BIOSYNTHETIC CONTROL OF THE DIURNAL RHYTHM OF TYROSINE α-KETOGLUTARATE TRANSAMINASE ACTIVITY IN RAT LIVER

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SUMMARY

Tyrosine α-ketoglutarate transaminase in rat liver shows diurnal rhythmic changes. Assay of the TAT antigen levels of liver extracts with antibody to TAT showed that at all times during the diurnal cycle there was a fixed relationship between enzyme activity and enzyme antigen levels. When rats were pulse labeled with isotopic amino acids and the labeled TAT isolated by immunoprecipitation, the kinetics of labeling the enzyme showed that maximal incorporation into TAT occurred several hours before peak TAT activity was reached. These studies indicate that the diurnal rhythm of TAT is controlled by a variable rate of TAT synthesis not involving the formation of a cross-reacting precursor or breakdown product.

Induction of the enzyme tyrosine aminotransferase (TAT) has been studied extensively in rat liver (1) and in hepatoma tissue culture cells (2). Adrenal steroid hormones enhance the rate of synthesis of TAT in both of these systems (1, 3) and glucagon and insulin increase the rate of synthesis in the liver (4). Recently, several groups showed that hepatic TAT activity varies four-fold throughout the day with the peak occurring approximately five hours after onset of the dark cycle (5). This rhythm, although shifted somewhat in phase, persists in the adrenalectomized or hypophysectomized animal. Therefore fluctuations in serum steroids are not responsible.

Although several etiological explanations have been offered, the basic question of whether this fluctuation in TAT activity is accompanied

by changes in the amount of TAT protein has heretofore not been investigated.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 150-200 g. were housed in a room with controlled lighting (light on between 3:00 PM and 3:00 AM) for a period of 3 weeks prior to the experiment. Animals were sacrificed at the indicated time by decapitation and 25% (w/v) liver homogenates were prepared in 0.1 M potassium phosphate buffer, pH 6.5. Following centrifugation for 30 min. at 30,000 x g., the supernatant fractions were further clarified by centrifugation at 55,000 x g. for 1 hour. These extracts were then frozen in a dry ice-acetone bath and stored at -20°C until tested. TAT was assayed by the method of Diamondstone (6) and protein by that of Lowry et al (7).

Addition of specific rabbit anti-TAT serum to an enzyme solution results in inactivation of the enzyme. The extent of inactivation is directly proportional to the amount of antiserum added until about 60% of the TAT activity is lost (3). Aliquots from each time point were assayed and diluted to a level of TAT activity (same for all extracts) which gave a linear neutralization curve with serial dilution of antiserum. The slope of the inactivation curves between 0.0 and 60.0% was taken as a measure of the antigenic content of the solution.

The pulse labeling and immunoprecipitation of TAT were carried out according to the methods developed by Granner, et al (3). Rats cycled as described above, were injected intraperitoneally with either a ^{3}H or ^{14}C amino acid mixture (NET-250 and NEC-445, New England Nuclear) 20 minutes prior to killing. Animals of the 9 PM group received 20 μCi of ^{14}C whereas those of all other groups received 50 μCi of ^{3}H. After aliquots for assays of TAT activity,

protein and protein specific radioactivity were removed, quantitative isolation of the labeled TAT by specific immunoprecipitation was accomplished as follows. Two and a half ml of each ^{3}H labeled extract were mixed with an equal volume of the ^{14}C extract (since TAT activity was lowest at 9 PM this time was taken as the basal group). This double isotope method was employed in order to increase the precision of our data by imposing the same experimental conditions in isolating the antigen-antibody precipitates of the samples to be compared. The combined extracts were then partially purified by heating (3) and each was adjusted to the same TAT activity by addition of a calculated amount of highly purified enzyme. A volume of rabbit anti-TAT serum that would give quantitative precipitation was added and the mixture was first incubated at 37° for 1 hour then at 4°C for 1 hour. Following centrifugation at 30,000 rpm for 20 minutes, the supernatant was removed and subjected to a repeat identical immunoprecipitation. The precipitates were washed 3 times with 2.0 ml of 0.1 M potassium phosphate buffer, pH 7.6, and then were dissolved in 10 ml of scintillation counting fluid (5% Biosolv-3 in Fluoralloy, Beckman Instruments). The aliquots of soluble extract for determination of total protein specific radioactivity were precipitated with cold 12% TCA, washed 3 times with 6% TCA, once each with alcohol and chlorofrom: alcohol (2:1), twice with ether, then dissolved in 2N NaOH and Biolsolv-2 and placed in Fluoralloy. Samples were counted in a Beckman liquid scintillation spectrometer and CPM were corrected for quenching, channel spill and efficiency. The second TAT immunoprecipitation represents non-specific counts so this was subtracted from the first to give net TAT counts. The latter was then divided by the appropriate total protein specific radioactivity to correct for differences in labeling of the respective amino acid pools. Since the pulse time was short in comparison to the turnover time, this number actually represents the rate of synthesis of TAT.

TABLE I

DIURNAL CHANGES IN TAT ACTIVITY AND SLOPES OF IMMUNOPRECIPITATION CURVES

Time ‡	TAT Activity m_{μ}moles/mg/min	Slope
9 PM	28.4 ± 3.4	0.2855 ± 0.0148
4 AM	72.6 ± 6.0	0.2978 ± 0.0080
7 AM	122.8 ± 10.6	0.2813 ± 0.0225
10 AM	88.2 ± 4.4	0.2508 ± 0.0076
2 PM	46.1 ± 4.8	0.2856 ± 0.0081

‡ Six or eight rats per group.

RESULTS AND DISCUSSION

Table I shows the results of an immunotitration experiment. The second column shows the typical diurnal variation in TAT activity. The third column shows the slopes computed by a least squares regression program, of the immunotitration curves at these times. No significant difference is noted among the groups, indicating that at all times there are parallel changes in TAT catalytic and antigenic activities. An increase in the slope of the immunotitration curve with an increase in activity would be expected if the increase in activity were due to activation of an antigenic cross-reacting material (CRM). Furthermore, during the declining phase of the diurnal curve, a decreasing slope would be expected if active enzyme were being converted to a catalytically inactive material which still had antigenic activity. The constant slope at all times of the cycle indicates that there is no significant accumulation of CRM precursor or breakdown products and implies that the changes in TAT catalytic activity are associated with net changes in TAT protein concentration. This is analogous to TAT induction by adrenal corticoids in rat liver and hepatoma cells (3).

TABLE II

DIURNAL CHANGES IN TAT ACTIVITY AND TAT SYNTHESIS

Time +	TAT Activity		TAT Synthesis
	m_{μ}moles/mg/min	% of activity at 9 PM	% of rate at 9 PM ‡
9 PM	43.7 ± 3.5	---	---
2 AM	63.6 ± 5.6	146*	326***
7 AM	149.3 ± 9.1	342***	259
12 Noon	82.7 ± 6.1	189***	180**

+ Five or six rats per group.

‡ $\dfrac{H^3 \text{ DPM}_{\text{TKT in immunoprecipitate}} \Big/ H^3 \text{ DPM}_{\text{Total protein}}}{C^{14}\text{DPM}_{\text{TKT in immunoprecipitate}} \Big/ C^{14}\text{DPM}_{\text{Total protein}}}$ time x x 100

*, **, ***, significant at p values of $<.05$, $< .01$, $< .001$ respectively.

Since hepatic TAT turns over with a half-time of about 2 hours (8), changes in TAT protein could be due to alterations in the rates of synthesis or degradation. The pulse labeling experiment, designed to answer this question, is reported in Table II. Each combined extract was considered individually in calculating whether the rate at a given time was significantly different from that at 9 PM which had the lowest rate. The mean values of the paired samples were expressed as the % of the rate of synthesis at 9 PM. After the minimum at 9 PM, the rate of synthesis increased rapidly, peaking at 326% of control at 2 AM; at this time the catalytic activity had only increased 46%. Following this there was a linear decline in the rate of synthesis to the basal level even though the catalytic activity peaked at 7 AM. The fact that the rate of synthesis increases prior to the increase in catalytic activity and that the magnitude of the increase exactly parallels the increase in catalytic activity indicates that the diurnal change of TAT activity is due

to a variable rate of synthesis of the enzyme. Kenney et al (4) reported a similar relationship between TAT activity and rate of synthesis following insulin, glucagon, and gluco-corticoid induction in liver, and a similar situation exists with gluco-corticoid induction of TAT in HTC hepatoma cells (9). This demonstration of a variable rate of synthesis, plus the evidence that no detectable CRM precursor or degradation pools of TAT exist through the cycle should help elucidate the mechanism of this rhythm.

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