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Circadian Oscillations of Serum Thyroid Hormones in the Laboratory Rat: the Effect of Photoperiods

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Summary
The seasonal influence on circadian oscillations of serum thyroid hormones has been confirmed in the laboratory rat, an animal exhibiting low photoperiodic activity. The aim of this paper was to study the influence of various photoperiods, applied in a single season, on circadian variations in the levels of thyroid hormones in male Wistar rats. After 6-weeks of adaptation to artificial light-dark regimens (LD) 08:16 h, 12:12 h, 16:08 h, and to the standard housing conditions, the rats were examined in 3 h intervals in the course of 24 h in December. The concentrations of thyroxine (T_4), triiodothyronine (T_3) and reverse T_3 (rT_3) were examined in the serum. The curves of T_4 circadian oscillations showed two peaks in all the photoperiods followed. Computative acrophases were localized between 07.00 and 08.00 h, the amplitude in the LD 12:12 regimen was twice that observed in LD 08:16 and 16:08, the rhythm was present and the mesors were approximately the same. Circadian oscillations of T_3 exhibited rhythmicity in all the photoperiods with computative acrophases localized between 07.30 and 09.00 h, and the values of mesors in LD 08:16 and 16:08 regimens were significantly lower in comparison with those in the LD 12:12 regimen. The rT_3 circadian variations in the LD 12:12 regimen showed rhythmicity with acrophase at 06.00 h. The rhythm in the LD 16:08 regimen was of borderline significance, the computative acrophase occurred at 8.16 h, and the mesor value was significantly higher than those in the LD 12:12 regimen. The decrease in the amplitude of T_4 oscillations and the lower T_3 mesors in LD 08:16 and 16:08 regimens in comparison with the LD 12:12 values indicated only minor modification in circadian oscillations of T_4 and T_3 resulting from artificial photoperiods. In comparison with our previous studies these data suggest that changes in circadian oscillations of serum thyroid hormones might reflect the effect of the season of the year rather than the effect of day duration, i.e. the photoperiod.

Key words
Serum thyroid hormones – Circadian oscillations – Photoperiods – Rats

Introduction

The levels of serum thyroid hormones exhibit circadian and circaannual oscillations dependent on the animal species (Smals *et al.* 1977, Rookh *et al.* 1979, Boissin-Agasse *et al.* 1981, Ryg and Jacobsen 1982). The data on circadian variations of thyroxine and triiodothyronine in laboratory rats varied particularly because of non-standard experimental conditions (Fukuda *et al.* 1975, Jordan *et al.* 1980, Ottenweller and Hedge 1982). In our previous studies, we followed the effect of time of the year on circadian oscillations of the concentrations of thyroxine (T_4), triiodothyronine (T_3) and reverse triiodothyronine (rT_3) in the serum of laboratory rats kept on an artificial regimen light:dark 12:12 h (Ahlersová *et al.* 1984), or in rats reared in parallel under natural and artificial light during different seasons of the year (Ahlersová *et al.* 1991). We observed marked seasonal changes in circadian concentrations of T_4 and T_3 in the serum which were more pronounced in the rats kept in natural light (Ahlersová *et al.* 1991).

In the present study, we attempted to elucidate the origin of the changing patterns in circadian

oscillations of thyroid hormones during various seasons, and whether the innate circaannual reactivity or the reaction to the changing duration of the photoperiod predominate. We therefore exposed the rats to artificial photoperiods (short, long) in a single season and examined their influence on circadian rhythm patterns of serum thyroid hormones.

Material and Methods

Three-month-old SPF male Wistar rats were divided into three groups and exposed in parallel to different light-dark (LD) regimens 08:16, 12:12 and 16:08 h for period of 6 weeks (November – December). The rats were kept under standard housing conditions (temperature 22±2 °C, relative humidity 60–70 %). Cold light from fluorescence lamps Tesla (40 W) was automatically switched on at 07.00 h in all light regimens, and switched off at 15.00 (LD 8:16), 19.00 (LD 12:12) and 23.00 h (LD 16:08). The intensity of light was approximately 150 lux in each cage where animals were kept in groups of four with free access to water and food (LD pellets, Velaz, Prague, CR). After adaptation to these conditions, the rats were killed by decapitation at 3 h intervals in the course of 24 h. Dim red light of less than 1 lux intensity was employed for decapitation during the dark part of the day. The thyroxine, triiodothyronine and reverse triiodothyronine concentrations were determined radioimmunochemically in the serum obtained from mixed blood using commercial Total T_4 and T_3 kits from the Institute for Research, Production and Application of Radioisotopes, Prague, CR, and Reverse T_3 kits from Hypolab, Switzerland. Each group consisted of 8 rats.

The results were evaluated by the cosinor analysis (Halberg *et al.* 1967) with a chosen 24 h rhythm period. The mesor (mesor is a mean value of the fitted oscillation curve) values were compared by the t-test. Two acrophases were used for the parameters tested: the computative acrophase, determined as the relation of the peak to 00.00 h local time (the values are given in a cosinor analysis table), and the external acrophase which is related to a given point on the synchronizing external cycle, in our experiment to switching on the light.

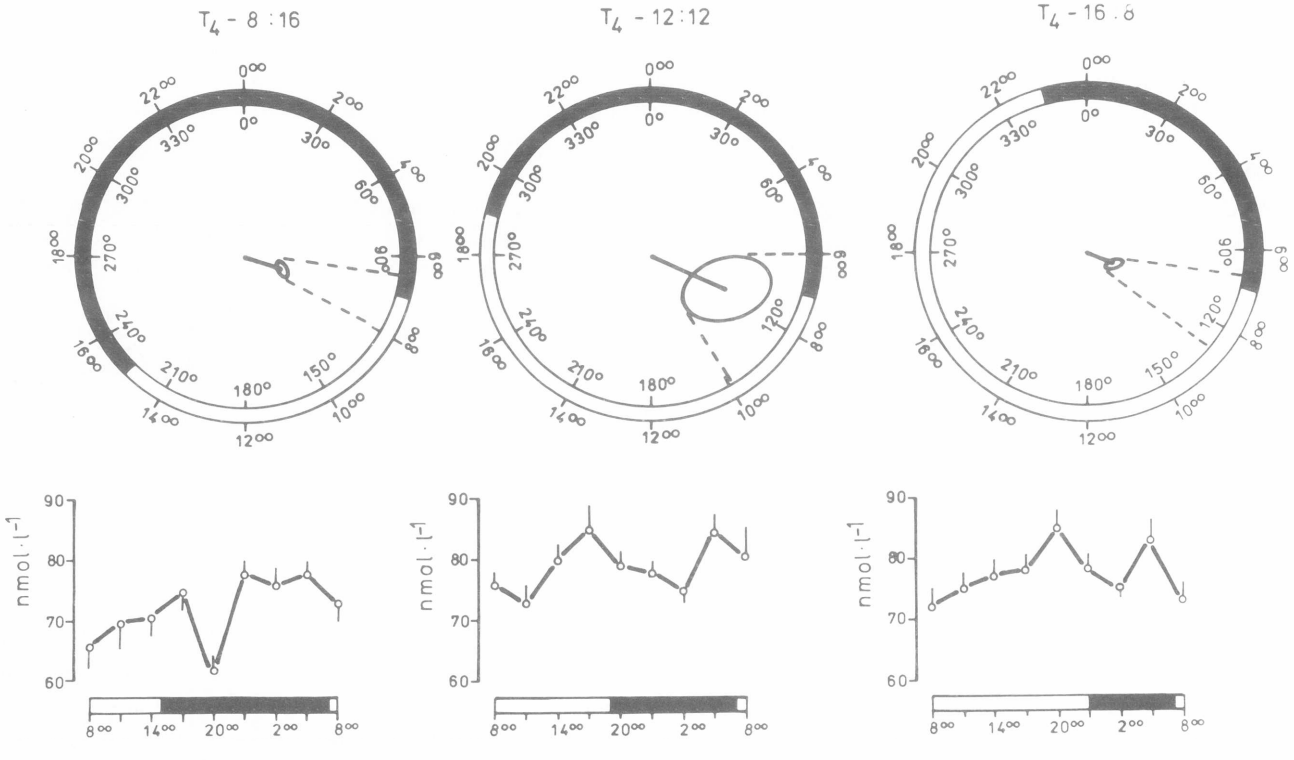

Fig. 1. *Circadian oscillations (below) and cosinor diagrams (above) of serum thyroxine (T_4) concentrations in rats from the photoperiods light-dark (LD) 08:16, 12:12, 16:08 h. The values are given as the arithmetical mean ± S.E.M. In the cosinor diagram, the vectors originating from the center of the circular system of coordinates represent the amplitude of oscillations; their position denotes the acrophase. The rhythm is present if the ellipse of the errors does not overlap the origin of the coordinates. Tangents to the ellipses represent the 95 % confidence interval for the acrophase. Light part of time data – light, dark part – darkness.*

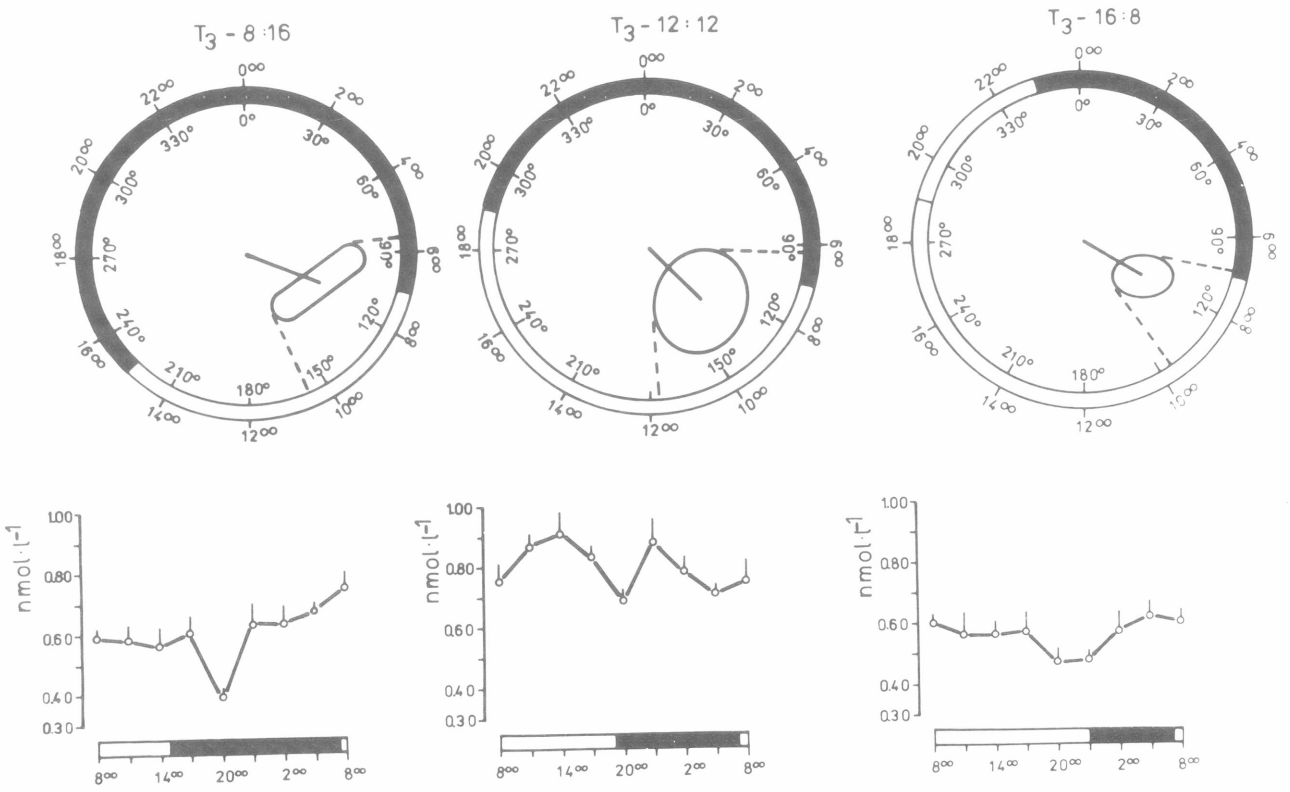

Fig. 2. *Circadian oscillations and cosinor diagram of the serum triiodothyronine (T_3) concentration in rats from the photoperiods LD 08:16, 12:12, 16:08 h. Other details as in Fig. 1.*

Results

The curves of circadian oscillations of serum T_4 concentrations in the rats had two peaks in all light regimens used. The amplitude of oscillations in the LD 12:12 h regimen was approximately twice the value that was observed for amplitudes in LD 08:16 and 16:08 h regimens. The computative acrophases were localized between 07.00 and 08.00 h for all the photoperiods. The onset of external acrophases was observed 52 min (LD 08:16), 44 min (LD 12:12) and 28 min (LD 16:08) after switching on the light. The values of mesors were approximately the same in all the photoperiods. A rhythm with 24 h period was detected in all light regimens used (Fig. 1, Table 1).

Circadian oscillations of T_3 concentrations in the serum were inconspicuous in LD 08:16 and 16:08 regimens and formed a two-peaked curve in the LD 12:12 h regimen. The amplitude of oscillations was approximately the same in all the photoperiods. The computative acrophases were localized between 07.30 and 09.00 and the external acrophases were observed 32 min (LD 08:16 h), 2 h (LD 12:12) and 1 h (LD 16:08 h) after switching on the light. The values of mesors were similar in LD 08:16 and 16:08 regimens and significantly lower in comparison with those in the LD 12:12 regimen. The T_3 oscillations followed rhythmically the 24 h period in all photoperiods examined (Fig. 2, Table 1).

Circadian oscillations of serum rT_3 concentrations were determined only in LD 12:12 and LD 16:08 regimens. For the LD 12:12 regimen a two-peaked oscillation curve with low amplitude and 24 h rhythmicity was observed. The computative acrophase was localized at 06.00 and the external acrophase was situated 11 h after switching off the lights. The curve of circadian oscillations in the LD 16:08 regimen was not sinusoidal in shape and the rhythm bordered on significance. The computative acrophase was localized at 08.16 h and the external acrophase was situated approximately one hour after switching on the light. The rT_3 mesor in the LD 12:12 h regimen was significantly lower than in the LD 16:08 h regimen (Fig. 3, Table 1).

Table 1. Characteristics of the cosinor test: the mesor (the mean values of the fitted curve) and amplitude are given in the employed units; the acrophase is given in an angular and a time interpretation. CI – confidence interval; its limits in the presence of rhythm are given in brackets.

Serum	Photoperiods (LD = light:dark)	Rhythm detection (95 % level)	Mesor ± S.E.M.	Amplitude ± CI (95 %)	Acrophase ± CI (95 %) Degrees	Time
Thyroxine	LD 08:16	+	71.18 ± 3.75	15.19(12.89;17.49)	118°(98°;120°)	$7^{52}(6^{32};8^{00})$
nmol/l	12:12	+	73.97 ± 8.26	30.23(16.93;43.53)	116°(90°;149°)	$7^{44}(6^{00};9^{56})$
	16:08	+	76.70 ± 2.97	12.20(9.76;14.64)	112°(100°;130°)	$7^{28}(6^{40};8^{40})$
Triiodo-	LD 8:16	+	0.59 ± 0.05^{b}	0.19(0.15;0.23)	113°(85°;158°)	$7^{32}(5^{40};10^{32})$
thyronine	12:12	+	0.81 ± 0.04	0.18(0.07;0.29)	137°(93°;178°)	$9^{08}(6^{12};11^{52})$
nmol/l	16:08	+	0.55 ± 0.04^{b}	0.17(0.11;0.23)	122°(102°;148°)	$8^{08}(6^{48};9^{52})$
Reverse						
triiodo-	LD 12:12	+	0.245 ± 0.007	0.03(0.02;0.04)	90°(46°;120°)	$6^{00}(3^{04};8^{00})$
thyronine	16:08	+	0.286 ± 0.018a	0.07(0.01;0.13)	124°(33°;194°)	$8^{16}(2^{12};12^{56})$
nmol/l						

Differences between LD 12:12 and other photoperiods designated as ^{a} ($P<0.05$) or ^{b} ($P<0.01$).

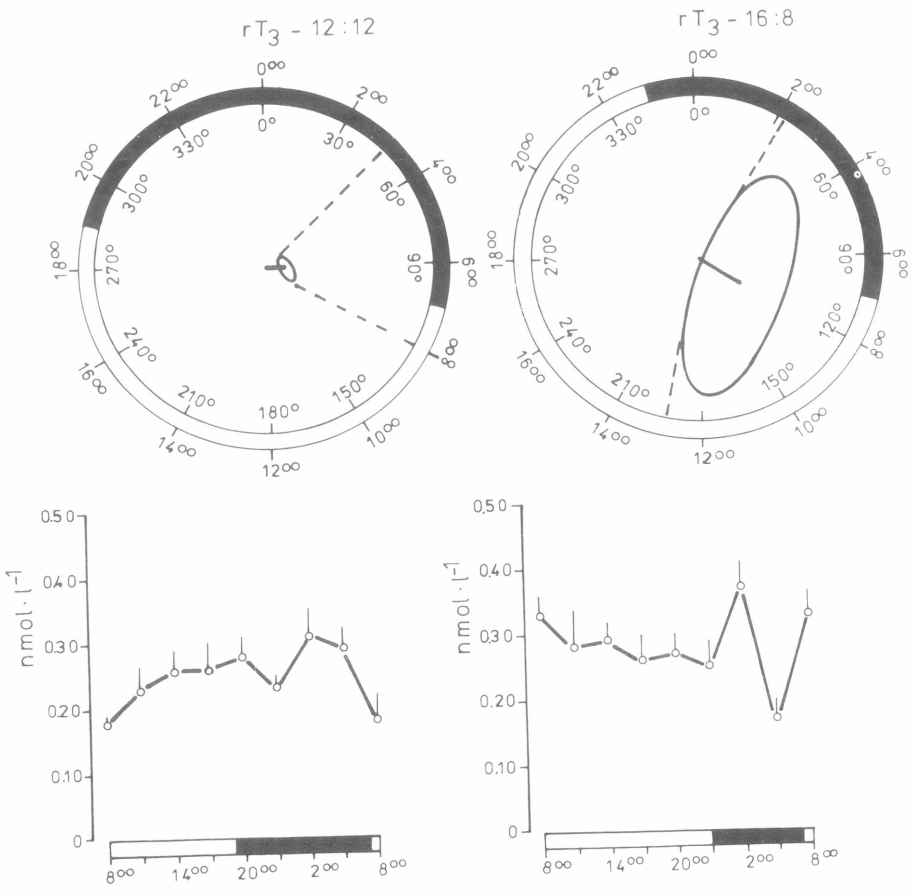

Fig. 3. *Circadian oscillations and cosinor diagram of the serum reverse triiodothyronine (rT_3) concentration in rats from the photoperiods LD 12:12, 16:08 h. Other details as in Fig. 1.*

Discussion

The data on circadian oscillations of thyroxine and triiodothyronine in the serum of laboratory rats vary in dependence on the strain used, the time of year and experimental conditions, such as the light regimen used, temperature, composition of feed, intensity of light during handling in the dark part of the day and the use of anaesthesia; the statistical evaluation of oscillations also varied (Fukuda *et al.* 1975, Rookh *et al.* 1979, Jordan *et al.* 1980, Ottenweller and Hedge 1982, Ahlersová *et al.* 1984). Our previous study dealt with the influence of the natural and artificial light regimen (LD 12:12) during various seasons on circadian oscillations of concentrations of T_4, T_3 and rT_3 in the serum of laboratory male rats (Ahlersová *et al.* 1991). Circadian oscillations of T_4 and T_3 showed two peaks and rhythmicity in the natural light regimen during all seasons while in the LD 12:12 regimen no rhythmicity was observed in spring (T_3) and summer (T_4, T_3). The concentration of T_4 (and to a smaller degree also T_3) culminated in natural light in dependence on the sunrise while, under the artificial light regimen, it showed irregular peaks after switching on the lights. The lowest T_4 and T_3 mesors found in rats exposed to natural and artificial light were those observed in winter, the highest in spring (T_4), and in the artificial photoperiod in T_3 in autumn. We have also described rhythmic circadian oscillations in serum rT_3 concentrations, the mesor values of which mirrored those of T_3. The results of the study cited confirmed the dominant influence of seasons on the levels of thyroid hormones in rats which was more pronounced under the conditions of natural lighting than under those of artificial lighting.

Circadian variations of serum T_4 and T_3 showed no relation to circadian oscillations in the intake of food and water by laboratory male rats that was investigated in an experimental model identical with those used in our recent papers (Ahlersová *et al.* 1992).

The effect of photoperiods on circadian oscillations of thyroid hormones was investigated mainly in photoperiodically active animals, namely in hamsters (Vaughan *et al.* 1982, 1985). Laboratory rats have been considered to exhibit little if any photoperiodic activity: the shortening of the light part of the day failed to cause involution of the testes in adult animals (Nelson *et al.* 1994). In our previous studies, we investigated the effect of various photoperiods on circadian oscillations of corticosterone (CS) and insulin in the serum of laboratory rats using the same experimental model as that described in the present study. The levels of circadian oscillations of CS culminated in dependence on the duration of light which suggest that a photoperiodical response of the hormone is involved. Circadian variations of insulin were less affected by the photoperiods (Ahlersová *et al.* 1992). Constant light and dark periods modified the circadian rhythms in corticotropin-releasing factor-like immunoreactivity in hypothalamus and of plasma adrenocorticotropic hormone and CS (Fischman *et al.* 1988).

The effect of artificial photoperiods on circadian oscillations of serum thyroid hormones in rats described in this study was moderate and was manifested by a decrease in the amplitude of thyroxine oscillations and mesors of triiodothyronine in the short and long days in comparison with the LD 12:12 h regimen. The mesor of rT_3 was, on the contrary, lower in the LD 12:12 h than in group kept on the long day.

The responsiveness of individual components of the neuroendocrine system in animals to the changing ratio of light and darkness varies. In this respect, either the endogenous circaannual mechanism (the clock) or the direct response to the photoperiodic stimulus from the environment can predominate. Both mechanisms can support one another; the two components could be distinguished under laboratory conditions using a constant light regimen (Hastings 1991). Our results mentioned above indicate that the oscillations in serum corticosterone, the hormone of activity, stress and adaptation, showed little changes in the course of the year, however, responded strongly to the photoperiodic stimulus. Intact suprachiasmatic nuclei are needed for circadian rhythm of CS; bilateral lesion of this central mammalian pacemaker resulted in the loss of the rhythm (Moore and Eichler 1972).

The cells in mammalian suprachiasmatic nuclei responded to the light with expression of immediate-early genes, like c-fos (Aronin *et al.* 1990). The duration of c-fos expression during the short photoperiod (LD 8:16 h) was about 5–6 h longer than under the long one and therefore through the sensitivity to changing photoperiod the rat suprachiasmatic nuclei could represent a "clock for all seasons" (Sumová *et al.* 1995). Oscillations in serum thyroid hormones exhibited marked changes over the year and only moderate response to the photoperiodic cue. With respect to the oscillations in the serum thyroid hormones in laboratory rats, the endogenous circaannual clock is obviously a more conserved mechanism than the photoperiodic signal.

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Reprint requests

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