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Structure of Rhythms of Blood Pressure, Heart Rate, Excretion of Electrolytes, and Secretion of Melatonin in Normotensive and Spontaneously Hypertensive Rats Maintained under Conditions of Prolonged Daylight Duration

M. L. Blagonravov, A. A. Bryk, E. V. Medvedeva, V. A. Goryachev, S. M. Chibisov, A. O. Kurlaeva, and E. D. Agafonov

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We studied the structure of rhythms of BP, HR (by telemetric monitoring), electrolyte excretion (by capillary electrophoresis), and products of epiphyseal melatonin (by the urinary concentration of 6-sulfatoxymelatonin measured by ELISA) in normotensive Wistar-Kyoto rats and spontaneously hypertensive SHR rats maintained at 16/8 h and 20/4 h light-dark regimes. In Wister-Kyoto rats exposed to prolonged daylight, we observed changes in the amplitude, rhythm power (% of rhythm), and range of oscillations of systolic BP; HR mezor decreased. In SHR rats, mezor of HR also decreased, but other parameters of rhythms remained unchanged. Changes in electrolyte excretion were opposite in normo- and hypertensive rats. Under conditions of 20/4 h light-dark regime, daytime melatonin production tended to increase in normotensive rats and significantly increased in SHR rats. At the same time, nighttime melatonin production did not change in both normotensive and hypertensive animals. As the secretion of melatonin has similar features in animals of both lines, we can say that the epiphyseal component of the "biological clock" is not the only component of the functional system that determines the response of the studied rhythms to an increase in the duration of light exposure.

Key Words: *arterial hypertension; biological rhythms; excessive exposure to light; melatonin*

Excessive exposure to artificial light at night, known as "light pollution", is becoming more and more common for the urban population in the world [7]. The influence of this adverse environmental factor is complemented by widespread use of devices with LCD screens during in the evening and at night, as well as energy-saving LED sources of visible light with predominant shortwave band light. During daytime, light with these properties has a positive effect on the body and prevents disorders of the structure of circadian rhythms [8,9]. However, chronic exposure to white and blue light, including the use of different electronic devices, in the evening and nighttime contributes to changes in circadian rhythm that lead to sleep disorders, cognitive functions of the CNS, changes in heart rhythm, *etc*. [5,6]. This is mainly due to the suppression of the production of epiphyseal melatonin [10].

Chronic disturbance of the circadian rhythm of melatonin secretion in the pineal gland is the leading mechanism of desynchronosis that provides the basis for the development of different types of pathology [2]. Excessive exposure to light is of special importance for persons engaged in night work. In particular, there is a correlation between sustained night work and exposure to artificial light at night on the development

V. A. Frolov Department of General Pathology and Pathophysiology, Institute for Medicine, Peoples' Friendship University of Russia, Moscow, Russia. ***Address for correspondence:*** blagonravovm@mail.ru. M. L. Blagonravov

and exacerbation of cardiac diseases, diabetes, obesity, and cancer [13]. Desynchronosis is also closely related to the pathogenesis of cardiovascular system diseases, in particular, primary arterial hypertension. Melatonin reduces excitability of the sympathetic nervous system and prevents the development of endothelial dysfunction [11]. One of the most important mechanisms of BP regulation is excretion of electrolytes and water. At the same time, the intensity of renal blood flow, glomerular filtration rate, and excretion of sodium and water are characterized by distinct circadian fluctuations [12]. In general, the rhythm-dependent contour of BP regulation can be described as follows: CNS (brain cortex, suprachiasmatic nuclei of the hypothalamus, and vasomotor center)—pineal gland—kidneys (excretion of water and electrolytes, secretion of renin). It should be noted that pathogenesis of arterial hypertension associated with the influence of excessive light exposure on the rhythm-dependent mechanisms of BP regulation is still poorly studied.

We studied the effect of prolonged exposure to visible light on biological rhythms of BP, HR, electrolyte excretion, and production of epiphyseal melatonin in rats with primary (genetically determined) arterial hypertension and in normotensive animals.

MATERIALS AND METHODS

The experiments were performed on male spontaneously hypertensive SHR and normotensive Wistar-Kyoto rats (age 38-40 weeks in the beginning of the experiment) in two parallel series. In series I (SHR, n=5; Wistar-Kyoto, n=5), 24-h continuous recording of BP and ECG was performed in standard lead II using telemetric monitoring technique. In series II (SHR, n=5; Wistar-Kyoto, n=5), urinary excretion of electrolytes (Na^+, K^+, Ca^{2+}) was assessed by capillary electrophoresis and the concentration of the metabolite of epiphyseal melatonin, 6-sulfatoxymelatonin (aMT6s) was measured by ELISA. The animals were kept and experiments were conducted in accordance with the Order No. 755 of the Ministry of Health of the USSR (August 12, 1977) and European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

In both series of experiments, the animals were sequentially exposed to three illumination regimes: standard artificial light mode (control) with 12/12 h light/dark phases (light from 7.00 to 19.00), prolonged 16-h light exposure (16/8 h light/dark phases; light from 5.00 to 21.00), and prolonged 20-h light exposure (20/4 h light/dark phases; light from 3.00 to 23.00). In all described light modes, illumination at level of animal eyes was 350 lux during the light phase and <0.5 lux during the dark phase. The animals were exposed to each mode for 7 days. The parameters were assessed on day 7.

During the experiment, the animals were caged individually at room temperature 23ºC. The food was given at the same time of day (19.00).

Continuous 24-h recording (7.00-7.00) of BP and ECG in standard lead II was conducted with the telemetric monitoring on the equipment of Data Sciences International. To this end, DSI HD-S11 radio-transmitters for monitoring BP, heart biopotentials, and motor activity and transmitting the signals to special radio receivers as a radio signal were implanted to animals under general anesthesia. BP was recorded via a catheter introduced into the abdominal aorta and fixed with tissue hemostatic adhesive. ECG was recorded via electrodes positioned under the chest muscles in the projection of the electrical axis of the heart. All parameters were recorded in 10 days after the implantation of radio transmitters. Thus, the animals during the experiment were moved freely and had free access to food.

The results were processed using the Dataquest A.R.T. 4.2 Gold. With the Chronos-Fit program [14], the 24-h profile of BP and HR was subjected to nonlinear analysis that represents a combination of partial Fourier analysis with step-by-step regression method. Mezor (the mean level of the studied parameter over a 24-h period), amplitude (the maximum deviation of the studied parameter from the mezor), range of oscillations (the difference between the maximum and minimum values of the studied parameter), power of oscillations (% of rhythm, a chronobiological indicator characterizing the proportion of oscillatory processes, *i.e.* the proportion of the values of the studied indicator that have oscillatory distribution during the day) were determined.

The urinary excretion of Na^+, K^+ and Ca^{2+} over 24-h (from 7.00 to 7.00) and during daytime (from 7.00 to 19.00) and nighttime (from 19.00 to 7.00) was measured. For urine collection, AE0906 metabolic cages for rats were used (OpenScience). The concentration of electrolytes in the urine was measured by capillary electrophoresis on a Kapel-105M instrument using the protocols and reagents manufactured by Lyumeks company. The amounts of secreted electrolytes were calculated per urine volume in appropriate portions.

The content of aMT6s was measured in daytime and nighttime portions of the urine by ELISA using ELISA kit for 6-Sulfatoxymelatonin (Buhlmann Laboratories AG). This parameter was used to estimate the concentration of epiphyseal melatonin in the blood, as it is known that the concentration of aMT6s directly correlates with the total level of melatonin in the blood [1,3,4].

Statistical data processing was conducted using Statistica 6.0 software (StatSoft, Inc.). For each parameter, the mean and error of the mean were calculated. Significance of differences was evaluated using the Mann—Whitney *U* test; the differences were significant at $p \leq 0.05$.

RESULTS

Telemetric monitoring of BP and HR. Mezor of systolic (sBP) and diastolic (dBP) BP in animals exposed to increased light phase (to 16 and 20 h) did not differ significantly from that in standard light regime (12/12 h) (Table 1). Thus, the main parameters characterizing systemic hemodynamics were resistant to photoperiod variations. Mezor of HR significantly decreased in animals exposed to 20/4 h illumination regime.

For parameters reflecting the rhythmic component of these functions (amplitude, range of oscillation, and % of rhythm), the following picture was observed. The amplitude, range, and power of the oscillations of sBP were significantly increased under conditions of light regimes of 16/8 h and 20/4 h in comparison with the standard light regime (12/12 h). The amplitude and range of HR oscillation were significantly higher at 16/8 h. The amplitude, range, and power of dBP oscillations tended to increase with increasing light phase duration, but the differences were insignificant. In general, considerable increase in light phase duration leads to significant changes in the structure of the rhythm of BP and HR in normotensive rats, which can be considered as reactions aimed at expansion the adaptive capacity of the cardiovascular system.

In SHR rats (Table 2), shifts in mezor were similar to those in normotensive animals are recorded. The mezor of sBP and dBP did not change after exposure to 16/8 h and 20/4 h light/dark cycles. However, the mezor of HR significantly decreased with lengthening of the daylight phase to 16 and 20 h. As Wistar-Kyoto and SHR rats are nocturnal animals, longer exposure to day light conditions, apparently, increased the duration of sleep, during which HR is lower than during wakefulness period. As a result, the mezor of HR decreases. Analysis of the range, amplitude, and power of the sBP, dBP, and HR oscillations revealed no significant differences in both regimes with increased light phase in comparison with standard 12/12 h light regime. This attests to rigid structure of the rhythms of these functions in SHR rats, in contrast to that in normotensive rats.

Urinary excretion of electrolytes. In standard light regime, a circadian rhythm of Na^+ and K^+ excretion was observed in normotensive rats; the nighttime values surpassed daytime ones (Table 3). For Ca^{2+}, the same trend was seen, but the differences were insignificant. With increasing light phase to 16 h (16/8 h cycle), the excretion of Na^+ and K^+ increased during daytime, the excretion of Ca^{2+} decreases during daytime, nighttime, and over 24 h. Due to increasing daytime excretion of Na^+ and K^+, circadian rhythm was also changed: the differences between day and nighttime Na^+ excretion disappear and the prevalence

TABLE 1. Indicators of a 24-h Profile of BP and HR, Determined on the Basis of a Nonlinear Rhythm Analysis in Wistar-Kyoto Rats at Standard Light Conditions and Excessive Light Exposure (*M*±*m*)

Parameter		Illumination regimes		
		12/12 h	16/8 h	20/4 h
Mezor	sBP, mm Hg	117.16±2.1	119.46±2.37	120.62±5.28
	dBP, mm Hg	83.64±3.69	84.66±4.51	90.5±11.1
	HR, bpm	252.30±7.12	245.67±3.09	222.95±2.79*
Amplitude	sBP, mm Hg	2.73±0.09	9.69±1.22*	8.81±1.62*
	dBP, mm Hg	4.73±0.03	7.99±1.76	5.65±0.71
	HR, bpm	42.34±1.45	76.17±5.89*	50.51±5.18
Range of oscillations	sBP, mm Hg	5.12±0.34	18.28±2.64*	17.12±3.29*
	dBP, mm Hg	9.45±0.05	14.38±3.79	11.15±1.38
	HR, bpm	75.58±1.39	120.94±6.73*	90.01±7.18
Rhythm power (% of rhythm)	sBP, %	9.69±1.36	30.32±5.40*	19.43±2.84*
	dBP, %	17.33±0.22	28.89±7.49	19.9±2.72
	HR, %	60.64±7.18	74.02±5.11	51.49±4.93

Note. Here and in the Table. 2: *$p \leq 0.05$ in comparison with the standard light mode (12/12 h).

TABLE 2. Indicators of the 24-h BP and HR Profile, Determined on the Basis of Nonlinear Rhythm Analysis in SHR Rats under Standard Light Conditions and Excessive Light Exposure (*M*±*m*)

Parameter		Illumination regimes		
		12/12 h	16/8 h	20/4 h
Mezor	sBP, mm Hg	192.83±10.03	193.7±7.76	190.28±6.00
	dBP, mm Hg	133.25±7.88	134.7±5.2	133.16±3.83
	HR, bpm	329.84±7.04	303.76±9.05*	295.09±6.68*
Amplitude	sBP, mm Hg	10.18±3.24	13.09±2.33	10.85±2.11
	dBP, mm Hg	9.67±2.31	11.14±6.5	8.89±1.74
	HR, bpm	42.75±6.88	46.9±5.01	46.9±4.42
Range of oscillations	sBP, mm Hg	18.29±4.95	24.43±3.95	19.72±2.84
	dBP, mm Hg	17.55±3.65	20.94±2.83	17.47±3.15
	HR, bpm	76.67±6.76	84.14±7.16	89.54±7.6
Rhythm power (% pf rhythm)	sBP, %	19.34±6.55	30.05±3.87	20.86±5.2
	dBP, %	28.03±5.48	33.15±3.4	21.12±5.28
	HR, %	52.30±9.07	54.76±6.68	56.69±5.51

TABLE 3. Indicators of Electrolyte Excretion with Urine in Rats of Wistar-Kyoto and SHR Rats under Standard Light Conditions and Excessive Light Exposure (mmol/l; *M*±*m*)

Parameter	Illumination regimes		
	12/12 h	16/8 h	20/4 h
Wistar-Kyoto rats			
Na^+_{24h}	1.70±0.25	2.76±0.51	9.04±1.26*
Na^+_{day}	0.16±0.06	1.82±0.61*	5.31±0.90*
Na^+_{night}	1.54±0.28$^+$	0.94±0.21	3.73±0.40*
K^+_{24h}	4.80±0.99	5.16±0.28	28.09±0.92*
K^+_{day}	1.04±0.22	3.21±0.08*	13.79±0.97*
K^+_{night}	3.76±0.84$^+$	1.95±0.29$^+$	14.30±0.78*
Ca^{2+}_{24h}	0.17±0.02	0.02±0.002*	0.56±0.06*
Ca^{2+}_{day}	0.07±0.02	0.01±0.001*	0.45±0.05*
Ca^{2+}_{night}	0.10±0.01	0.02±0.002*$^+$	0.11±0.01$^+$
SHR rats			
Na^+_{24h}	3.94±1.50	1.31±0.53	1.07±0.09
Na^+_{day}	1.07±0.19	0.38±0.11*	0.26±0.09*
Na^+_{night}	2.86±1.34	0.93±0.47	0.81±0.11$^+$
K^+_{24h}	7.96±2.31	3.43±1.37	3.13±0.45
K^+_{day}	3.92±0.62	1.13±0.30*	1.45±0.39*
K^+_{night}	4.04±1.76	2.30±1.14	1.68±0.08
Ca^{2+}_{24h}	0.23±0.08	0.09±0.05	0.12±0.02
Ca^{2+}_{day}	0.08±0.02	0.01±0.001*	0.03±0.007*
Ca^{2+}_{night}	0.15±0.06	0.08±0.05	0.09±0.01$^+$

Note. $p \leq 0.05$ in comparison with *the standard light mode (12/12 h), $^+$with daily values.

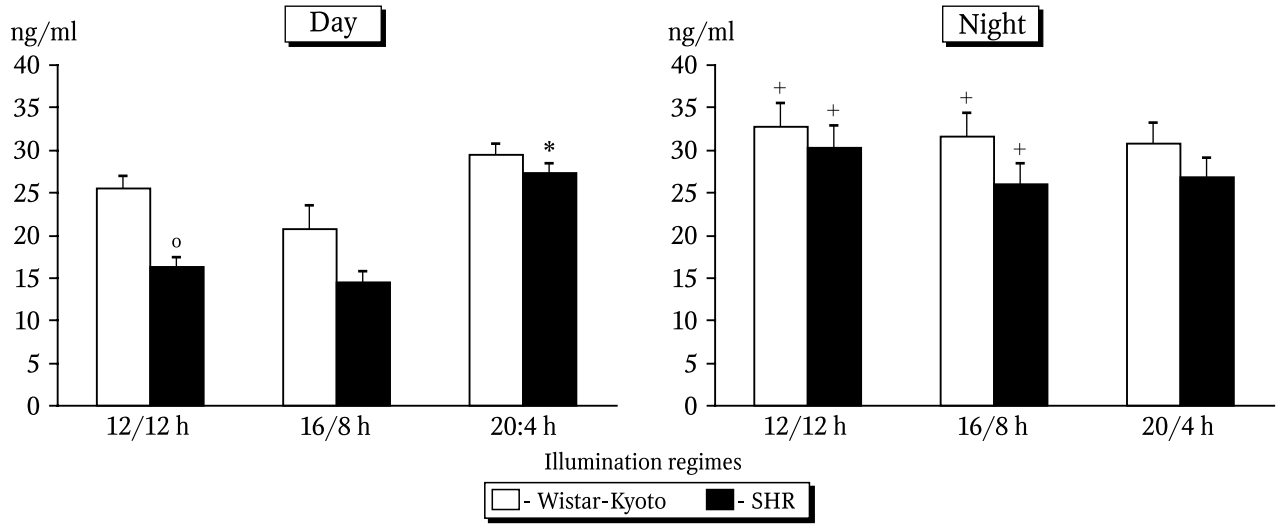

Fig. 1. Urinary concentration of aMT6s in Wistar-Kyoto and SHR rats kept under different light conditions. $p \leq 0.05$ in comparison with *12/12 h light cycle, +with daily values, and °values in Wistar-Kyoto rats.

of daytime excretion of K^+ over nighttime values became characteristic. In 20/4 h light cycle, the excretion of all studied electrolytes during daytime and at night sharply increased, except for nighttime excretion of Ca^{2+} that did not differ from the corresponding parameters at standard light cycle. At the same time, the circadian rhythm of excretion of K^+ and Na^+ completely disappears: almost the same amount of electrolytes was excreted during daytime and at night. It should be noted that the described changes were accompanied by a significant increase in the volume of day and night diuresis (by 5.73 and 4.48 times, respectively).

Electrolyte excretion in SHR rats (Table 3), in contrast to normotensive Wistar-Kyoto rats, was the same during daytime and nighttime at a standard light regime of 12/12 h. Therefore, under conditions of long-term arterial hypertension, the circadian rhythm of urinary excretion of ions was smoothened. Lengthening of the light phase to 16 (16/8 h light cycle) and 20 h (20/4 h light cycle) led to a decrease in the daytime excretion of all studied electrolytes. Nighttime values tended to decrease, but the differences were insignificant. These changes in electrolyte excretion were associated with a decrease in diuresis, more pronounced during the daytime: the volume of urine released during the increased light phase decreased by 3.63 times during the daytime hours and by 3.84 times during the nighttime hours. Exposure to 20/4 h light cycle led to the appearance of daily circadian rhythm of Na^+ and Ca^{2+} excretion with predominance of nocturnal excretion of these ions. However, the overall changes in electrolyte excretion in SHR rats exposed to longer light phase are deleterious, because Na^+ and Ca^{2+} retention contribute to the development of hypertension.

The secretion of epiphyseal melatonin. In normotensive rats kept under standard light regime, a distinct circadian rhythm of aMT6s concentration in the urine was observed with the prevalence of night values over daytime values (Fig. 1). With increasing the duration of the light phase to 20 h, a pronounced tendency to an increase of this parameter during the daytime occurred, however, the night level did not change. As a result, the differences between the nighttime and daytime levels of aMT6s in the urine disappears in animals exposed to 20/4 h light cycle. Similar regularities were revealed for SHR rats kept under 20/4 h regime; during daytime, the level of aMT6s becomes significantly higher than at standard light regime. It should also be noted that urinary concentration of aMT6s during the daytime in SHR rats kept at 12/12 h light cycle was significantly below the level of Wistar-Kyoto rats.

Based on the results of aMT6s concentration in the urine, we can conclude that the production of epiphyseal melatonin in SHR rats increased during the daytime and did not change at night upon significant lengthening of the light phase. In normotensive animals, melatonin secretion during the daytime tended to increase, while nocturnal secretion did not change. As a result, the circadian rhythm of this function in animals of both lines disappears. It can be assumed that with a longer light exposure, the tendency to increased secretion of melatonin in the daytime is associated with an increased formation of serotonin, which is a substrate for its synthesis.

Thus, with increasing the duration of the light phase of the day, the structure of HR and BP rhythms undergoes significant changes in normotensive rats and remained unchanged in SHR rats. Changes in

electrolyte excretion were opposite in the control and SHR rats. As secretion of melatonin has similar patterns in animals of both lines, it can be concluded that the epiphyseal component of "biological clock" is not the only component of the functional system that determines the response of the studied rhythms to lengthening of the light phase.

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