DOI 10.1007/s10517-018-4312-6

GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

24-Hour Profile of Blood Pressure, Heart Rate, Excretion of Electrolytes, and Locomotor Activity in Wistar-Kyoto and SHR Rats under Conditions of Free-Run Rhythm

M. L. Blagonravov, E. V. Medvedeva, A. A. Bryk, V.A. Goryachev, A. E. Rabinovich, A. S. Letoshneva, and E. A. Demurov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 166, No. 8, pp. 146-151, August, 2018
Original article submitted May 28, 2018

We presented the results of our study of chronostructure of BP, HR, electrolyte excretion, and locomotor activity under conditions of "free-run rhythm" (light deprivation). In adult male Wistar-Kyoto (normotensive) and SHR (spontaneously hypertensive) rats, BP, biopotentials of the heart (ECG), and locomotor activity were recorded over 24 h by telemetric monitoring and the rate of excretion of electrolytes (Na^+, K^+, Ca^{2+}, and Mg^{2+}) during the nighttime and daytime hours was measured. It was found that under free-run rhythm, 24-h profiles of BP, HR, excretory function of the kidneys, and locomotor activity underwent more considerable changes in normotensive Wistar-Kyoto rats in comparison with hypertensive SHR rats. However, hypertensive rats demonstrated pronounced changes in rhythmic characteristics of HR, which can restrict adaptation reserves of the cardiovascular system.

Key Words: *arterial hypertension; electrolytes; spontaneously hypertensive rats; "free-run rhythm"; telemetric monitoring*

Many people all over the world live and work under conditions of artificial light rhythm characterized by lengthening of the daylight period. At the same time, numerous groups suffer from deficiency of daytime light which may have an unfavorable impact on the higher nervous activity and on somatic health. This primarily concerns people living in the North and Subarctic regions and experiencing disturbances of normal circadian BP structure under conditions of modified photoperiod (both people with normal vascular tone and with hypertension) [2]. The incidence of depressive disorders is also higher under conditions of chronic deficit of light, which is predominantly attributed to a decrease in the production of 5-hydroxytryptamine in the CNS [5,6,8]. However, even in the middle latitudes, many people are prone to the so-called seasonal affective disorders during the autumn-winter period, which can be due to the effect of the photoperiod on hippocampal activity [9]. It should be stressed that the researchers working in this field are mainly focused on mental activity, while the response of the cardiovascular system, including the structure of its biological rhythms, to changes in the light regime parameters receive little attention. As the BP regulation system includes various mechanisms, estimation of its rhythmic component requires analysis of at least several indices reflecting their activity. In particular, among factors contributing to the development of hypertension, disorders of the water-salt metabolism are of spe-

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

cial interest. It should be noted that renal mechanisms of BP regulation are characterized by 24-h periodicity determined by circadian rhythms of water and electrolyte exchange [4,7]. It was also shown that 24-h dynamics and the rate excretion of Na^+, K^+, and Ca^{2+} also depends on the season and lighting conditions [1].

In the present study, an attempt was undertaken to evaluate the state of 24-h profile of BP, HR, excretion of electrolytes, and locomotor activity in normotensive and hypertensive rats using the model of "free-run rhythm" [3], *i.e.* under conditions of long-term 24-h light deprivation. It is obvious that this situation is unusual for humans. We used this model to simulate extreme conditions of visible light deficit for estimation of possible short-term responses of the cardiovascular and excretory systems, which could be imperceptible under a milder impact.

MATERIALS AND METHODS

The experiments were performed on 20 male SHR (spontaneously hypertensive rats) and Wistar-Kyoto rats (animals with normal BP) in two parallel series. The animals were kept and all manipulations were performed in accordance with the Order No. 755 of the Ministry of Health of the USSR, (August 12, 1972) and European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

In series I (SHR, n=5; Wistar-Kyoto, n=5), BP, ECG, and locomotor activity were continuously recorded over 24 h using the method of telemetric monitoring. In series II, excretion of electrolytes (Na^+, K^+, Ca^{2+}, and Mg^{2+}) was evaluated by capillary electrophoresis (the animals were divided into groups according to the same principle as in series I). At the beginning of the experiment, the animals aged 36-38 weeks.

In both series, the animals were successively kept under 2 types of light regimens. Standard artificial 12/12 h light/dark regimen (control); light period from 07.00 to 19.00, light intensity 350 lux at level of animal eye; dark period from 19.00 to 07.00 (light intensity <0.5 lux). Free-run rhythm: absolute darkness (<0.5 lux) for 24 h. The animals lived under each of the described regimens for 7 days. The studied parameters were assessed on day 7.

During the experiment, the animals were kept in individual cages at constant temperature 23°C. The animals were fed regularly at 19.00.

In series I, BP, ECG in standard lead II, and locomotor activity were recorded over 24 h (07.00-07.00) by the method of telemetric monitoring using Data Sciences International equipment. To this end, TL11M2-C50-PXT radio transmitters, devices monitoring BP, biopotentials of the heart, and locomotor activity and transmitting the data in the form of the radiosignal to special receivers, were implanted to animals under general anesthesia. BP was recorded through a catheter introduced into the abdominal aorta and fixed with a tissue hemostatic adhesive. For ECG monitoring, electrodes were fixed under the chest muscles in projection of the electrical axis of the heart. Registration of all parameters was started in 10 days after implantation of transmitters. Thus, the animals were freely moving and eating during the experiment.

The data were stored in the computer and processed using Dataquest A.R.T. 4.2 Gold software. The following parameters were evaluated: mean daily (07.00-07.00), mean daytime (07.00-19.00), and mean nighttime 19.00-07.00) values of systolic BP (SBP_{24h}, SBP_{day}, and SBP_{night}), diastolic BP (DBP_{24h}, DBP_{day}, and DBP_{night}), HR (HR_{24h}, HR_{day}, and HR_{night}), and locomotor activity (LA_{24h}, LA_{day}, and LA_{night}).

Using Chronos-Fit software [10], 24-h profiles of BP, HR, and locomotor activity were also subjected to nonlinear rhythm analysis which represents a combination of partial Fourier analysis and stepwise regression technique. The following indices were determined: mesor as the mean value of the studied index over 24-h period, magnitude as the maximum deviation of the investigated index from the mesor, amplitude as the difference between the maximum and minimum value of the parameter, power of oscillations (% rhythm) as a chronobiologial index characterizing percentage of oscillations (percentage of values of the studied parameter with oscillatory distribution within 24 h).

In series II, urinary excretion of Na^+, K^+ and Ca^{2+}, Mg^{2+} over 24 h (07.00-07.00), daytime period (07.00-19.00), and nighttime period (19.00-07.00) was evaluated. For urine collection, metabolic cages for rats AE0906 (Open Science) were used. Electrolyte concentration in the urine was assessed by the method of capillary electrophoresis on a Kapel-105M instrument using technologies and reagents from Lyumeks Company. The amount of excreted electrolytes was calculated for the volume of urine in the corresponding samples.

The results were processed using Statistica 6.0 software. For each parameter, the mean and error of mean were calculated. Significance of differences was assessed using Mann—Whitney U test; the difference between the means was considered significant at $p \leq 0.05$.

RESULTS

Comparison of the data of telemetric monitoring of BP, HR, and locomotor activity in animals of the two strains revealed the following peculiarities (Table 1).

Under standard light regimen (12/12 h), practically no differences between the mean daytime and mean nighttime SBP and DBP were revealed in normotensive Wistar-Kyoto rats. In SHR rats, the mean nighttime SBP was significantly higher than the mean daytime SBP; for DBP, a tendency to an increase during nighttime was revealed. Thus, spontaneously hypertensive rats, in contrast to normotensive animals, demonstrated an accentuated circadian rhythm of BP. In rats of both strains, nighttime of HR and locomotor activity surpassed the corresponding daytime values and the daily distribution of the studied parameters had similar patterns. It should be noted, that their levels were somewhat different. In SHR rats, HR was higher than in Wistar-Kyoto rats at any time of day or night. On the contrary, locomotor activity in SHR rats was lower at night and within 24 h on the whole, but during daytime, locomotor activity in rats of both strains was at the same minimal level.

In animals kept under conditions of 24-h darkness (free-run rhythm), the following results were obtained on day 7. In normotensive rats, SBP tended to decrease during both the day and night hours, but the differences were insignificant. In SHR rats, SBP and DBP were the same as under standard light regimen. The difference only involved a wider range of SBP, due to which the difference between its daytime and nighttime levels became insignificant. Analysis of heart rhythm revealed substantial peculiarities. In Wistar-Kyoto rats, HR_{24h}, HR_{day}, and HR_{night} significantly decreased in comparison with standard light regimen. In SHR rats, these parameters increased under conditions of 24-h darkness. At the same time, a clear-cut circadian rhythm persisted and nighttime values were still higher than the daytime levels in both groups. It is important to note that the revealed features of HR were not associated with changes in animal locomotor activity.

The results of nonlinear analysis of the rhythm of 24-h profiles of BP, HR, and locomotor activity are shown in Table 2. No significant differences of the parameters characterizing the circadian rhythm of SBP under free-run rhythm in comparison with standard light regimen were noted in both normotensive and hypertensive animals. In Wistar-Kyoto rats, a significant increase in the magnitude, amplitude, and power of oscillations of DBP were revealed under conditions of 24-h light deprivation. In SHR rats, these changes were absent. As for HR, the mesor, magnitude, amplitude, and power of oscillations were increased in Wistar-Kyoto rats. In SHR rats, on the contrary, all these parameters decreased under conditions of 24-h light deprivation in comparison with standard light regimen. The rhythm of locomotor activity had the following features. The magnitude, amplitude, and power of oscillations increased under free-run rhythm in normotensive rats, but remained unchanged in SHR rats. Thus, rhythmic parameters in normotensive rats more flexibly responded to changes of photoperiod. These changes were probably aimed at increasing adaptation capacities of the body. SHR rats are characterized by more stable and rigid rhythms, which manifested in the absence of significant changes in rhythmic parameters of SBP, DBP, and locomotor activity under conditions of 24-h light deprivation.

TABLE 1. BP, HR, and Locomotor Activity of Wistar-Kyoto and SHR Rats under Standard Light Regimen (12/12 h) and under Conditions of Free-Run Rhythm (24-h Light Deprivation; 24/0 h) (*M*±*m*)

Parameter	Wistar-Kyoto rats		SHR rats	
	12/12 h	24/0 h	12/12 h	24/0 h
SBP_{24h}, mm Hg	117.29±2.17	109.18±3.31	202.72±3.83	201.37±5.69
SBP_{day}, mm Hg	116.54±2.12	110.80±2.25	196.43±4.83	196.2±6.19
SBP_{night}, mm Hg	118.05±2.32	108.43±4.36	209.01±2.94^{+}	207.03±5.21
DBP_{24h}, mm Hg.	83.69±3.82	79.50±3.12	146.37±4.14	144.77±4.69
DBP_{day}, mm Hg	82.74±3.23	78.24±2.37	140.88±4.46	140.43±5.18
DBP_{night}, mm Hg	84.48±4.20	78.95±3.89	151.86±3.95	149.52±4.20
HR_{24h}, bpm	252.33±6.93	220.79±7.15*	299.64±4.86	324.56±6.15*
HR_{day}, bpm	238.42±7.70	214.15±4.67*	282.63±5.09	310.74±5.67*
HR_{night}, bpm	266.22±4.23^{+}	226.71±3.52*$^{+}$	316.64±6.63^{+}	339.67±6.69*$^{+}$
LA_{24h}, score	2.91±0.43	2.66±0.37	1.62±0.18	1.42±0.14
LA_{day}, score	1.10±0.15	1.14±0.13	0.99±0.06	0.99±0.03
LA_{night}, score	4.72±0.89^{+}	3.71±0.72^{+}	2.26±0.34^{+}	1.89±0.28^{+}

Note. $p \le 0.05$ in comparison with *standard light regimen (12 h:12 h), $^{+}$mean daytime values.

TABLE 2. Parameters of 24-h Profile of SBP, DBP, HR, and Locomotor Activity Calculated using Nonlinear Rhythm Analysis in Wistar-Kyoto and SHR Rats under Standard Light Regimen (12/12 h) and under Conditions of Free-Run Rhythm (24-h Light Deprivation; 24/0 h) (*M*±*m*)

Parameter	Wistar-Kyoto rats		SHR rats	
	12/12 h	24/0 h	12/12 h	24/0 h
Mesor				
SBP, mm Hg	117.16±2.18	109.18±3.31	202.94±3.77	201.5±5.5
DBP, mm Hg	83.48±3.81	79.39±3.12	146.47±4.06	144.90±4.59
HR, bpm	252.35±6.93	220.65±7.15*	298.06±6.16	325.59±7.40*
Locomotor activity, score	2.94±0.40	2.45±0.32	1.58±0.15	1.46±0.15
Magnitude				
SBP, mm Hg	10.69±0.41	12.31±1.23	14.95±2.45	12.99±2.04
DBP, mm Hg	8.94±0.02	16.46±2.78*	13.63±2.27	10.28±0.84
HR, bpm	47.99±4.98	59.18±6.19	47.0±2.69	33.17±1.28*
Locomotor activity, score	1.87±0.22	4.29±0.49*	1.07±0.07	1.11±0.22
Amplitude				
SBP, mm Hg	20.17±0.10	22.07±0.34*	29.69±4.94	24.23±3.16
DBP, mm Hg	16.90±0.85	28.35±2.20*	27.14±4.58	20.29±1.60
HR, bpm	122.93±6.14	137.07±7.05	93.75±4.61	65.52±2.00*
Locomotor activity, score	3.73±0.43	7.74±0.14*	2.21±0.15	2.15±0.39
Power of oscillations (% of rhythm)				
SBP, %	43.13±10.30	52.49±3.64	33.93±7.25	28.91±2.86
DBP, %	32.71±12.01	75.03±13.83*	33.15±6.93	22.53±0.78
HR, %	49.47±3.31	64.93±4.24*	42.52±5.56	20.94±1.69*
Locomotor activity, %	12.31±1.17	23.80±4.34*	10.37±0.68	9.76±1.72

Note. *$p \le 0.05$ in comparison with *standard light regimen (12/12 h).

The pattern of changes in the HR rhythm (decrease in all parameters) attests to a certain impairment of the adaptation potential.

In series II, urinary excretion of electrolytes was evaluated (Table 3). In normotensive Wistar-Kyoto rats, excretion of K^+ and Mg^{2+} during the night period under standard light regimen was significantly higher than during daytime. For Na^+ and Ca^{2+}, the differences were insignificant. However, under conditions of 24-h light deprivation, the excretion rate of all studied electrolytes was significantly higher during night hours. In SHR rats, statistically significant differences in the excretion of all electrolytes between day and night hours were observed under conditions of standard light regimen and free-run rhythm.

Under standard light regimen, urine output in Wistar-Kyoto rats in day and night hours was 1 and 5 ml, respectively. In SHR rats, the corresponding volumes were 1.35 and 12.5 ml. Under free-run rhythm, the volumes of day-time and night-time diuresis virtually did not change, while in Wistar-Kyoto rats, diuresis increased to 1.64 ml during daytime and 21.2 ml at night. In Wistar-Kyoto rats maintained under conditions of 24-h light deprivation, excretion of electrolytes at night increased in comparison with that observed under standard light regimen. In SHR rats, no significant differences in electrolyte excretion between the two regimens were found despite considerable increase in diuresis under conditions of 24-h light deprivation. This is probably related to compensatory increase in the rate of osmotic dilution of the urine aimed at prevention of excessive loss of electrolytes in the animals of this strain. In normotensive rats, the concentration function of the kidneys increased. Thus, metabolism of the main electrolytes under conditions of light deprivation seems to be more stable in rats with genetically determined hypertension in comparison with normotensive animals. Opposite dependence was observed for water exchange.

TABLE 3. Parameters of Electrolyte Excretion (mmol) in Wistar-Kyoto and SHR Rats under Standard Light Regimen (12/12 h) and under Conditions of Free-Run Rhythm (24-h Light Deprivation; 24/0 h) (*M*±*m*)

Parameter	Wistar-Kyoto rats		SHR rats	
	12/12 h	24/0 h	12/12 h	24/0 h
Na^+_{24h}	2.12±0.44	6.22±1.67*	3.51±1.29	5.15±1.77
Na^+_{day}	0.85±0.07	1.09±0.24	0.48±0.16	0.50±0.16
Na^+_{night}	1.27±0.37	5.13±1.60*+	3.03±1.14+	4.65±1.65+
K^+_{24h}	4.51±0.56	8.68±1.24*	6.35±1.44	11.00±1.97
K^+_{day}	1.08±0.17	0.99±0.09	1.04±0.42	0.88±0.15
K^+_{night}	3.43±0.42+	7.69±1.30*+	5.31±1.17+	10.12±1.88+
Ca^{2+}_{24h}	0.06±0.01	0.50±0.11*	0.15±0.03	0.24±0.09
Ca^{2+}_{day}	0.03±0.01	0.030±0.003	0.03±0.01	0.02±0.01
Ca^{2+}_{night}	0.030±0.003	0.47±0.11*+	0.12±0.02+	0.22±0.09+
Mg^{2+}_{24h}	0.82±0.08	1.49±0.29*	0.96±0.03	0.70±0.09*
Mg^{2+}_{day}	0.27±0.03	0.09±0.02*	0.31±0.04	0.10±0.01
Mg^{2+}_{night}	0.55±0.07+	1.40±0.28*+	0.65±0.06+	0.6±0.05+

Note. $p \leq 0.05$ in comparison with *standard light regimen (12/12 h), +daytime values.

Taking into account the increase in HR and the absence of changes in BP in hypertensive rats under free-run rhythm, we can hypothesize that animals responded to 24-h light deprivation by enhancement of the excitability of the sympathetic nervous system that was partially attenuated for BP by the activation of diuresis.

Our findings suggest that daily rhythms of BP, HR, excretory function of the kidneys, and locomotor activity undergo more considerable changes under conditions of 24-h light deprivation in Wistar-Kyoto rats in comparison with SHR rats. Hypertensive rats have a relatively more stable and rigid rhythm of BP, electrolyte excretion, and locomotor activity. However, changes in chronostructure of HR can limit the adaptation reserves of the cardiovascular system.

REFERENCES

1. Zamoshchina TA, Ivanova EV. Peculiarities of the daily dynamics of sodium, potassium, calcium, and lithium content in rat blood, brain, and urine depending on the season and illumination conditions. Vestn. Orenburg. Gos. Univer. 2006;(12, Suppl.):104-106. Russian.
2. Simonenko VB, Solovieva KB, Dolbin IV. 24-hr monitoring arterial pressure in outpatients with cardiovascular risk factors in the Far North. Klin. Med. 2013;91(10):38-43. Russian.
3. Aschoff J, Wever R. Human circadian rhythms: a multioscillatory system. Fed. Proc. 1976;35(12):2326-2332.
4. Bonny O, Vinciguerra M, Gumz ML, Mazzoccoli G. Molecular bases of circadian rhythmicity in renal physiology and pathology. Nephrol. Dial. Transplant. 2013;28(10):2421-2431.
5. Dell'Osso L, Carmassi C, Mucci F, Marazziti D. Depression, Serotonin and Tryptophan. Curr. Pharm. Des. 2016;22(8):949-954.
6. Fakhoury M. Revisiting the serotonin hypothesis: implications for major depressive disorders. Mol. Neurobiol. 2016; 53(5):2778-2786.
7. Gumz M.L, Rabinowitz L. Role of circadian rhythms in potassium homeostasis. Semin. Nephrol. 2013;33(3):229-236.
8. Gupta A, Sharma P.K, Garg V.K, Singh A.K, Mondal S.C. Role of serotonin in seasonal affective disorder. Eur. Rev. Med. Pharmacol. Sci. 2013;17(1):49-55.
9. Miller M.A, Leckie R.L, Donofry S.D, Gianaros P.J, Erickson K.I, Manuck S.B, Roecklein K.A. Photoperiod is associated with hippocampal volume in a large community sample. Hippocampus. 2015;25(4):534-543.
10. Zuther P, Gorbey S, Lemmer B. Chronos-Fit 1.06, 2009. URL: http://chronos-fit.sharewarejunction.com.