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Reactivity of stress-implementing system under conditions of light regime change in experiment

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Influence of light exposure duration (model 18 : 6) on hormonal indices of stress-implementing system in laboratory animals blood is studied. Activity of stress systems central link was evaluated based on data on concentration of adrenocorticotrophic hormone, melatonin and β-endorphin in blood serum, observed during enzyme immunoassay. Reaction of peripheric link of the stress-implementing system was evaluated in blood smears based on results of qualitative count of catecholamine granules in erythrocytes. It was established, that variations of hormonal indices of the stress systems for laboratory animals depend on trigger factor activity periods. With increase of the experiment duration to 21-st days the most pronounced reduction of melatonin, β-endorphin and increase of adrenocorticotrophic hormone concentration, as well as sharp increase of catecholamines level are observed in the blood serum. These hormonal changes develop as a result of failure of compensation mechanisms of the stress-implementing systems in connection with melatonin rhythms disturbance, that indicates the gradual development of the general adaptation syndrome.

Keywords: endocrine profile, stress-implementing system, general adaptation syndrome, biorhythms, light desynchronosis.

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1. Introduction

Living organism functioning is subject to cyclicity and is regulated as a result of combined operation of nervous, endocrine and immune systems [1]. External factors have a large influence on consistency support of an organism internal environment. Natural alternation of day and night, solar activity, climatic conditions and other parameters constantly impact the circadian rhythms generation in organism [2,3]. Circadian rhythms participate in regulation of various physiological and behavior processes: hormones synthesis, metabolic processes intensity, sleep and wake duration, etc.

Human in the modern world is often under conditions of constant influence of various stress factors of external nature, which result in homeostatic disruption and development of dysfunctional and morphological changes in organs. Under exposure of a stress stimulus the development of the general adaptation syndrome is induced and cascade of non-specific neurohumoral reactions is started, focused on activation of homeostatic mechanisms for organism adaptation to activity under new conditions. One of such stress factors is a light exposure. Change of light regime, occurring, for instance, as a result of day and night alternation periods breaking during jet lag or at night working, results in organism biorhythms desynchronization [2,4,5].

Severity of changes of physiological functions in organism depends on duration of abnormal exposure, external oscillator power and light regime option. On the works [6–12] it was demonstrated, that in connection with long-term light exposure the gnawing animals experience reduction of waking hours, degradation of sleep duration and quality, development of functional and structural changes in organs, obesity and type II diabetes mellitus. However, until recently, the issues of activity of the stress-implementing system hormones secretion under conditions of the extended photoperiod, accompanied with alternation of light and dark lighting regimes, remain understudied. Therefore the purpose if this work is to study the stress-implementing system and melatonin (hormonal „pacemaker") concentration change in response to stimulus action under conditions of light regime change in the experiment.

2. Materials and methods

The experimental study was carried out on the base of scientific laboratories of histology and hominal physiology faculties named after I.A. Chuevsky of the Saratov SMU named after V.I. Razumovsky. The experiment was carried out in accordance with the international ethical norms of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) and „International Guiding principles for Biomedical Research Involving Animals" (2012), and based on the recommendations of the Ethics Committee of the Federal State-Owned Publicly-Funded Institution of Higher

Education Saratov SMU named after V. I. Razumovsky of the Ministry of Health of the Russian Federation (protocol № 4 dated 06.12.2016).

Long-term light exposure modeling was performed using differential approach to alternation of light and dark regimes Light-Dark (18:6). Light-Dark model assumes 18 h of continuous light exposure with light intensity of 50 w and 6 h of dark hours regime. The experiment was performed using 36 white mongrel male rats with weight of 225 ± 25 g, which were divided into 3 groups: two experimental groups and one control group. The first experimental group of animals was under conditions of extended light regime for 10 days, the second — for 21 days. The animals from the control group were under natural lighting conditions for 21 days. Duration of time periods in the experiment was defined based on data on stress reactions staging formation in organism: 10-th day is characterized with development of the general adaptation syndrome stage, 21-st day — with adaptation mechanisms failure [13]. The experiment was carried out during fall period. The animals of all experimental groups had free access to water and food.

The animals were removed from the experiment during the first half of the day (from 9 a.m. to 1 p.m.) by narcosis preparation overdosing. For manipulations performing the intramuscular combination of Telazol (Zoetis Inc, USA) at a dose of 8 ml/kg and Xylanit (Nita-Farm, Russia) at a dose of 8 mg/kg was used.

Study of the central link of the stress-implementing system of laboratory animals was carried out using enzyme immunoassay (EIA) with application of a reagents kits ELISA Kit For Melatonin (MT), ELISA Kit For Adrenocorticotropic Hormone (ACTH) made by CLOUD-CLONE CORP. (USA) at automatic microtablet spectrophotometer „EpochBioTek Instruments“ (USA). Blood sampling for EIA was carried out with a puncture from the pulmonary heart into plastic tubes BD Vacutainer SSTTM II Advance

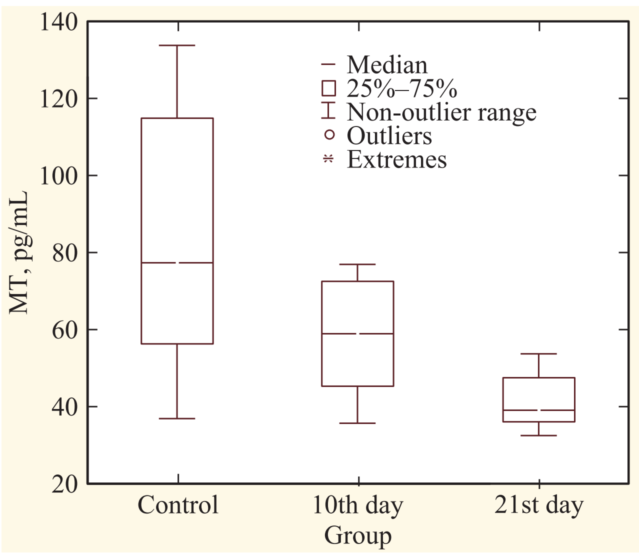

Figure 1. Dynamics of melatonin concentration in blood serum, pg/mL.

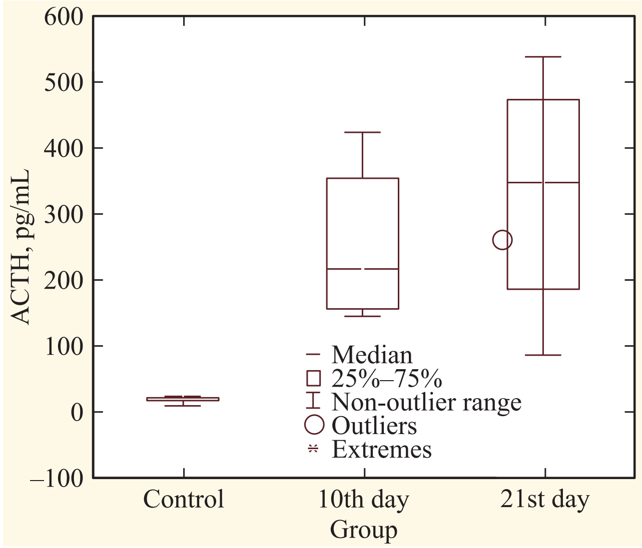

Figure 2. Dynamics of ACTH concentration in blood serum, pg/mL.

REF with yellow cap Brand Vacutainer in a volume of 5 ml. For biochemical study the blood serum was prepared by centrifugation for 20 min and not later than 3 h after samples acquisition. Determination of concentrations of melatonin, adrenocorticotrophic hormone (ACTH) and β-endorphin (bEP) in the blood serum was performed using EIA.

Reaction of peripheric link of the stress-implementing system on the long-term light exposure was evaluated using a cytochemical method of A.I. Mardar and D.P. Kladienko [14]. Blood smears were applied to albumen-coated microscopic slides and fixed with 2% water solution of potassium dichromate at temperature of 37□ for 2 h. Then the smears were washed in distilled water and colored with 5% water solution of silver nitrate. After 5 min the smears were washed in distilled water and colored with 1% alcohol solution of eosin. Qualitative evaluation of catecholamine granules content, occluded on erythrocytes in the blood smears, was performed using medical microvisor μ Vizo-101 LOMO (OOO „LOMO FOTONIKA“ RF). During study the number of granules per 100 erythrocytes in a lens field of $\times 40$ was counted.

Statistical processing of the study results was performed using Statistica 10.0 software (Stat Soft Inc, USA). In case of difference of values distribution in a sample from the normal, median and quartiles were calculated. Significance of differences (p) was calculated using a non-parametric test of Mann–Whitney. Changes at $p < 0.05$ were considered significant.

3. Study results

It was established, that the variations of hormonal indices of the stress systems of the laboratory animals, that were

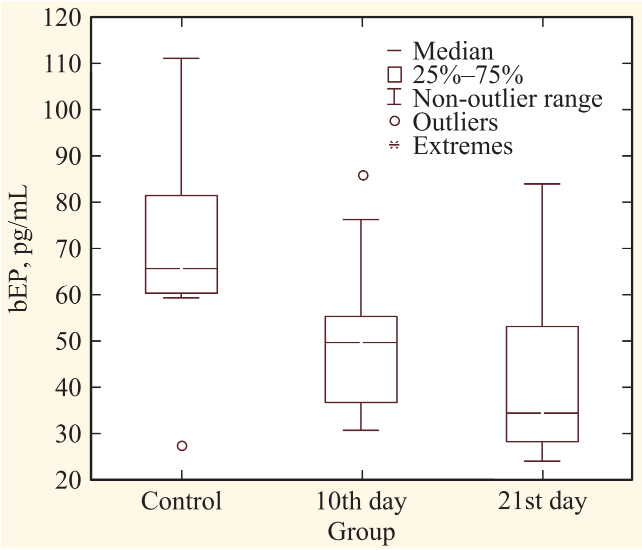

Figure 3. Dynamics of β-endorphin concentration in blood serum, pg/mL.

under light exposure conditions, depended on duration of trigger factor activity. As a result of EIA performing the changes in concentrations of hormones of melatonin, β-endorphin and ACTH in response to light exposure duration increase were observed in the blood serum (fig. 1–3).

In the group of animals, that were under conditions of the experiment for 10 days, the insignificant reduction of melatonin concentration in blood serum to 59 pg/mL [49; 68] was observed, while control group values were 77 pg/mL [64; 113]. Increase of exposure duration to 21 days was accompanied with reduction of this index values in half compared to the results, observed in the control group — to 39 pg/mL [37; 47].

Changes of concentration of ACTH hormone blood serum were irreversible and appeared as sharp increase of values. On the 10-th day of the experiment the increase of this index to 215 pg/mL [158; 336] was observed, while the control group values were 20 pg/mL [17; 22]. Increase of experiment duration to 21 days was accompanied with the further increase of ACTH titer to 346 pg/mL [242; 445], exceeding the results in the control group by a factor of 17.3.

In connection with corticotropin content increase the reduction of β-endorphin level in laboratory animals blood in all experimental groups was registered: on the 10-th day of the experiment to 49.6 pg/mL [39.1; 54.9] and on the 21-st day to 34.5 pg/mL [28.1; 48.7].

During analysis of catecholamines content in erythrocytes on the 10-th day of the experiment the number of catecholamine granules was not statistically different from the control group and was equal to 33 [26; 43]. It should be noted, that on the 21-st day of light exposure the sharp increase of this index to 483 [455; 525] was observed, exceeding the value in the control group by a factor of 20.

Adaptive response of organism is defined with the intensity of trigger factor activity and can be presented with a simple limited reaction or development of a generalized system response. Determination of level of melatonin and hormones of the stress system allows to evaluate the developing reaction in organism and monitor the time of regulatory mechanisms failure [16].

In the experiment using Light-Dark (18:6) model the changes of titers of hormones of melatonin, β-endorphin and ACTH in laboratory animals blood depended on duration of light exposure and developed in accordance with the stages of the general adaptation syndrome formation. The main criterion of biorhythms disturbances in organism is reduction of melatonin generation, which is in inverse proportion to the light exposure duration. Reduction of melatonin content in the blood serum on the 10-th day of the experiment is a physiological reaction to the long-term light exposure and primarily is of regulatory nature. With experiment duration increase in connection with the extended photoperiod, the activation of light-sensitive suprachiasmatic nuclei in the hypothalamus occurs, accompanied with increase of hypophysiotropic hormones synthesis and the further reduction of dark secretion of melatonin with epiphysis [16]. As a result, the level of melatonin on the 21-st day of exposure reduces in half compared to the control group, resulting in stable activation of stress-implementing components and reduction of stress-limiting factors, development of biorhythms desynchronization and start of the chronic stress reaction. One of the main links of the stress-limiting system is ?-endorphin, that limits the influence of the stress-implementing system, preventing from living organism tissues damage [17]. On the 21-st day of the experiment the largest reduction of β-endorphin concentration is observed, indicating the lowering of resistance to the trigger factor activity. This reaction is accompanied with increase of concentration of hormones of central origin (ACTH), as well as increasing growth of neurotransmitters (catecholamines) level — known markers of intensive activity of a stress agent [18].

Thus, the long-term light exposure exerts the significant negative influence on organism hormonal state and results in failure of stress system mechanisms. Without a doubt, it defines the development of adverse effects of the light exposure, appearing as deviations both in behavior reactions of animals and morphofunctional changes in visceral organs [3–5].

Conclusion

As a result of this study it was established, that at long-term light exposure using differential approach to alternation of light and dark regimes Light-Dark (18:6), the reduction of level of melatonin, β-endorphin and increase of concentration of ACTH and catecholamines occur. Severity of hormonal disorders increases with increase of experiment duration and indicates the gradual development of the general adaptation syndrome stages.

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3.1. Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Kalsbeek A., Palm I.F., La Fleur S.E., Scheer F.A.J.L., Perreau-Lenz S., Ruiter M., Kreier F., Cailotto C., Buijs R.M. // J. Biol. Rhythms 2006. V. 21. № 6. P. 458–469 doi.org/10.1177/0748730406293854.

[2] LeGates T.A., Fernandez D.C., Hattar S. // Nat Rev Neurosci. 2014. V. 15. № 7. P. 443–454. doi.org/10.1038/nrn3743

[3] Cedernaes J., Waldeck N., Bass J. // Genes Dev. 2019. V. 33. № 17–18. P. 1136–1158. doi.org/10.1101/gad.328633.119

[4] Snezhinskij V.A., Pobivanceva N.F. // Zhurnal Grodnenskogo gosudarstvennogo medicinskogo universiteta. 2013. № 1. S. 9–13. (in Russian)

[5] Koch C.E., Leinweber B., Drengberg B.C. et al. // Neurobiol. Stress. 2017. V. 6. P. 57–67. doi.org/10.1016/j.ynstr.2016.09.001

[6] Rapoport S.I., Chibisov S.M., Blagonravov M.L. // Klinicheskaya medicina. 2013. № 9. S. 71–73 (in Russian)

[7] Zlobina O.V., Pahomij S.S., Bugaeva I.O., Maslyakova G.N., Ivanov A.N. // Vestnik novyh medicinskih tekhnologij. Elektronnoe izdanie. 2018. № 5. S. 245–249. (in Russian)

[8] Zlobina O.V., Bugaeva I.O., Pahomij S.S., Ivanov A.N., Slyusarenko Yu.A., Usol'ceva E.D. // Vestnik novyh medicinskih tekhnologij. Elektronnoe izdanie. 2018. № 5. S. 250–254. (in Russian)

[9] Fonken L.K. et al. // PNAS. 2010. № 107. P. 18664–18669.

[10] Coomans C.P. et al. // FASEB journal. 2013. № 27. P. 1721–1732.

[11] Casiraghi L.P., Alzamendi A., Giovambattista A., Chiesa1 J., Golombek A.D. // Physiological Reports. 2016. V. 4. № 8. E 12743. doi. 10.14814/phy2.12743

[12] Phillips D.J., Savenkova M.I., Karatsoreos I.N. // Brain, Behavior, and Immunity. 2015. № 47. P. 14–23. doi.org/10.1016/j.bbi.2014.12.008.

[13] Morozov V.N., Hadarcev A.A. // Vestnik novyh medicinskih tekhnologij. 2010. № 1. S. 15–17. (in Russian)

[14] Mardar A.I., Kladienko D.P. Cytochemical method for detecting catecholamines in erythrocytes. Laborat. Delo. 10: 586–588. 1986. (In Russ)

[15] Zlobina O.V., Moskvina A.O., Ivanov A.N., Bugaeva I.O. // Rossijskij fiziologicheskij zhurnal im. I.M. Sechenova. 2021. № 107(3). S. 312?320. (in Russian) https://doi.org/10.31857/S0869813921030109

[16] Krupatkin A.I. // Regionarnoe krovoobrashchenie i mikrocirkulyaciya. 2014. T. 13, № 1. S. 83–99. (in Russian)

[17] Lasukova T.V., Nizkodubova S.V., Muhtobarova E. Yu. Opioidnaya sistema, yeye vozmozhnaya rol' v mekhanizme adaptacii organizma sportsmenov k dejstviyu gipoksii. Vestnik TGPU.140(12): 215–221. 2013. (in Russian)

[18] Poryadin G.V., Zelichenko L.I. Stress i patologiya. Metodicheskoe posobie Ros. gos. med. univer. 2009. (in Russian)