http://dx.doi.org/10.35630/2199-885X/2021/11/1.8

EFFECT OF CHRONIC ALCOHOL INTOXICATION AND CONSTANT LIGHTING ON CARDIOVASCULAR PARAMETERS IN MALE RATS

Received 25 January 2021;
Received in revised form 23 February 2021;
Accepted 25 February 2021

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ABSTRACT — The aim of the research was to study the effect of chronic alcohol intoxication and constant il-lumination on the circadian rhythms (CR) of some parameters of the cardiovascular system in rats separately, as well as to study the rhythms of these parameters under the combined action of chronic alcohol intoxication (CAI) and constant illumination. It was found that chronic alcohol intoxication CAI at a fixed light regime causes a decrease in heart rate, an increase in SBP and PP; no changes were noted at CAI under constant lighting. At the same time, constant illumina-tion without ethanol exposure results in a decrease in heart rate and an increase in PP.
At the same time, CAI with a fixed light regime leads to the destruction of CR of all parameters, except for MBP; at constant illumination with CAI no circadian rhythms of HR, DBP, PP and MBP are detected. Constant illumination leads to the destruction of the CR of PP.
Among the remaining CRs, the heart rate rhythm, which is extant in the second group, persists practically unchanged, but the characteristics of all other CRs change significantly in comparison with control.

KEYWORDS — circadian rhythms, heart rate, blood pressure, desynchronosis.

INTRODUCTION

The rhythmicity of functioning is a fundamental, integral property of all living systems, which plays an important role in ensuring of normal vital functions. Based on biological rhythms, periodic programs are built that provide the necessary order for the course of bioprocesses, the optimal level of the functioning of organism at any given moment in time. Daily, or circadian rhythms (CR) are among the most significant rhythms for mammals. The cycles of life processes, which consequently replace each other, differ in their parameters, such as the duration of the period, amplitude, phase [3, 5, 16].

The temporal organization of the systems of mammalian organism is endogenous and ge-netically determined, but, nevertheless, it is modulated under the influence of periodic environmental factors — synchronizers, or pacemakers; and the light is one of the strongest synchronizers of daily biological rhythms in mammals. The rhythm of the course of adaptation processes is also of great practical importance, because it opens a reliable way to predict the dynamics of the state of the organism in acute and chronic stress induced by both internal and external causes. In cases of successful adaptation processes, the degree of influence of stressors on circadian rhythms is insignificant. Otherwise, the rhythmic processes of the organism lose their correctness, regularity, and state of desynchronosis occurs, which can lead to the development of one pathology or another, especially if there is a predisposition to it or the adaptive capabilities of the organism are weakened [9].

Currently, a fairly large number of people in the world are exposed to light pollution (in other words, lighting at night). Such impact may be related to the profession, may be due to habit and lifestyle [1]. Exposure to light at night has become an essential part of modern lifestyles and is associated with many serious behavioral and health conditions, including cardiovascular diseases and cancer [6, 7]. It is shown [10], that in the dynamics of the development of the diseases the general desynchronosis is one of the first disorders.

According to the hypothesis of *circadian destruction*, exposure to light at night disrupts the endogenous circadian rhythm, suppresses the nighttime secretion of melatonin by the pineal gland, which leads to a decrease in its concentration in the blood [14]. Disruption of CR during shift work leads to an increased risk of cardiovascular diseases, metabolic syndrome, type II diabetes mellitus [12, 15].

Another of the anthropogenic environmental factors to which the organism has to adapt is alcohol, or rather, alcohol intoxication. The chronotoxicity of alcohol and chronoesthesia to it were described in the works of Erhard Haus and Franz Halberg back in 1959. Even a single in-take of alcohol can cause significant chronobiological shifts: desynchronosis, amplitude-phase rhythm disturbances. Signs of desynchronosis persist after complete elimination of alcohol for several days [11, 17].

In some patients with alcohol dependence, even with prolonged abstinence, the normali-zation of circadian biorhythms does not occur; in this regard, another hypothesis was put forward — about the primacy of desynchronosis itself in the pathogenesis of the development of alcoholism. Chronic alcohol consumption alters the normal functioning of both central and peripheral rhythm-organizing structures, disrupting the normal functioning of systems of organism [2, 13].

The toxic effect of alcohol directly on the myocardium is manifested in the appearance of functional heterogeneity - one part of the muscle fibers atrophies, and the other hypertrophies. Due to the melting of the Z-discs of sarcomeres by acetaldehyde, diffuse focal cardiosclerosis progresses, while the normal propagation of excitation through the myocardium is disrupted. Subsequently, fatty degeneration in the heart tissue and arteriosclerosis occur, which are accompanied by a decrease in vascular tone in the microvasculature against the background of a progressive decline of the cardiac contractile function [8].

The most important parameters of cardiac activity — heart rate (HR), blood pressure (BP), etc., have their own clear biological rhythms, synchronized in time in accordance with the period of wakefulness and sleep. The mismatch of biorhythms of various CVS parameters due to CAI can precede the development of pathological conditions with subsequent informational, energetic, metabolic and structural changes.

In this regard, we found it relevant to study the effect of chronic alcohol intoxication and constant illumination on the CR of some parameters of the cardiovascular system of rats separately, as well as to study the rhythms of these parameters under the combined action of CAI and constant illumination.

MATERIALS AND METHODS

Animals

The study was conducted on 160 male Wistar rats at age of 6 months, weighing 300±20 g. Animals were taken from the Stolbovaya nursery of laboratory animals (Moscow Region, Russia).

Design of experiment

All animals were kept in plastic cages with free access to food and water within 3 weeks. Animal were divided on 4 equal groups.

Control group was kept in standard laboratory conditions at fixed light regime (light:dark/10:14 hours, with lights on at 8:00 and off at 18:00).

1st group (n=40), was kept in the same conditions as control, but received as a drink a 15% aqueous solution of ethanol ad libitum.

2nd group (n=40), was kept in standard laboratory conditions at constant lighting (24 hours).

3rd group (n=40), was kept in standard laboratory conditions, but also at constant lighting (24 hours) and received as a drink a 15% aqueous solution of ethanol ad libitum.

The criterion for the selection of rats in the 1 and 3 groups, along with the absence of vis-ible deviations in the state and behavior, was the initial preference for a 15% solution of ethyl alcohol to a tap water. For this, a preliminary experiment was carried out for 3 days in individual cages with free access to both liquids.

Measurement of the parameters of the cardiovascular system was carried out at 9:00, 15:00, 21:00 and 3:00 using the "Systola" device (Neurobotics, Russian Federation). Heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded directly. The pulse pressure (PP) was calculated by formula PP=SBP-DBP. For calculation of mean blood pressure (MBP) we used the formula: MBP= DBP+0,43PP; the index of energy costs was determined by the formula I=HR×SBP/1000.

All animal experiments were performed in according to the compliance with EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Methods of statistical processing

The obtained data were analyzed using the GraphPad Prism 6.0 program by calculating average values, standard deviation, and arithmetic mean error. The numerical rows characterizing the diurnal fluctuations of the studied physiological rhythms of animals were subjected to mathematical processing, on the basis of which group chronograms were drawn. The statistical difference was determined using the Kruskal-Wallis test. Differences were considered statistical-ly significant at $p < 0.05$.

For statistical estimation of amplitude and acrophase of CR the cosinor analysis, which is an international, recognized method for the unified study of biological rhythms, was performed using the Cosinor-Ellipse2006-1.1 program.

Cosinor analysis is intended for the analysis of wave processes and the processing of chronobiological data. In the course of the analysis, the experimental data are approximated by the least squares sinusoidal parameter estimation. The presence of a reliable circadian rhythm, as well as its acrophase and amplitude, were determined. The output information of the cosinor analysis are the main parameters of the rhythms:

mesor, i.e. the value of the average level of the sinusoid (h), the amplitude of the sinusoid (A) and acrophase (Phi), that is the time of the onset of the maximum of the function. Mesor coincides in magnitude with the daily average value of the investigated function. Acrophase is a measure of the peak time of total rhythmic variability over a 24-hour period, i.e. the time when the function reaches its maximum. The amplitude corresponds to half of the total rhythmic variability in the cycle. Acrophase is expressed in hours; amplitude values are expressed in the same units as the studied variables.

The second stage is the construction of an error ellipse, which is necessary to determine the validity of the existence of rhythms at the accepted confidence level (for example, at the level of 0.95). A sinusoid is depicted on a plane by a point, the polar coordinates of which are amplitude and acrophase. All points obtained in this way in Cartesian coordinates are considered as realizations of a two-dimensional random variable with a hypothetically normal distribution law, and an ellipse of dispersion of errors of the general mean is constructed. The circadian rhythm is considered reliable when two conditions are met: the averaged sinusoid, approximating chronograms (depicted by a cross), must enter the ellipse, and the ellipse itself must not pass through the center of coordinates (because in this case, acrophase will fall on the entire 24 hour period) [4].

RESULTS

As a result of conducted study it is established that in 1st and 2nd experimental groups there is the decrease of HR in comparison with control, but the value of this parameter in the 3rd group is higher, than in other experimental groups, and does not reliably differ from the values of control. At the same time it is noted the reliable increase of SBP at 1st experimental group, and also the increase of PP in animals of 1st and 2nd experimental groups (Table 1, 2).

When considering the results of the cosinor analysis of the daily dynamics of the studied parameters, the presence of reliable CR for all parameters in the control was established. At the same time, CR of heart rate in groups 1 and 3 is destroyed, remaining in the 2nd group with char-acteristics practically indistinguishable from control (Table 3).

Reliable CR of SBP is not observed in 1st group, and the rhythm parameters in groups 2 and 3 differed significantly from control indicators. In the case of DBP, the rhythm, as in the case of HR, is maintained only in the second group, but at the same time the amplitude-phase characteristics of the rhythm differ significantly from the control.

CR of PP is observed only in the control, being destroyed in all three experimental groups, and CR of MAP is destroyed only in 3rd group, although CR of groups 1 and 2 differ in phase-amplitude characteristics from the control.

The CR of index of energy cost is destroyed in the 1st experimental group, while the acrophase of this rhythm occurs in the control at night hours, and in the second and third groups — in the daytime.

CONCLUSION

As a result of the study, it was found that chronic alcohol intoxication (CAI) at a fixed light regime causes a decrease in heart rate, an increase in SBP and PP; no changes were noted at CAI under constant lighting. At the same time, constant illumination without ethanol exposure results in a decrease in heart rate and an increase in PP.

At the same time, CAI with a fixed light regime leads to the destruction of CR of all pa-rameters, except for MBP; at constant illumination with CAI no circadian rhythms of HR, DBP, PP and MBP are detected. Constant illumination leads to the destruction of the CR of PP.

Among the remaining CRs, the heart rate rhythm, which is extant in the second group, persist practically unchanged, but the characteristics of all other CRs change significantly in comparison with control.

***Table 1.** Parameters of cardiovascular system in rats*

	Control	1st group	2nd group	3rd group
HR, bpm	431.4±34.72	390.1±46.23	367.9±41.07	433.3±32.86
SBP, mm Hg	113.9±10.75	132.8±21.68	118.9±19.72	118.1±13.51
DBP, mm Hg	96.1±10.35	104.4±24.24	93.1±16.15	94.0±11.07
PP, mm Hg	17.75±9.53	28.40±11.68	25.74±10.35	24.26±9.68
MBP, mm Hg	108.6±25.27	166.6±22.44	104.2±17.02	104.3±11.22
Index of energy costs of heart	49.21±6.74	51.83±10.82	43.5±8.79	51.27±1.60

Table 2. *Significance of intergroup differences in the studied parameters of the cardio-vascular system in rats.*

	C×1EG	C×2EG	C×3EG	1EG×2EG	1EG×3EG	2EG×EG
HR, bpm	<0,005	<0,0001	>0,05	>0,05	<0,005	<0,0001
SBP, mm Hg	<0,005	>0,05	>0,05	>0,05	<0,05	>0,05
DBP, mm Hg	>0,05	>0,05	>0,05	>0,05	>0,05	>0,05
PP, mm Hg	<0,005	<0,05	>0,05	>0,05	>0,05	>0,05
MBP, mm Hg	>0,05	>0,05	>0,05	>0,05	>0,05	>0,05
Index of energy costs of heart	>0,05	>0,05	>0,05	<0,05	>0,05	<0,005

Table 3. *Results of the cosinor analysis of the diurnal dynamics of the cardiovascular pa-rameters in rats*

Group	HR			SBP		
	Acrophase	Amplitude	Mesor	Acrophase	Amplitude	Mesor
Control	1601	7.18	431.35	436	1.12	113.9
1st group	No reliable CR			No reliable CR		
2nd group	16.02	8.99	369.0	1424	21.34	117.0
3rd group	No reliable CR			11.18	7.98	117.82
Group	DBP			PP		
	Acrophase	Amplitude	Mesor	Acrophase	Amplitude	Mesor
Control	2144	4.22	96.10	1436	4.89	20.88
1st group	No reliable CR			No reliable CR		
2nd group	1408	15.05	91.22	No reliable CR		
3rd group	No reliable CR			No reliable CR		
Group	MBP			Index of energy costs of heart		
	Acrophase	Amplitude	Mesor	Acrophase	Amplitude	Mesor
Control	1418	18.01	102.23	136	1.67	48.57
1st group	1148	11.88	115.21	No reliable CR		
2nd group	342	14.35	102.32	1425	8.18	43.23
3rd group	No reliable CR			1207	5.90	51.07

ACKNOWLEDGEMENTS

Financial support for this study was carried out by Moscow State Regional University.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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