DIURNAL INCORPORATION OF ^{3}H-LEUCINE INTO LIVER PROTEIN

A.V.LEBOUTON and S.D.HANDLER

Department of Anatomy, The Center for the Health Sciences, University of California, Los Angeles, California 90024, USA

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1. Introduction

Most studies on various aspects of rat liver protein synthesis are done at a time of day that is convenient for the investigator, not the rat. Yet there are diurnal variations in liver enzyme synthesis and activity [1, 2] as well as changes of protein synthesis in liver cell organelles [3]. Aware that the synthesis of some proteins in rat liver is periodic we decided to engage in preliminary studies to investigate the possibility that total hepatic protein synthesis might exhibit fluctuations during a 24 hr period. We report here that a rhythm in total protein synthesis does appear to exist in rat liver, with a peak synthesis rate occurring midway through the dark phase of a 12-12 light-dark cycle, a lighting regimen not too different from that used in many research vivariums.

2. Methods

Male Sprague-Dawley rats (155 g) were caged in groups in a vivarium with automatically controlled lighting conditions (lights on 6 a.m. to 6 p.m., lights off 6 p.m. to 6 a.m.) for 7 days prior to experimentation. The light source was fluorescent bulbs of the warm white type and light intensity was not measured. To duplicate conditions used by most investigators rats were allowed Purina Rat Chow and water *ad libitum*. Starting at 6 a.m. and at 2 hr intervals threreafter, rats were injected with 5 μCi/g of L-leucine-4,5-^{3}H (specific radioactivity, 58.1 Ci/mmole, lot 40903, Schwarz BioResearch, Orangeburg, New York, USA). The radioisotope was 97% chromatographically pure and was injected during light ether anesthesia via the external jugular vein in a volume of about 0.4 ml.

Rats were dispatched 20 min after the radioisotope injection when radioactivity in liver proteins is near its peak value [4]. Livers were perfused free of blood through the portal vein *in situ* [5] and the left median (LM) and right anterior lateral (RAL) lobes were dissected free. These lobes receive their portal blood supply from different viscera [4]. The lobes were separately homogenized in 0.25 M sucrose – 0.001 M EDTA (4 ml/g wet wt) and centrifuged at 15,000 $g_{av.}$ for 10 min as described previously [4]. The supernatant fluid containing microsomes, soluble proteins and free amino acids was fractionated with 10% trichloroacetic acid – 0.5% sodium tungstate (TCA-T) into TCA-T soluble (free amino acids) and TCA-T insoluble (proteins) fractions [4]. Whole blood was also obtained from the inferior vena cava just prior to the liver perfusion. The blood was allowed to clot and serum was recovered by centrifugation. TCA-T soluble and insoluble fractions were then prepared from the serum.

Radioactivity in the liver and serum samples was estimated by liquid scintillation spectrometry [6]. Data were plotted as histograms using the method of six-hr moving averages [7] to minimize individual variations and reveal any general trends. Each point is the average of triplicate determinations on each animal.

3. Results and discussion

The results of determinations on both liver lobes were essentially similar (fig. 1). Incorporation of

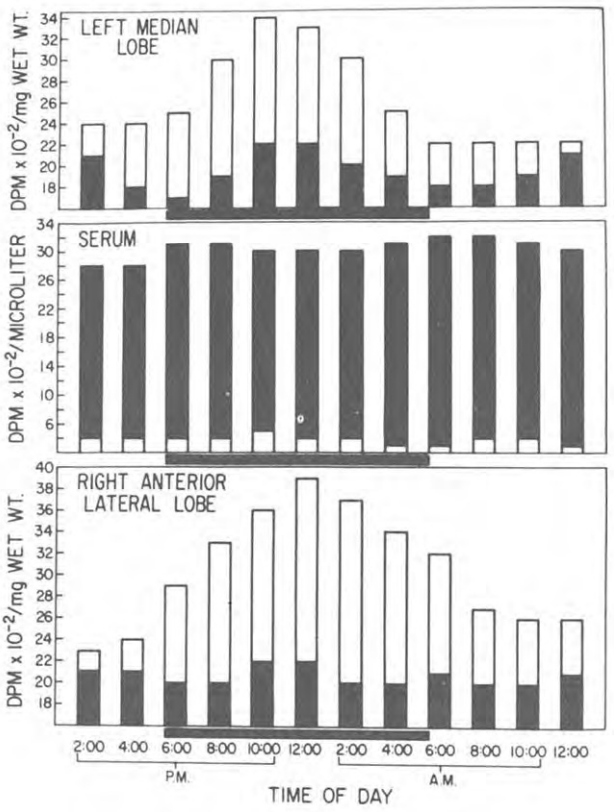

Fig. 1. Histograms of radioactivity in two liver lobes and serum during a 24-hr period. Values are 20 min after ^{3}H-leucine injection. Open block is the TCA-T insoluble fraction, solid block is the TCA-T soluble fraction. Both values read from the base line which actually starts above zero on the histograms to save space. Precision of the measurements was so close that calculated standard errors were small (5% to 7%) and are omitted from the histograms for clarity. Lights were off from 6 p.m. to 6 a.m.

^{3}H-leucine into the TCA-T insoluble fraction was low between noon and 2 p.m., began to increase at 6 p.m. (when the light was turned off), attained the highest value between 10 p.m. and midnight, slowly decreased during the early morning hours and finally dropped to low levels shortly after the light went on at 6 a.m. Radioactivity in the TCA-T soluble fraction was usually in phase with the insoluble fraction except midway through the light period (noon) when it rose slightly, relative to the TCA-T insoluble fraction which was near its lowest point (fig. 1).

Values of the serum TCA-T insoluble fractions were low since 20 min were not enough time for adequate quantities of labeled plasma proteins to be secreted into the blood by the liver [6] (fig. 1). Values of the TCA-T soluble serum fractions indicated a possible damped rhythm with two relative high points that occurred near the beginning and end of the dark phase, and two low points midway through both the light and dark periods (fig. 1).

The data in fig. 1 demonstrate that a rythm does exist in the incorporation of ^{3}H-leucine into hepatic proteins, with highest radioactivity occurring midway through the dark phase of a 12-12 light-dark cycle. This peak radioactivity could be due to either a faster rate of protein synthesis or to insertion of more labeled leucine residues into any given protein that is synthesized at a constant rate. The latter alternative implies a higher specific radioactivity of the amino acid pool, which the TCA-T soluble values in fig. 1 do not seem to indicate since the TCA-T soluble value at midnight (when the TCA-T insoluble radioactivity is high) is only 200 dpm higher than the corresponding value at noon (when the TCA-T insoluble radioactivity is low). However, we must stress that in the absence of data on the intracellular specific radioactivity of leucine and total leucine concentration the arguement at this point at best be tentative. Even so since it is known that rats begin eating at darkness [8, personal observations] and the concentration of amino acids in portal blood rapidly increases after eating [9] the ^{3}H-leucine in the hepatic amino acid pool should be diluted, thereby decreasing radioactivity of proteins synthesized from this pool, especially if they were synthesized at the same rate as prior to eating. On this basis we presume that the higher values of radioactivity in protein obtained near the middle of the dark period represent a possible increased rate of protein synthesis by the liver at this time.

Among other factors this presumed faster rate of protein synthesis could be associated with prior formation of RNA. In line with this reasoning is the report [10] that in rats on an identical 12-12 light-dark cycle there is rhythmic incorporation of ^{32}P into liver microsomal RNA with a peak value occurring at 8 p.m., which is just 2 to 3 hr prior to the time of peak protein synthesis found in the present report.

A previous study [4] has shown that the two liver lobes studied here conduct general protein synthesis in an identical fashion even through the LM lobe

receives its blood from the jejunum while the RAL lobe is perfused with blood from the spleen. As seen here these lobes are also similar in the periodicity of incorporation of ^{3}H-leucine into proteins, emphasising that liver lobes are functionally identical entities. This is even more interesting when one considers that after a meal the amount of amino acids entering the LM lobe from the jejunum must be higher than the amount entering the RAL lobe from the spleen, an organ that does not directly participate in absorption of ingested food.

We do not know whether the rate of synthesis of all species of hepatic proteins might be increased during the dark phase. The presumed increased rate of protein synthesis seen here may involve non-export proteins such as tryptophan pyrrolase [1] and alpha-ketoglutarate transaminase [2] which exhibit highest activity at 10 to 11 p.m. In the case of export proteins such as albumin it is known that it takes about 2 hr after injection of ^{3}H-leucine for the specific radioactivity of albumin to attain its highest value in plasma [6]. We find peak labeling here of liver proteins at near midnight whereas 2 hr later at 2 a.m. is when the absolute concentration of albumin in plasma reaches a high point in its cyclic behavior [11].

Thus depending on the species of protein under investigation, results might be different if experiments were done on rats at varying times during their light-dark cycle. This could be achieved by shifting the lighting sequence in the vivarium so that a convenient time of day for the investigator would correspond to any chosen point in the light-dark cycle of the rat.

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