DIURNAL VARIATION IN THE ACTIVITY OF CHOLESTEROL 7α-HYDROXYLASE IN THE LIVERS OF FED AND FASTED RATS

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1. Introduction

The introduction of a hydroxyl group at the 7α-position of cholesterol is the first step in the major pathway for the conversion of cholesterol into cholic acid and other bile acids. Cholesterol 7α-hydroxylase, the enzyme catalyzing this step, is confined to the microsomal fraction of the liver cells and there is evidence to suggest that cytochrome P-450, together with an NADPH-dependent flavoprotein, are involved in this hydroxylation [1].

Cholesterol 7α-hydroxylase is probably an important rate-limiting enzyme in bile acid biosynthesis [2], and therefore the study of changes in the activity of this enzyme under different physiological conditions may be expected to provide information on the regulation of bile acid synthesis in liver. In view of this fact, it would be of interest to know whether the activity of cholesterol 7α-hydroxylase measured *in vitro* is sensitive to the nutritional, hormonal and other experimental conditions under which the experimental animals may be kept.

In this communication it is shown that the activity of cholesterol 7α-hydroxylase exhibits diurnal rhythmicity in the livers of both fed and fasted rats. The pattern of the cyclic rise and fall observed in the activity of this enzyme is similar to that of tyrosine transaminase measured in the soluble part of liver homogenates. The rise in the activity of cholesterol 7α-hydroxylase is prevented by treating the rats with inhibitors of protein synthesis, such as cycloheximide or actinomycin D. A brief account of part of this work has been given elsewhere [3].

2. Experimental

The rats used were males of the Wistar strain weighing 180–220 g when killed. The animals were kept at 24° in wire cages in a room that was artificially illuminated from 9:00 a.m. to 7:00 p.m., and they had free access to food [4] during the dark periods. The animals were adapted to their new environment for at least 15 days and showed normal weight increases. Fasted rats were maintained without food for various periods of time as indicated for the particular experiment. The activities of cholesterol 7α-hydroxylase and other enzymes were measured, at each specific time, in groups of three fed or fasted animals which were killed within 10 min of the given time.

The preparation of the subcellular fractions, incubation conditions and measurement of the mass of 7α-hydroxycholesterol produced during the incubation have been described previously [1]. Under the conditions of our experiments, the rate of synthesis of 7α-hydroxycholesterol was constant up to 20 min and all the incubations were carried out for this length of time. An estimate of the size of the microsomal pool of cholesterol which acts as substrate for cholesterol 7α-hydroxylase was obtained using the equation

$$M = \frac{F \times R}{S_{7\alpha}}$$

where M = mass of cholesterol in the substrate pool (nmole); F = fraction of added [^{14}C]cholesterol that enters the pool; R = the amount of ^{14}C added (dpm); and $S_{7\alpha}$ = specific radioactivity of 7α-hydroxycholes-

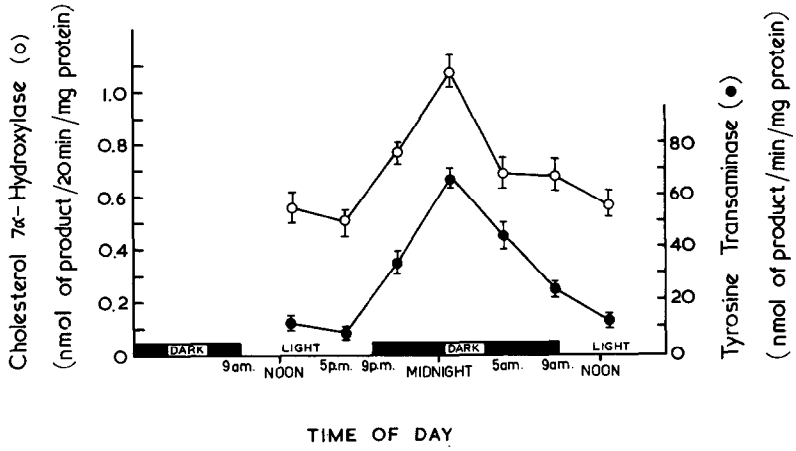

Fig. 1. Diurnal changes in the activity of hepatic cholesterol 7α-hydroxylase and tyrosine transaminase in rats adapted to controlled lighting and feeding. The rats were supplied with food only between 7:00 p.m. and 9:00 a.m. Each point shows the mean and SD of values obtained from 3 groups of rats.

terol formed after addition of [^{14}C]cholesterol (dpm/nmole). F was assumed to be equal to unity and hence M is only a maximum estimate of the actual size of the substrate pool in the microsomal fraction [5]. Tyrosine transaminase activity in the soluble part of the liver homogenates was assayed by the method of Diamondstone [6].

3. Results and discussion

In order to ensure that cholesterol 7α-hydroxylase activities could be accurately compared under different conditions, an estimate of the activity of the enzyme was obtained by incubating [4-^{14}C]cholesterol with a microsomal preparation at various concentrations of protein and then assaying the incorporation of [^{14}C]-cholesterol into the 7α-hydroxycholesterol fraction. When the amount of protein in the incubation was varied from 0 to 10 mg, the amount of radioactive 7α-hydroxycholesterol formed increased linearly. In all subsequent comparisons, therefore, the incubation mixture contained between 6 and 10 mg of microsomal protein.

In order to observe the diurnal variation of cholesterol 7α-hydroxylase, three groups of rats, each containing three rats, were killed every 4 hr and the activity of the enzyme was assayed in the microsomal preparation from the combined livers of each group. The activity of tyrosine transaminase was assayed in the soluble part of the same homogenates. From fig. 1 it may be seen that the activity of cholesterol 7α-hydroxylase is maximal during the middle of the dark period. The activity then declines to a minimum around noon, before rising again in the evening. This is in agreement with earlier findings of Gielen [7]. The activity of tyrosine transaminase also exhibits a similar pattern of variation (fig. 1). The activity of cholesterol 7α-hydroxylase expressed in nmole of 7α-hydroxycholesterol formed per g of liver per 20 min shows a diurnal rhythm very similar to that expressed on a basis of the microsomal protein.

It is not possible to explain the rhythmicity in the activity of the enzyme on the basis of changes either in the level of total liver microsomal cholesterol or in the size of the pool of microsomal cholesterol that acts as substrate for cholesterol 7α-hydroxylase. The total cholesterol content of the microsomal preparations, expressed either per mg of protein or per g wet weight of the liver, does not change significantly during the 24-hr cycle. However, the estimated size of the substrate pool reaches a minimum around midnight when the activity of cholesterol 7α-hydroxylase is at its peak value (fig. 2). If substrate availability has any influence

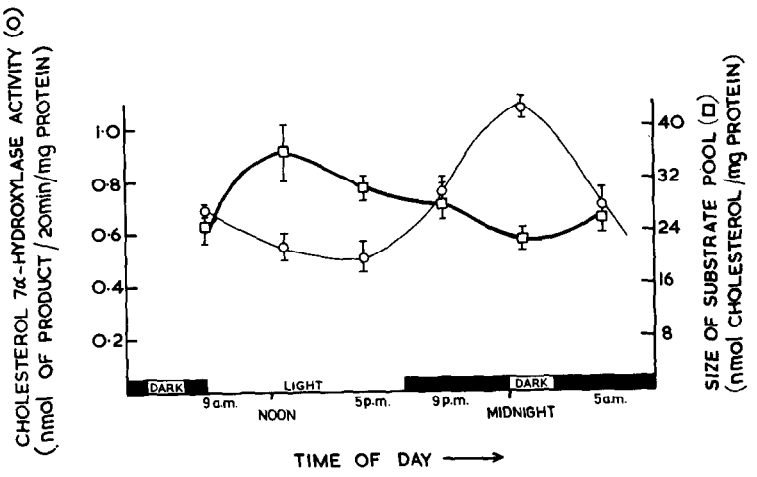

Fig. 2. Diurnal changes in the activity of hepatic cholesterol 7α-hydroxylase and in the size of the microsomal substrate pool for this enzyme in rats adapted to controlled lighting and feeding. The substrate pool of cholesterol was estimated by a method based on measurement of the specific activity of the 7α-hydroxycholesterol formed from [^{14}C]cholesterol during incubation of liver microsomes [5]. Each point shows the mean and SD of values obtained from 3 groups of rats.

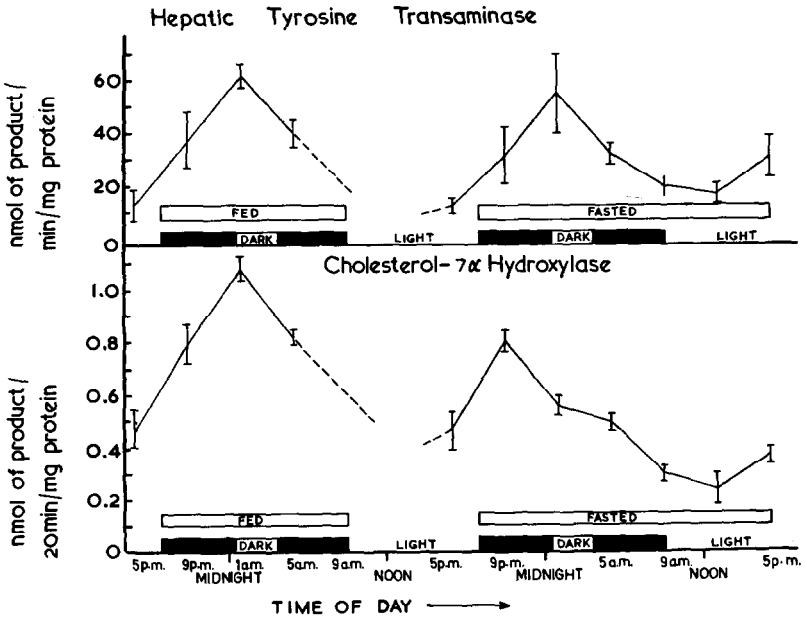

Fig. 3. Effect of fasting on diurnal changes in the activities of hepatic tyrosine transaminase and cholesterol 7α-hydroxylase in rats adapted to controlled lighting and feeding. Each point shows the mean and SD of values obtained from 3 groups of rats.

on the enzyme rhythm then the amplitude of the oscillations observed in enzyme activity might have been even higher, if the size of the substrate pool had remained constant. It is worth noting that the content of tyrosine in the liver also approaches a minimum at the point where the activity of tyrosine transaminase exhibits a maximum [8].

In order to investigate the effect of food intake on the diurnal rhythm of cholesterol 7α-hydroxylase, the activity of the enzyme was assayed during a 24-hr cycle of normal feeding, followed by assay of the activity of the enzyme every 4 hr in the next 24 hr during which the rats were deprived of food. In fig. 3 it may be seen that the activity of cholesterol 7α-hydroxylase exhibits a diurnal rhythm even during the fasting state. The pattern of variation of enzyme activity is broadly similar to that observed with fed rats, but the minimum and maximum values of activity are lower than that in the fed state. Furthermore, there is a shift in the peak of maximum activity to the early part of the dark period, whereas during feeding the peak activity of the enzyme occurs around midnight. In confirmation of previous results [9], tyrosine transaminase activity varies during fasting in a manner similar to that observed in the fed state (fig. 3).

There is strong evidence to suggest that diurnal rise and fall in the activity of a number of enzymes is controlled at the level of enzyme synthesis and degradation [10, 11]. In order to investigate such a possibility in the case of cholesterol 7α-hydroxylase, rats which were adapted to a controlled feeding and lighting schedule were injected intraperitoneally with a solution of actinomycin D or cycloheximide at 7:00 p.m. and the animals were killed 4 hr later. Control rats were injected with solvents only. In the control rats, the activity of cholesterol 7α-hydroxylase in the liver microsomal preparations was twice as high at 11:00 p.m. as at 7:00 p.m. (table 1). However, in the rats with actinomycin D or cycloheximide at 7:00 p.m., the activity of the enzyme at 11:00 p.m. did not rise above that observed 4 hr earlier. These observations indicate that an increase in enzyme synthesis was responsible for the diurnal rise in the activity of cholesterol 7α-hydroxylase observed during the early part of the dark period.

Table 1

Effect of administration of inhibitors of protein synthesis on the diurnal variation in the activity of cholesterol 7α-hydroxylase.

Treatment of animals	Activity of cholesterol 7α-hydroxylase (nmole/20 min/mg protein)	
	7:00 p.m.	11:00 p.m.
Control	0.40	0.89
Actinomycin D	0.40	0.38
Cycloheximide	0.40	0.25

The rats were adapted to controlled feeding (7:00 p.m.–9:00 a.m.) and lighting (9:00 a.m.–7:00 p.m.) for 2 weeks before the experiment. Actinomycin D (0.5 mg/kg) or cycloheximide (0.5 mg/100 g) was injected intraperitoneally at 7:00 p.m. and the rats were killed 4 hr later. Control animals were killed at 7:00 p.m. and 11:00 p.m. Each value shows the results obtained from a group of three rats.

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References

[1] K.A. Mitropoulos and S. Balasubramaniam, Biochem J. 128 (1972) 1.
[2] H. Danielsson, K. Einarsson and G. Johansson, European J. Biochem. 2 (1967) 44.
[3] S. Balasubramaniam, K.A. Mitropoulos and N.B. Myant, in: Bile Acids in Human Diseases, ed. P. Back (F.K. Schattauer Verlag, Stuttgart, New York, 1972) in press.
[4] H.M. Bruce and A.S. Parkes, J. Hygiene 44 (1946) 501.
[5] S. Balasubramaniam, K.A. Mitropoulos and N.B. Myant, European J. Biochem., submitted for publication.
[6] T.I. Diamondstone, Anal. Biochem. 16 (1966) 395.
[7] J. Gielen, Mémoire présenté pour l'obtention du grade de docteur en sciences biomédicales expérimentales, Université de Liège, 1969.
[8] R.J. Wurtman, W.J. Schoenmaker and F. Larin, Proc. Natl. Acad. Sci. U.S. 59 (1969) 800.
[9] M. Watanabe, V.R. Potter and H.C. Pitot, J. Nutrition 95 (1968) 207.
[10] R.T. Schimke, E.W. Sweeney and C.M. Berlin, J. Biol. Chem. 240 (1965) 322.
[11] M. Higgins, T. Kawachi and H. Rudney, Biochem. Biophys. Res. Commun. 45 (1971) 138.