The expression of TATmRNA also changes during the day indicating circadian fluctuations which change with age. In 3-month old rats the TATmRNA peak is at 19.00 hrs, preceding that of the enzyme activity. In 12-month old rats the TATmRNA synthesis reaches a maximum at midnight and in 24-month old rats at 03.00 hrs. The results show that the circadian rhythm of tyrosine aminotransferase activity is due to a different gene expression throughout the day, which is influenced by age.

Tyrosine aminotransferase (EC 2.6.1.5; TAT) is a specific liver enzyme, which is rate-limiting for tyrosine catabolism (1). The activity of TAT changes during hepatocyte maturation (2) and is regulated by several hormones (3-5) and dietary factors (6). Also during the day there are marked variations in the activity of the enzyme related to the time of the day, with the features of a circadian rhythm (7). The circadian rhythm appears in 10-day old rats and is maintained in the following ages. The peak of TAT in the liver of 10-day old rats is at 03.00 hrs; after weaning it is anticipated to midnight and remains unchanged up to the age of 18-months; thereafter the peak shifts again to 03.00 hrs (8). The variations in activity represent probably changes in enzyme synthesis because TAT inactive form was not found (9).

The mechanisms responsible for this circadian rhythm are not completely understood. Glucocorticoid influences have been excluded, since the rhythm

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is still present in adrenalectomized and hypophysectomized rats (10). Diet composition seems to play a role because carbohydrates reduce TAT activity (11), whereas proteins increase it (12). In an artificial dark/light cycle in which the animals are fasted for 22 hrs (light period) and food is available only for 2 hrs during the dark, the increase from 12 to 60% of protein content in the diet causes a sharp increase in TAT activity , which is preceded by an increase in TATmRNA occurring 30 min after feeding (9). The dietary induction cannot, however, be held responsible for the spontaneous TAT rhythm, since fasting for 48 hrs influences the basal level of TAT, but the midnight peak is maintained (8).

Moreover, up to now there is no evidence to show whether the diurnal variations in enzyme activity are accompanied by variations in TATmRNA expression. In the present study the correlation between the circadian rhythm of TAT and gene expression is addressed in order to clarify whether the regulatory mechanisms act at the transcriptional or translational levels.

MATERIALS AND METHODS

Male BN rats 3-, 12- and 24-month old were used. The animals and the food were kindly provided by Dr. Knook of the Institute for Experimental Gerontology TNO, Rijswijk, Holland, under the Eurage program. The rats were conditioned for 20 days to a controlled light/dark cycle (light from 07.00 hrs to 19.00 hrs.) and allowed free access to food only during the dark period, with water ad libitum. The animals of each age were divided into 5 groups of 3 rats each and sacrificed at 03.00, 06.00, 09.00, 19.00 and 24.00 hrs respectively. The liver was quickly removed from animal in deep anaesthesia and divided into two parts for the evaluation of enzyme activity and TATmRNA respectively.

Enzyme assay

The enzyme activity was determined by the Diamondstone reaction (13) modified by Granner and Tomkins (14) and expressed as μM of p-hydroxyphenilpyruvate/h/g of fresh liver. The detailed protocol is described by Magni and Viola (15).

Poly(A)$^+$ RNA purification

The frozen tissue was ground in 4M guanidium isothyocyanate (Boehringer Mannheim Gmbh), 5mM sodium citrate, 0.1M ß-mercaptoethanol, 0.5% sarkosyl (Sigma Chemical Co.). After the addition of $CsCl_2$ (Boehringer Mannheim Gmbh), 1 g/ 2.5 ml of homogenate, the sample was layered on a cushion of 5.7M $CsCl_2$ in 0.1M EDTA pH 7.5. Separation of total RNA was performed by centrifugation at 20°C for 14 hrs at 105,000 x g (16). The pellet was resuspended in 10mM Tris HCl pH 7.4, 5mM EDTA, 1% SDS (Sodium Dodecyl Sulphate), and the RNA was extracted twice with chloroform-isoamylic alcohol 4:1 (17). The poly(A)$^+$ RNA was purified by oligo dT cellulose (Sigma Chemical Co) chromatography (17).

Northern blot analysis

Poly(A)$^+$ RNA (2 μg) was fractioned by electrophoresis on 1% agarose (Sigma Chemical Co)/2.2M formaldehyde (Carlo Erba) and then blotted on nitrocellulose membrane (Schleicher & Schuell). The filter was baked for 2 hrs at 80°C and then prehybridized for 4 hrs at 42°C in a solution containing 50% formammide (Carlo Erba), 3X SSC (0.15M NaCl, 0.015 sodium citrate), 100mM TrisHCl pH 7.5, 5X Denhardt's solution made with 0.1% Ficoll (Sigma Chemical Co.), 0.1% polyvinylpyrrolidone (Merck), 0.1% Bovine Serum Albumine (Sigma Chemical Co.), 10% dextran sulfate (Sigma Chemical Co), 100 μg/ml denaturated salmon sperm DNA (Sigma Chemical Co). Hybridization was carried for 16 hrs at 42°C in the same solution plus the pcTAT-3a probe (600 bp Pst I fragment kindly provided by Dr. W. Schmid) labelled with (α^{32}P)dCTP (Amersham), specific activity 2-5 x 10^8 dpm/μg DNA, by the "nick translation" method (Boehringer Nick Translation system). The filters were washed 3 times at room temperature for 20'and then 3 times at 60°C for 30' with 0.1X SSC, 1% SDS. The autoradiographs were performed for 18 hrs at -80°C using Kodak X Omat AR films.

RESULTS AND DISCUSSION

TAT activity

In 3-month old rats the TAT activity changed during the day showing a circadian rhythm with a peak at midnight (Fig. 1A). This result was the same as that obtained with other strains, as previously reported (8). The same rhythm was evidenced in 12-month old rats (Fig.1B), whereas in 24-month olds the TAT peak was around 03.00 hrs (Fig.1C).

In a previous work with 18-month old Sprague Dawley rats it was shown that the TAT activity at 03.00 hrs did not differ significantly from that found at midnight. This result was confirmed in 36-month old rats of the same strain (8).

TATmRNA

The TATmRNA was not expressed at the same level during the day. In 3-month old rats the peak was observed at 19.00 hrs, whereas the lowest values were observed at 03.00 hrs and midnight (Fig.2A). This behaviour was similar to that reported for 50-day old of Sprague Dawley animals (18).

In 12-month old rats the TATmRNA peak was at midnight, then decreased strongly at 03.00 hrs and reached an almost steady value at 06.00 hrs and 09.00 hrs (Fig.2B). An evident increase of transcription was observed at 19.00 hrs just before the midnight peak. Comparison of the data of 3- and 12-month old rats shows that aging delaies the TATmRNA peak.

This result was confirmed in 24-month old rats in which the peak was at 03.00 hrs (Fig.2C). The expression of mRNA was strongly reduced at 06.00

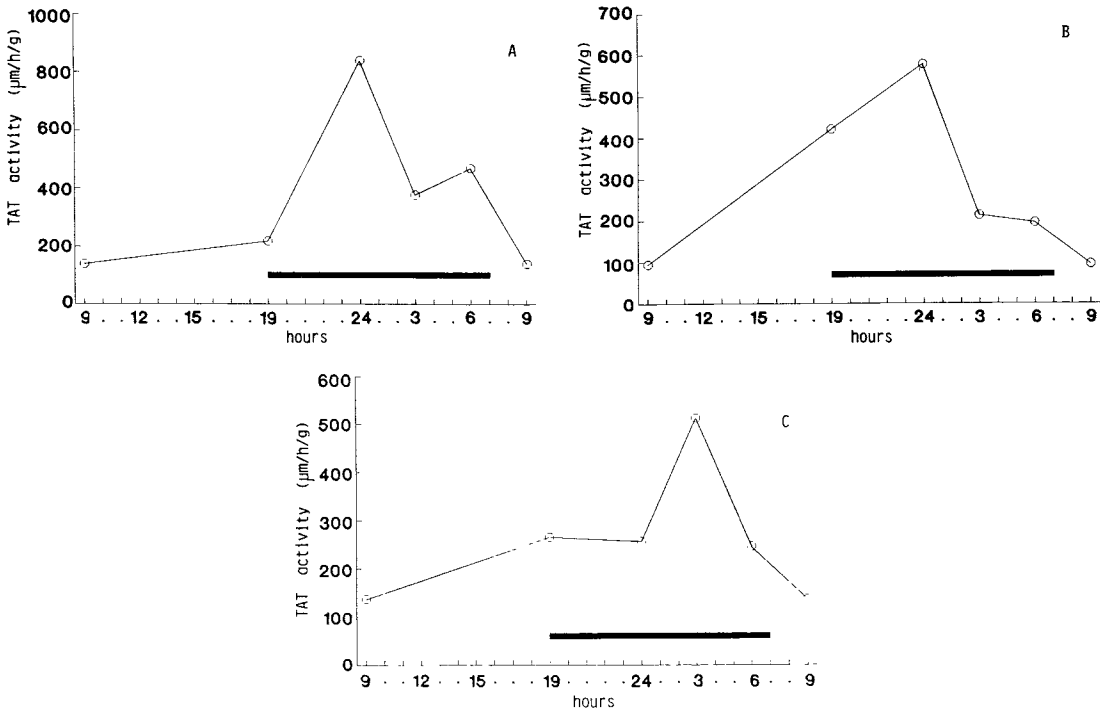

Fig. 1
TAT activity circadian rhythm. TAT activity was evaluated at different times of the day in 3-month old (Panel A), 12-month old (Panel B) and 24-month old rats (Panel C). TAT activity was expressed as μM of p-hydroxyphenilpyruvate/h/g of fresh liver. Black bar indicates the dark period.

hrs, then began to increase at 09.00 hrs. A further increase was evident at 19.00 hrs, followed by a small decrease at midnight. It was also evident that the TATmRNA values at 19.00 hrs were elevated at all ages even if the peak was shifted to midnight or 03.00 hrs.

The results of the present investigation show that the TAT circadian rhythm is accompanied by differences in mRNA synthesis during the day. The influence of age, which was previously found in studying enzyme activity, appears more evident with the analysis of TAT expression. In fact, the peak of mRNA is at 19.00 hrs in 3-month old rats, shifts to midnight in 12-month old rats and to 03.00 hrs in 24-month old rats, whereas the peak of enzyme activity is at midnight and only in 24-month old animals there is an evident increase at 3 with respect to midnight. The shift of the peak appears better described by the mRNA behaviour than by the enzyme activity.

These results also contribute to the clarification of the possible role of the regulatory mechanisms. The influence of feeding can be excluded, since

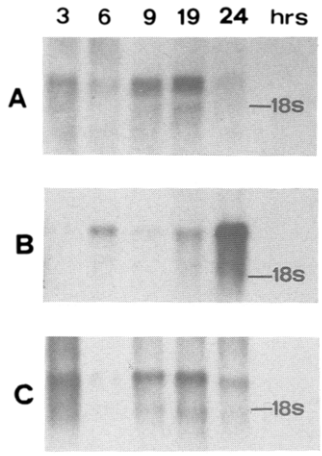

Fig. 2
Detection of TAT messenger RNA in rat liver during the day in 3-month old (Panel A), 12-month old (Panel B) and 24-month old rats (Panel C). Poly(A)$^+$RNA (2µg/lane) from rat at 3 (lane 1), 6 (lane 2), 9 (lane 3), 19 (lane 4) and 24 (lane 5). Poly(A)$^+$RNA was subject to Northern blot analysis and hybridized with radiolabeled rat TAT cDNA. Location of 18 S is indicated.

in 3-month old rats the peak of mRNA is at 19.00 hrs just before the dark feeding period. The possibility that hypoglycemia, developed as a consequence of fasting, stimulates secretion of glucagon which in turn could influence the expression of TATmRNA, cannot be applied to the 12-month old animals because the mRNA peak is at midnight, 4 hrs after feeding. This is also in agreement with the results that fasting for 48 hrs does not abolish the circadian rhythm (8). Other regulatory mechanisms must therefore be taken into account.

A possible endogenous rhythm cannot be excluded, since it has been demonstrated that the TAT circadian rhythm is also present in hepatocyte cultures (19). In the light of this hypothesis the peak variation during aging could be caused by modifications of some endogenous modulating factors.

Whathever the mechanisms implicated in the modulation of TAT activity during the day, the present results indicate clearly that they act at the level of TAT gene expression.

Furthermore, our observation suggest that the evaluation of enzyme activity gives only a coarse estimate of changes occurring with age, and that a prerequisite for elucidating this phenomena is a fine investigation at the gene expression level.

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REFERENCES

1. Tanaka, K. and Ichihara, A. (1975). Biochim. Biophys. Acta, **399**, 302-312.
2. Greengard, O. (1970). In Mechanism of Hormon Action, G.Litwack.Vol I pp. 3-85, New York: Academic Press.
3. Lin, E.C.C. and Knox, W.E., (1957). Biochim. Biophys. Acta, **26**, 85-88.
4. Nichol, C.A. and Rosen, F., (1964). In Action of Hormones on Molecular Processes. Litwack G. and Kritchevsky D. Eds. Wiley New York pp 234-256.
5. Csanyi, V., Greengard, O. and Knox, W.E. (1967). J. Biol. Chem. **242**, 2688-2692.
6. Watanabe, M., Potter, V.R. and Pitot, H.C. (1968). J. Nutr., **95**, 207-227.
7. Wurtman, R.J. (1974). Life Science, **15**, 827-847.
8. Viola-Magni, M.P. and Magni, F. (1986). In Modern Trends in aging research. **147**, 141-146, John Libbey Eurotext Ltd.
9. Granner, D.K. and Hargrove, J.L. (1983). Mol. Cell. Biochem., **53/54**, 113-128.
10. Wurtman, R.J. and Axelrod, J. (1967). Proc. Natl. Acad. Sci., USA, **57**, 1594-1598.
11. Peraino, C., Lamar, C. Jr. and Pitot, H.C. (1966). Adv. Enz. Reg. **4**, 199-217.
12. Yanage, S., Campbell, H.S. and Potter, V.R. (1975). Life Science, **17**, 1411-1422.
13. Diamondstone, T.I. (1966). Anal. Biochem., **16**, 395-401.
14. Granner, D.K. and Tomkins, G.M. (1970). In Methods in Enzymol. **17A**, 633-637.
15. Magni, F. and Viola, M.P. (1985). J. Auton. Nerv. Syst., **13**, 245-254
16. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979). Biochemistry, **18**, 5294-5299.
17. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1989). Molecular cloning: A laboratory manual. Cold Spring Harbor, New York Cold Spring Harbor Laboratory.
18. Della Fazia, M.A. Servillo, G. and Viola-Magni, M.P. (1990). In Metabolismo della cellula in Patologia spontanea e sperimentale. XX Congresso Nazionale. Società Italiana di Patologia pp. E12-E14.
19. Hardeland, R. (1972). J. Interdis. Cycle Res. **3**, 109-114.