THE INFLUENCE OF BILATERAL SUPERIOR CERVICAL GANGLIONECTOMY, CONTINUOUS LIGHT AND CONTINUOUS DARKNESS ON THE DIURNAL RHYTHM OF HYDROXYMETHYLGLUTARYL-COENZYME A REDUCTASE IN RAT LIVER

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1. Introduction

The activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (mevalonate: NADP-oxidoreductase CoA-acylating, EC 1.1.1.34; HMG-CoA reductase), the rate limiting enzyme of cholesterol biosynthesis in rat liver [1], shows a marked diurnal rhythmicity [2–5]. The maximum of enzyme activity is reached in the middle of the dark period and a broad minimum occurs during the light period. Similarly, the food intake changes periodically, i.e. rats eat much more during the dark period than during the light period [6]. If rats have access to food only for a brief fraction of the light period, the maximum of activity of HMG-CoA reductase is found in the light period [4]. The rhythm is maintained even when the animals did not have access to food during the last feeding period scheduled [4]. Similarly, the rhythm of the enzyme persists with rats fasted for 24 hr, albeit with a strongly reduced amplitude [2]. This suggests that periodic signals other than cyclic food intake may be a cause of the rhythm. After a 12 hr shift of the lighting cycle the phase of the rhythm of the enzyme is reversed [7]. This demonstrates that light is a synchronizer of the rhythm and that other rhythmically changing physical influences have no effect. However, the experiment does not answer the question whether the rhythm is endogenously generated or whether a light–dark change (LD) is necessary to evoke the rhythm. Therefore one object of the present paper is the study of the activity of the enzyme with rats kept in constant light (LL) or constant darkness (DD).

Involvement of neuroendocrine pathways in photoregulation of diurnal rhythms of biochemical parameters has been described [8]. The content of serotonin and melatonin in the pineal gland varies periodically with the environmental lighting cycle [9, 10]. In the pineals of adult rats this response depends on the intactness of the sympathetic innervation of the organ by fibers originating from the superior cervical ganglia [11]. The rhythm of HMG-CoA reductase activity appears to be in phase with the cyclic change of the melatonin concentration and in inverse phase to the pineal serotonin rhythm. As another object of the present contribution we examined the possibility of a pineal regulation of the rhythm of the enzyme using bilaterally superior cervical ganglionectomized rats.

It was found that the diurnal rhythm of HMG-CoA reductase persists in ganglionectomized rats and in rats kept under constant light or constant darkness.

2. Methods

Male Sprague-Dawley rats (Fa. Wiga, Sultzfeld/Königshofen, Germany) of 40–60 g initial body weight were housed in plastic cages (4 animals per cage, area 36 cm × 20 cm) at 23°. Throughout all experiments the animals had free access to rat chow Altromin R10 (Fa. Altrogge, Lage/Lippe) and tap water. After adaptation to a normal light–dark cycle (L: 7:00 to 19:00 hr, D: 19:00 to 7:00 hr) for at least 7 days the animals were i) maintained in continuous light or

ii) maintained in continuous darkness (also after enucleation of the eyes) or iii) returned to the normal lighting schedule after ganglionectomy or sham operation. At the time of surgery the rats weighed 80–120 g. The animals were anesthesized by ether. Enucleation of the eyes was performed by bilateral disconnection of the optic nerves. Bilateral superior cervical ganglionectomy was carried out as described [12, 13]. The postoperative occurrence of ptosis in all ganglionectomized animals indicated the successful gangionectomy [12].

All animals gained weight at a normal rate of 5.9 to 7.3 g per day, with the exception of the ganglionectomized and enucleated animals, whose weights declined during the first two postoperative days, then recovered and showed normal increase until the time of sacrifice. Groups of animals (body weight 140–190 g) treated in the specific ways indicated were killed at various times of day. Their livers were excised and assayed for HMG-CoA reductase activity as reported previously [14].

3. Results and discussion

3.1. *Influence of continuous light*

Since the activity of HMG-CoA reductase is strongly reduced in fasted rats [1] the intake of food was measured throughout all experiments to assure that a reduction of the enzyme activity after a given treatment could not be due to a fasting effect evoked concomitantly.

As expected [6], the animals kept under a normal light–dark cycle ingested food periodically during the day, with 79% of the food (17.3 g/120 g body weight/day) consumed during the dark period. During exposure to continuous light the consumption of food remained unchanged for the following 8 days (average: 16.5 g/120 g body weight/day). On day 8 of continuous light 58% of the food ingested was eaten during the original dark period. The decrease from 79% to 58% is gradual and probably due to desynchronization of the individual rhythms [15]. On day 9 groups of rats were killed at intervals during a 24 hr period and the HMG-CoA reductase activity was determined. With the same animals the food intake was measured for the individual period extending from 7:00 hr to each time of

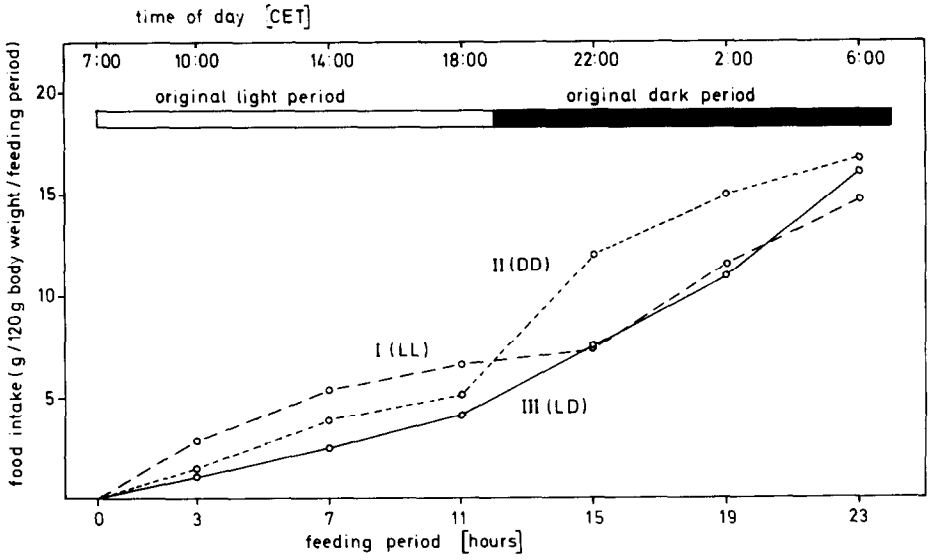

Fig. 1. Food intake of rats at day 8 of exposure to continuous light or continuous darkness and food intake of rats kept under normal lighting conditions. 4 Animals were kept per cage. Food intake was measured per cage (4 animals per cage) and calculated per animal. Curve I: food intake in continuous light (LL); curve II: food intake in continuous darkness (DD); curve III: food intake under normal lighting conditions (LD; [6]).

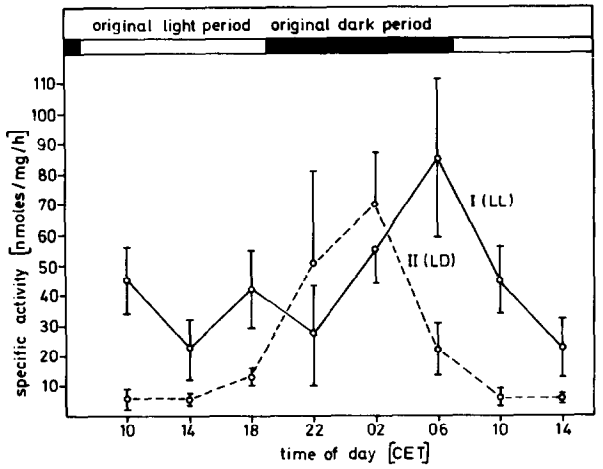

Fig. 2. Rhythm of HMG-CoA reductase activity in continuous light (curve I; LL) and under a normal light–dark cycle (curve II; LD). The values of curve I were measured at day 9 of exposure to continuous light. Each point of curves I and II represents the average of data obtained with 4 animals. Curve II is given for comparison (quoted from [2]). The vertical lines represents the standard errors of the mean. To provide a better impression of the rhythmic process the values at 10:00 hr and 14:00 hr are given twice. The values at 6:00 and 14:00 hr (fig. 2, curve I) are significantly different ($P < 0.01$).

sacrifice. After 9 days of exposure to continuous light the rats still ate periodically (fig. 1, curve I). The total food intake on day 9 of continuous light (fig. 1, curve I) was comparable to that found with rats kept under the light-dark cycle (fig. 1, curve III).

As seen from fig. 2 (curve I) the rhythm of HMG-CoA reductase activity persists in animals exposed to continuous light for 9 days. The peak of activity is reached at 6:00 hr; the minimum is found between 14:00 and 22:00 hr.

The rhythm of enzyme activity observed with rats subjected to normal lighting conditions is shown for comparison (fig. 2, curve II). While the peak activities are of comparable size with both experimental groups, the minimal enzyme activity with rats kept under continuous light is notably elevated (compare curves I and II, fig. 2). Interestingly, the peak of activity found under continuous light is reached at a later time of day than with the control group.

3.2. *Influence of continuous darkness*

Rats kept in continuous darkness for 9 days consumed regular amounts of food (17.3 g/120 g body weight/day) in a cyclic pattern of intake (fig. 1, curve II).

Table 1
Specific activity of HMG-CoA reductase (± standard error of the mean) of blinded or untreated rats kept in continuous darkness for 9 days.

Treatment	Specific activity (nmoles/mg/hr)	
	10:00 hr	22:00 hr
Blinded + DD	8.0 (± 2.5)	36.5 (± 22.5)
Untreated + DD	10.0 (± 4.3)	46.2 (± 17.9)

The data are the mean of values obtained with 4 to 5 rats. The control animals ate normal amounts of food (16.2 g/120 g body weight/24 hr while enucleated rats consumed 21% more. The values at 10:00 and 22:00 hr are highly significantly different ($P < 0.01$).

As in continuous light, in continuous darkness the rhythm of HMG-CoA reductase persists (fig. 3, curve I). The maximum of activity is found at 22:00 hr; the minimum between 6:00 and 10:00 hr. This curve (fig. 3, curve I) resembles the control curve (fig. 3, curve II, normal lighting conditions) closely, except that the maximum seems to be reached earlier in the experimental than in the control group.

At the time of feeding and weighing (7:00 and 19:00

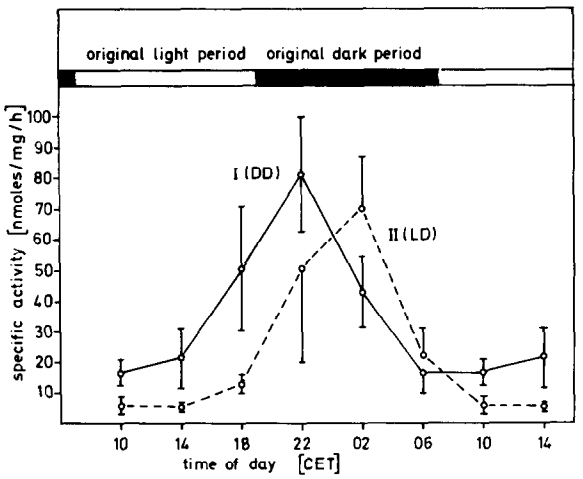

Fig. 3. Rhythm of HMG-CoA reductase activity in continuous darkness (curve I; DD) and under a normal light–dark cycle (curve II, LD). For other details see fig. 2.

hr) the animals were exposed for 15 min to the dim red light used for illumination and the green light from the scale of the balance. To eliminate the possible influence of these light signals experiments in continuous darkness were performed using rats blinded by enucleation of the eyes. Groups of blinded animals and untreated controls, which had been kept in darkness for 9 days, were killed at 10:00 and 22:00 hr. At 22:00 hr the HMG-CoA reductase activities of enucleated and untreated rats were significantly higher than at 10:00 hr (table 1). This confirms that the rhythm persists in absence of light stimuli. The difference between the control values obtained at 22:00 hr and values obtained 6 months earlier under comparable conditions (fig. 3, curve I) might be due to seasonal changes of the amplitude of the rhythm. It was not investigated further.

3.3. *Influence of ganglionectomy*

Superior cervical ganglionectomized, sham operated and untreated rats were kept under a normal light–dark cycle for 14 days. One to 2 days before sacrifice the ganglionectomized rats (body weight approx. 170 g) gained weight (6.1 g/day) about at the same rate as sham operated and untreated animals (5.9 g/day). At this time the daily food intake of ganglionectomized rats (12.1 g/120 g body weight) was only 10% lower than that of sham operated (13.3 g/120 g body weight) and untreated rats (13.5 g/120 g body weight; all values are the mean values of data obtained with 4 animals). At day 14 animals of each group (45 days old) were killed at 12:00 and 24:00 hr. As the table shows, ganglionectomy does not influence the rhythm of the reductase activity. The difference betwen the noon and the midnight values is highly significantly ($P < 0.01$) with all 3 groups. As essentially identical results were obtained with 28 day old rats, the data are not given here. The result suggests, that the pineal is not involved in the regulation of the rhythm of the enzyme. It has been reported that the rhythm of pineal serotonin is dependent on the sympathetic innervation of the gland with 60 day old (adult) rats, but not with animals 15 days of age (immature rats) [11]. Therefore, the status of the pineal innervation in the rats used in our experiment is not clear (intermediate age of 45 days). Let us assume that the rats of our experiment behaved like immature animals. Then the pineal serotonin rhythm would still be independent of innervation and therefore would not have been affected by the ganglionectomy. However, with immature rats a causal relationship between the rhythms of HMG-CoA reductase and pineal serotonin appears improbable, since only the latter is abolished in continuous light [11]. On the other hand, let us assume that the rats of our experiment can be compared with adult animals. In adult animals the pineal serotonin rhythm persists in constant darkness but not in constant light [16]. As the HMG-CoA reductase rhythm continues under constant light and constant darkness, the pineal serotonin cannot be responsible for the regulation of the reductase rhythm. Similarly, the pineal melatonin rhythm cannot be its cause either. The rhythm of the activity of the rate limiting enzyme of melatonin synthesis is suppressed in constant light but not in constant darkness [17]. The persistence of the reductase rhythm in animals kept under constant light or darkness demonstrates that the light–dark cycle is not required to maintain the rhythm. While the cyclic input of light does act as a synchronizer of the enzyme rhythm, other physical signals from the environment are eliminated as possible regulators [7]. We conclude that cyclic signals of endogenous origin must be responsible for the regulation of the diurnal change of the reductase activity. Experiments are in progress to prove this hypothesis. The conservation of the rhythm in fasted rats [4] shows that food intake does not generate the rhythm. However, cyclic availability of food can act as a synchronizer of the rhythm [4] that overrides the "Zeitgeber" effect of the light–dark cycle [18].

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