Physiol. Res. 41: 307–314, 1992

Influence of Light Regimen and Time of Year on Circadian Oscillations of Insulin and Corticosterone in Rats

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Received January 16, 1992
Accepted May 20, 1992

Summary

Male SPF bred Wistar rats were adapted to natural light (N) and to a 12 : 12 h (light-dark) artificial light (A) regimen in the course of the year. The rats were analyzed at 3 h intervals during 24 h approximately at the time of the vernal and autumnal equinox and at the winter and summer solistice. Serum insulin circadian oscillations depended on the season, being different in various light regimens. The mesors were the highest during summer, the lowest during winter in both regimens. The external acrophases of insulin in the N differed from those in the A group, contrary to the computative ones. The annual mean of serum insulin concentration was lower in the N than in the A group. The circadian oscillations of corticosterone were influenced primarily by the time of year. The mesors were the highest during summer, lower in winter and spring in N and A group. The computative acrophases were similar in both groups in all seasons except spring. The external acrophase was similar in both regimens during the year. The response of insulin, a major anabolic hormone, to various light regimens during the day and year was different from that of corticosterone, a major hormone of the stress reaction.

Key words

Rat insulin and corticosterone – Natural and artificial light regimens – Time of day and year

Introduction

A light-dark variation within a day and year is one of the dominant synchronizers of biological rhythms. The effect of natural (solar) and artificial (fluorescent) light on seasonal variability in circadian oscillations of thyroid hormones was studied in our previous work (Ahlersová *et al.* 1991). Besides thyroid hormones, the concentrations of insulin and corticosterone were also examined. The results obtained are discussed in this paper.

Material and Methods

Male SPF rats of the Wistar strain were exposed from their birth to an artificial lighting regimen (light-dark LD 12 : 12 h). Immediately after delivery from Velaz (Prague), they were divided into two groups and allowed to adapt to artificial (LD 12 : 12) and natural light for 5 weeks during each season. The animals weighing 180 g at the begginning of the experiments were given LD food (Velaz, Prague) and water *ad libitum*. They were housed in cages of four rats each under constant environmental conditions: temperature, 23±2 °C; humidity 60–70 %. Cool light (fluorescent lamps Tesla, 40 W) of 150 lux intensity in each cage was automatically switched on at 07.00 h. The rats adapting to natural light were kept in a room 3x4 m, in cages placed about 1.5 m from the windows. Two windows facing the west were not shaded. After 5 weeks of adaptation to natural (N) and artificial (A) lighting regimens, the rats were killed by quick decapitation at 3 h intervals over the 24 h period. The experiments were carried out in the last week of March, June, September and December, i.e. at about the time of vernal and autumnal equinox, summer and winter solistice. The time of sunrise and sunset recorded on the examination days was as follows:

Days of examination time

Sunrise and Sunset
March, 23 (05.31 h, 17.51 h)
June, 26 (04.34 h, 20.42 h)
September, 29 (05.31 h, 17.21 h)
December, 22 (07.25 h, 15.43 h)

Table 1

Characteristics of the cosinor test: the mesor (the mean value 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. Sp – spring, Su – summer, Au – autumn, Wi – winter, N – natural, A – artificial light regimen

Serum		Rhythm detection (95 % level)	Mesor ±S.E.M.	Amplitude ±CI (95 %)	Acrophase ±CI (95 %)	
					Degrees(°)	Hours/minutes
Glucose	N_{Sp}	+	7.62±0.348	1.40(1.10;1.70)	107(97;114)	$7^{08}(6^{28};7^{36})$
	A	+	8.04±0.348	1.43(0.93;1.72)	114(103;125)	$7^{36}(6^{52};8^{20})$
	N_{Su}	+	8.33±0.17	0.75(0.25;1.25)	36(292;86)	$2^{24}(19^{28};5^{44})$
	A	-	8.46±0.15	0.77(– ; –)	340(– ; –)	22^{40}(– ; –)
	N_{Au}	+	7.29±0.29	1.22(0.66;1.78)	129(84;150)	$8^{36}(5^{36};10^{00})$
	A	+	7.35±0.24	0.98(0.54;1.42)	106(66;170)	$7^{04}(4^{24};11^{20})$
	N_{Wi}	+	7.17±0.22	0.90(0.30;1.50)	118(40;182)	$7^{52}(2^{40};12^{08})$
	A	+	7.92±0.26	1.07(0.57;1.57)	118(83;148)	$7^{52}(5^{32};9^{52})$

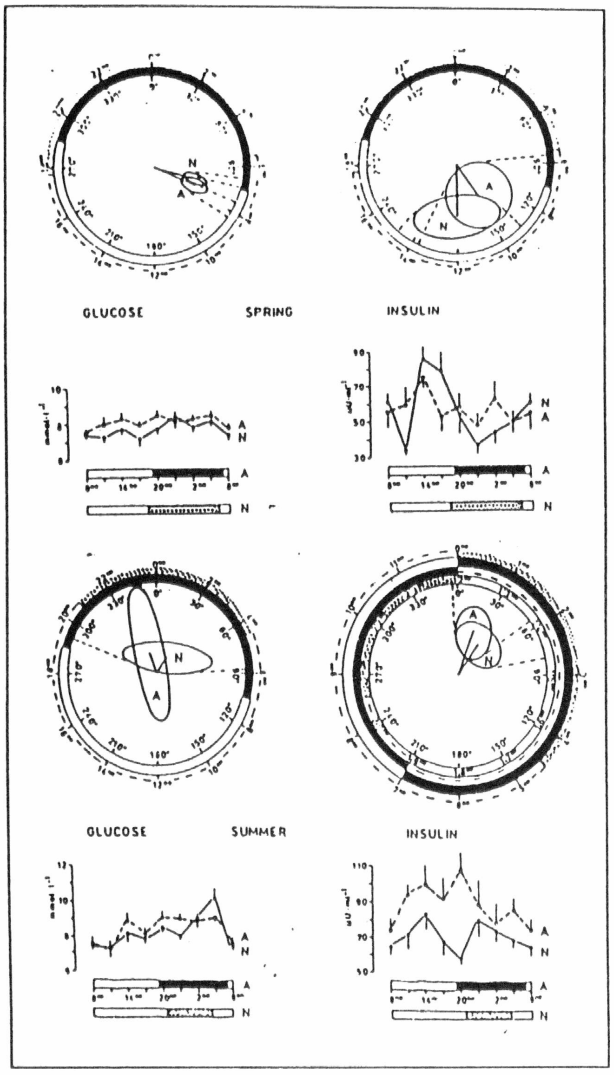

Fig. 1

Circadian oscillations (curves) and cosinor diagram (circular plot) of serum glucose and insulin concentrations in rats kept on natural (N) and artificial (A) lighting regimens during spring and summer. The oscillations are given as arithmetical means ± S.E.M. White part of time data – daylight, black or dotted part – darkness. Abscissa A – A regimen, abscissa N – N regimen. Time data in cosinor diagram illustrate the A (black part) and N (dotted part) regimen. The basal characteristics of oscillations are illustrated in the cosinor diagram; the vector originated from the centre of circular system of coordinates, represents the amplitude of oscillations. The computative acrophase is represented by the angle contained by the vector and hour 0. The rhythm is present if the ellipse of the errors does not overlap the origin of coordinates; the tangents to the ellipse depict 95 % confidence interval for the acrophase.

The concentration of insulin was determined radioimmunologically (Radioisotope Centre Swierk, Poland), of glucose enzymatically (Hugget and Nixon 1957) and of corticosterone fluorimetrically (Guillemin *et al.* 1958). There were 8 rats in each group. Statistical evaluation was done by cosinor analysis (Halberg *et al.* 1967) with a period of 24 h and by the non-paired t-test. The effect of different photoperiods on circadian oscillations of parameters followed was evaluated using two acrophases. The computative acrophase given in a cosinor analysis table, expresses the relation of the peak to 0.00 local time. The external acrophase allowing more exact evaluation, is a computative acrophase related to a certain point in synchronizing the external cycle – in our experiment to the light onset (sunrise, lighting up).

Results

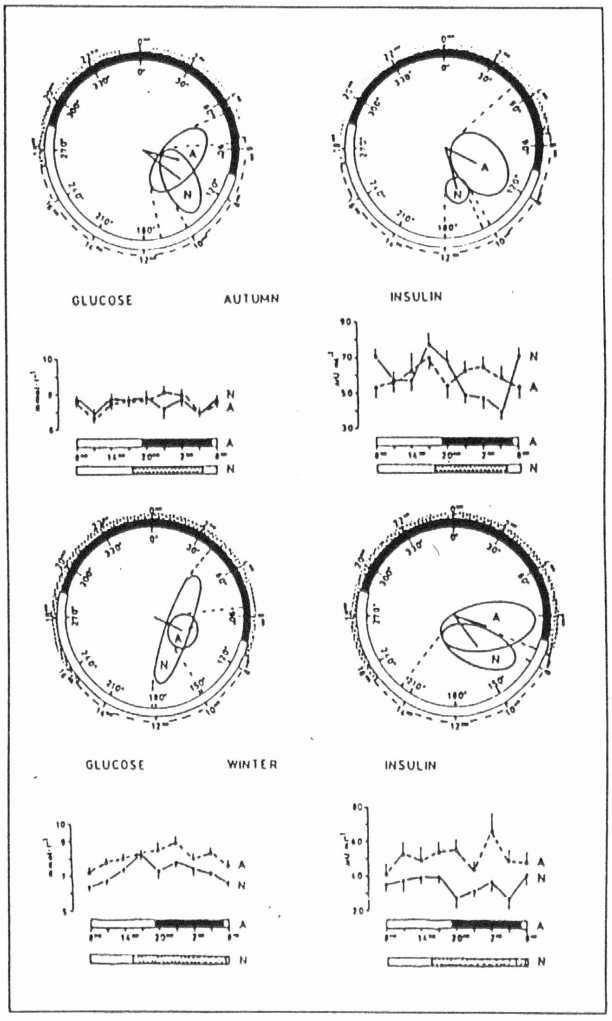

Fig. 2

Circadian oscillations and cosinor diagram of serum glucose and insulin concentration during autumn and winter. Other details as in Fig. 1.

Circadian oscillations of serum glucose concentrations were rhythmic in their occurrence during the 24 h period in all seasons excepting summer in the A group, where no rhythm was found. Computative acrophases in both N and A groups were situated between 07.00 and 08.30 h in all seasons except

Table 2

Characteristics of the cosinor test: the mesor (the mean value 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. Sp – spring, Su – summer, Au – autumn, Wi – winter, N – natural, A – artificial light regimen

Serum		Rhythm detection (95 % level)	Mesor ±S.E.M.	Amplitude ±CI (95 %)	Acrophase ±CI (95 %)	
					Degrees(°)	Hours/minutes
Insulin	N_{Sp}	+	57.45 ± 4.22	17.48(11.45;2351)	180(135;222)	$12^{00}(9^{00};14^{48})$
	A	+	57.74 ± 3.09	13.09(2.38;23.80)	146(85;207)	$9^{44}(5^{40};13^{48})$
	N	+	71.38 ± 3.01	13.11(6.21;20.01)	35(356;80)	$1^{10}(11^{52};2^{40})$
	(12 h) Su					$13^{10}(23^{52};14^{40})$
	A	+	101.85 ± 4.60	17.14(7.94;26.34)	20(356;55)	$0^{40}(11^{52};1^{50})$ $12^{40}(23^{52};13^{50})$
	N_{Au}	+	59.79 ± 3.39	14.32(10.32;18.32)	164(150;180)	$10^{56}(10^{00};12^{00})$
	A	+	60.71 ± 2.86	11.84(2.24;21.44)	118(50;155)	$7^{52}(3^{20};10^{20})$
	N_{Wi}	+	34.12 ± 2.22	9.43(2.63;16.23)	144(112;218)	$9^{36}(7^{28};14^{32}$
	A	-	51.29 ± 2.18	8.80(– ; –)	109(– ; –)	7^{16}(– ; –)
Cortico-sterone	N_{Sp}	+	337.72 ± 17.74	74.92(52.42;97.42)	344(319;27)	$22^{56}(21^{16};1^{48})$
	A	+	257.43 ± 22.44	90.63(34.84;146.43)	14(358;45)	$0^{56}(23^{52};3^{00})$
	N_{Su}	+	482.61 ± 43.25	252.2(189.2;315.2)	258(237;295)	$17^{12}(15^{48};19^{40})$
	A	+	426.86 ± 48.03	197.64(152.0;243.2)	283(255;309)	$18^{52}(17^{00};20^{36})$
	N_{Au}	+	354.00 ± 23.20	89.05(35.62;142.48)	241(350;205)	$16^{04}(23^{20};13^{40})$
	A	+	362.80 ± 27.66	107.95(29.98;185.9)	270(238;21)	$18^{00}(15^{52};1^{24})$
	N_{Wi}	+	320.60 ± 12.16	47.86(29.96;65.76)	270(213;346)	$18^{00}(14^{12};23^{04}$
	A	+	256.12 ± 34.65	146.26(122.86;169.7)	318(265;355)	$21^{12}(17^{40};23^{40})$

summer when the peak was shifted. External acrophases in the N group occurred between 0.5–3 hours after sunrise and 2 h before sunrise in summer only. In the A group, they were localized 8 h before lighting onset in summer and shortly after lighting in other seasons. In rats from both lighting regimens, the amplitudes of glucose oscillations were higher in spring and lower in summer comparing to other seasons. The highest mesor was found in summer in both regimens; in winter it was lower in the N group (Figs. 1, 2, 4, Table 2).

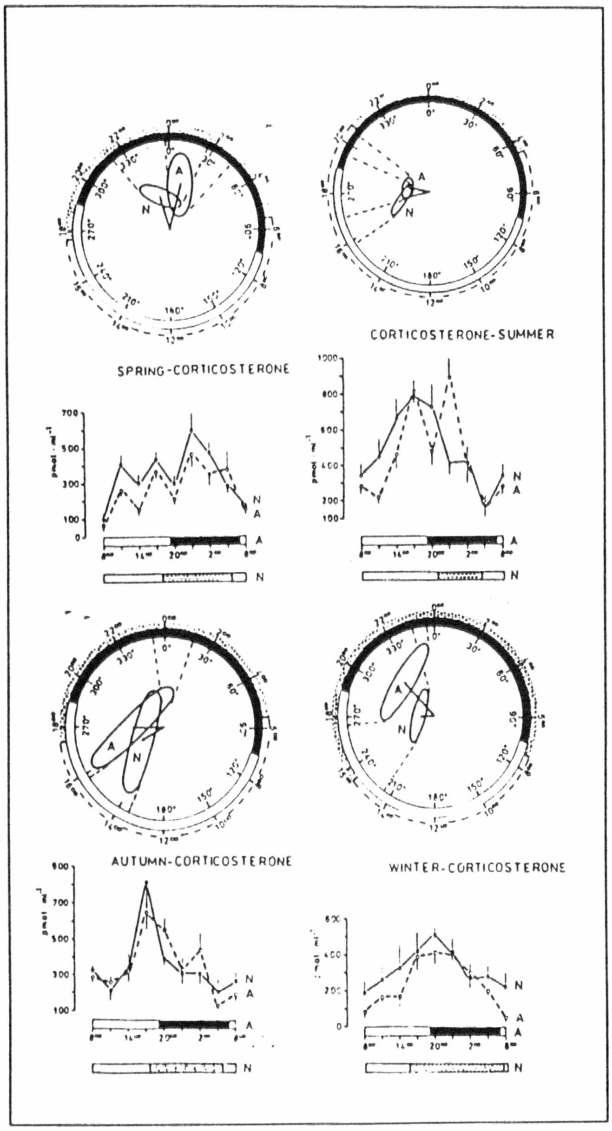

Fig. 3

Circadian oscillations and cosinor diagram of serum corticosterone concentration in the course of the year. For other details see Fig. 1.

Circadian oscillations of serum insulin concentration showed a 24 h rhythmicity in both regimens except the winter oscillations under artificial lighting conditions, when no rhythm occurred. In summer, the rhythm with the 12 h period was observed in both groups. Computative acrophases were localized in N and A regimens between 07.00 to 12.00 h. External acrophases, however, were localized at a different time. In natural light 2 h (winter) and 5–6 h (spring and autumn) after sunrise and 3.5 h before sunrise (summer). In artificial light, they culminated within three hours after lighting up in all seasons but not in summer, when the peak 6 h before the lights were turned on was seen. The amplitudes of insulin oscillations were lower in winter than in other seasons in both regimens. In the course of the year, no difference was found in the values between the two groups. The mesors, lower in winter, were almost doubled in summer in both groups. In the N group, the summer and winter mesors were considerably lower than in the other groups. The annual mean of insulin concentration was lower in rats kept in natural light (Figs. 1, 2, 4, Table 1).

Circadian concentrations of serum corticosterone (CS) showed a 24 h cycle in all seasons. In natural light, the maximal concentration was achieved between 16.00-18.00 h. Artificial light shifted the maximum to 18.00-21.00 h in summer, autumn and winter, and to midnight in spring (computative acrophases). The external acrophases were the same in both regimens. They were situated before sunrise (N) and lighting up (A) in spring 6.5 and 6 h, in summer 11.5 and 12 h respectively, and after sunrise and lighting up in autumn 10.5 and 11 h, in winter 11 and 10 h respectively. The amplitudes of CS oscillations were higher in the rats from the artificial lighting regimen in all seasons except the summer when reverse values were found. In both groups the amplitudes and the mesors were high during summer, low during winter (N) and spring (A). In spring, the mesor was considerably higher in the N group (Figs. 3, 4, Table 1).

The rats weighing 180 g at the beginning of the experiments, exhibited a different gain in weight during individual seasons and lighting regimens. In comparison with 100 % initial weight, rats in A gained in weight from winter to autumn by 123, 89, 87 and 71 % respectively. The gain of weight in rats kept under natural conditions was lower namely in winter (by 25 %) and summer (by 11 %) as compared to the A group.

Discussion

In previous work the dependence of circadian oscillations of blood glucose, tissue glycogen and pancreatic hormones on the seasons were followed in laboratory rats. In rats kept on LD 12 : 12 regimen, mean daily values of insulin (mesors) were found to be similar in all seasons except the autumn (October), when the lowest concentrations were observed (Ahlersová *et al.* 1984).The absence of the rhythmicity

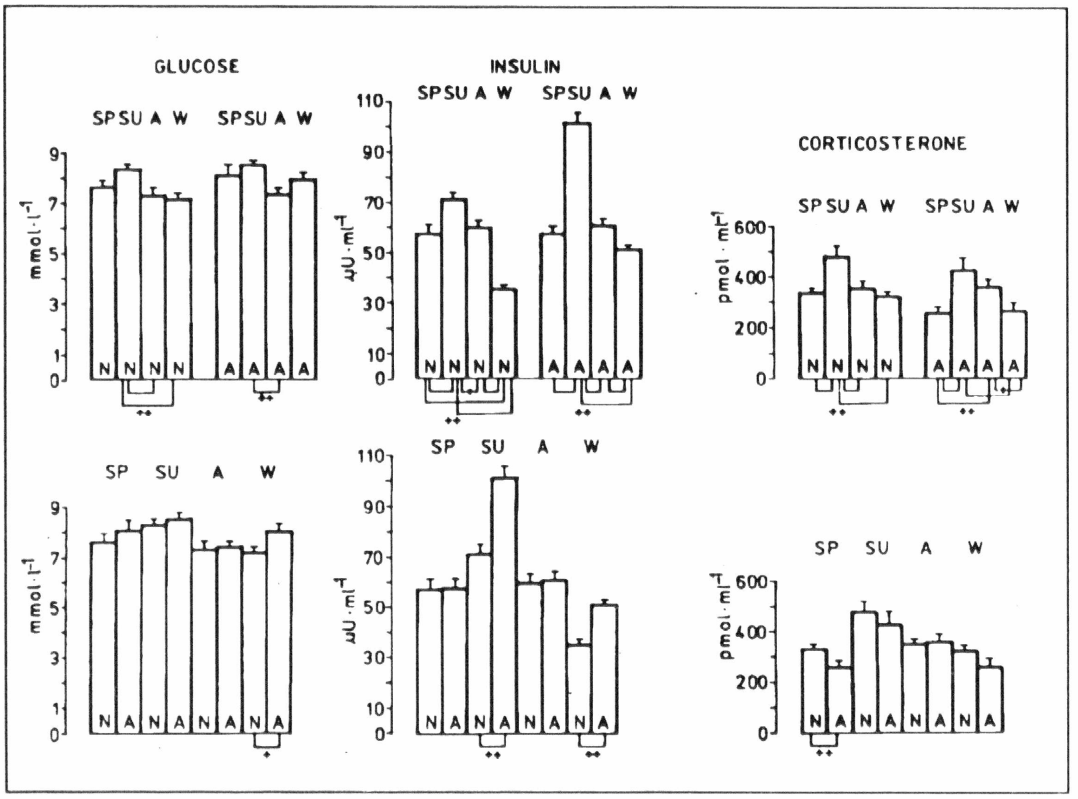

Fig. 4

The columns illustrate the mean values (± S.E.M.) of fitted curves (mesors) of glucose, insulin and corticosterone serum concentrations in the course of the year. SP – spring, SU – summer, A – autumn, W – winter. The significance of differences between seasons and light regimens: + + for $P<0.01$, + for $P<0.05$. Other details as in Fig. 1.

in blood glucose circadian oscillations and a decrease in mesor values during autumn were described for rats exposed to LD 12 : 12 regimen (Ahlersová *et al.* 1982). In the present work, the lowest mesor of glucose concentration was again seen in the A group, in autumn (September).

The effect of various artificial lighting regimens on circadian rhythms of blood glucose and insulin in laboratory rats has been reported in many studies. A light-dark (LD 12 : 12) regimen increased the mesor of blood glucose markedly as compared to the other lighting regimens such as dark-light (DL 12 : 12), DD (continuous dark) or LL (continuous light). In general, the oscillation changes were mostly minimal. However, the curve inversion in the DL should be noted. This implies that glucose circadian rhythms are, to some extent, independent of lighting regimen changes (Pauly and Scheving 1967). Weinert *et al.* (1986) found that the conversion of LD to a DL regimen did not influence insulin circadian oscillations in mice plasma, but was shown to invert the curve of food intake circadian rhythms and to decrease the meal size in comparison to the LD regimen. Male Sprague-Dawley rats exposed to LD 12 : 12 cycle with a light of 90 lux intensity displayed a higher level of blood glucose and meal size in the dark than rats exposed to 1.2 lux light intensity (Jarleblad and Smith 1983). The stability of daily rhythms in serum glucose, insulin and corticosterone was found in scotoresistant Syrian hamsters (*Mesocricetus auratus*) after changing LD 14 : 10 to LD 10 : 14 (De Souza and Meier 1987). In scotosensitive hamsters, after conversion from a longer to a shorter photoperiod, besides gonad atrophy, about a 2-fold decrease in mean daily insulin level was found in a short day. In our experiments, various photoperiods (LD 8 : 16, 12 : 12, 16 : 8) did not modify serum glucose circadian rhythms in male Wistar rats. However, a long (16 : 8) photoperiod shifted the phase of serum insulin circadian variations toward the dark (0500 h) while in shorter photoperiods, the acrophases were localized in the light part of the day (11.00 h) (Ahlersová, unpublished data). In this work, natural light decreased the glucose mesor in winter and the insulin mesor in summer and winter as compared to artificial light.

Little is known about seasonal changes in corticosterone circadian oscillations in laboratory rats. Golikov and Golikov (1973) have reported the highest CS concentration in the blood plasma of female rats during summer and minimal during autumn with the highest binding ability of transcortin found during spring. Circa-annual rhythms of CS in the serum and adrenals in male Wistar rats exposed to LD 12 : 12 regimen were followed in the study of Ahlers *et al.* (1980). The acrophases were situated at about 20.00 h during the whole year. The mesors tended to be similar except for higher values of mesors and amplitude in the adrenals during spring. No difference in mean plasma CS concentration during summer and winter was observed in 3-months-old Sprague-Dawley rats kept in LD 14 : 10 regimen (Wong *et al.* 1983). Unfortunately, examinations during spring and autumn were not done. In our present work, values of CS mesors and external acrophases were shown to be season-dependent in both light regimens. The effect of various light regimens on CS circadian rhythms was also studied by Krieger (1973) in plasma of new-born and old Sprague-Dawley rats. The author found that the circadian periodicity of plasma CS stabilized approximately 25 days after birth and is irreversibly abolished by blinding. The CS circadian rhythm in healthy rats kept from their birth in continuous light or continuous dark was altered reversibly since it normalized after exposure to the LD regimen. The author confirmed that the visual sense is necessary for maintaining the CS circadian rhythm and light-dark alternation for synchronizing the CS rhythms. The response of the axis hypothalamus-hypophysis-adrenals to various lighting regimens in Sprague-Dawley rats was assessed by Fishman *et al.* (1988). Synchronous oscillations of corticotropin-releasing factor-like immunoreactivity (CRF-LI) in the hypothalamus and ACTH and CS in the plasma were observed during LD 12 : 12 regimen. In the DD regimen, an increase in CRF-LI concentration and a decrease in ACTH level occurred with a phase shift of all three parameters. In the LL regimen, ACTH and CS rhythms were desynchronized while CRF-LI and ACTH rhythms still remained synchronized. In our experiment, CS circadian oscillations were only little affected by various types of light. Of note is a higher mesor in rats from the N regimen during spring as compared to the A regimen.

The rats from both regimens in our experiment exhibited the highest mean body weight in winter. This corresponds with a maximum of food intake in winter and early spring found in the rats exposed to LD 12 : 12 (Rietfeld *et al.*1980). Distinct lighting regimens did influence the body weight in the course of the year. The rats in the A group weighed more on the average during winter, summer and autumn than in the N group.

We suggest that the response of laboratory rats to the time of year under natural light conditions differs from that under a stable photoperiod of artificial light. Marked differences between summer and winter characteristics, between individual seasons during exposure to both lighting regimens, point to the substantial role played by the time of the year in forming the circadian rhythms of the followed parameters. The difference in the external acrophases in individual seasons, as seen in serum insulin, can be considered as clear evidence of the dependence on the type of lighting regimen. On the other hand, the external acrophases in serum CS circadian rhythms tended to be similar in both lighting regimens throughout the whole year. Then, in our experiments, neither insulin nor CS were chosen by chance. The first as a major anabolic hormone, is closely associated with the food intake pattern, the latter as one of stress hormones with a catabolic effect, related to locomotor activity. Among the differences induced by distinct types of light, most important are the higher CS mesors, lower insulin and glucose mesors (in this study) and thyroid hormone mesors (Ahlersová *et al.* 1991) found in rats maintained on natural light in some seasons. However, in three seasons these animals showed a lower increase in weight than those from the A lighting regimen. Similarly, the mean annual insulin concentrations were lower in the N group.

It is apparent that there is an interrelationship between seasons and changes in light-dark proportion in the course of the year. The specificity of the effect of light as a dominant external synchronizer, implies a possible difference in the characteristics of circadian oscillations in laboratory rats under a natural and an artificial lighting regimen. We suppose that different circadian rhythms seen in the course of seasons, primarily in the natural light, reflect a different state of the controlling circadian pacemaker during the year and its interaction with the changing photoperiod.

Acknowledgements

The authors wish to thank Mrs. E. Balušíková, Mrs. L. Milárová and Mrs. M. Štefková for outstanding technical assistance.

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