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MORPHOFUNCTIONAL PARAMETERS OF SEGMENTED NEUTROPHILS IN HEN BLOOD DURING ADAPTATION TO DIFFERENT LIGHTING REGIMES

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Abstract.

The analysis of the morphofunctional state of neutrophils in hen blood (geometric parameters, the parameters of surface protrusions, the modulus of membrane elasticity, surface potential) was performed using atomic-force microscopy during the adaptation to the lighting regime change. According to the study results it was found that hen demonstrate significant and reliable changes in the parameters of the protrusions and invaginations of the plasma membrane surface during the adaptation process to light regimes. These changes can characterize the decrease of neutrophil secretory activity and the activation of their motor capacity.

Keywords: *Adaptation, atomic force microscopy, stress, neutrophils, illumination mode, cytometry.*

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INTRODUCTION:

The studies of recent years have shown a significant role of white blood cells in the adaptation of the organism to the effects of adverse environmental conditions. Neutrophilic leukocytes are the first protective barrier of an organism against infection, and also they rid the body of its own transformed and damaged cells. Currently, neutrophils are considered as the main cells that play a key role in the anti-infection protection of a body not only due to its ability to absorb pathogens, but also to release the spectrum of microbicidal components. They accumulated a large amount of experimental and clinical material that makes it possible to consider the activation of neutrophils as an emergency reaction determining an organism resistance. The study of morphological and functional parameters of blood cells provides valuable information about their participation in homeostasis maintaining [1; 2; 3]. The method of atomic force microscopy makes it possible to study the morphofunctional changes occurring at the level of cellular structures [4, 5], which opens the possibility of their use during the analysis of organism adaptive reactions and stress reaction evaluation.

The purpose of the study: to study the dynamics of some morphometric and functional parameters of hen blood neutrophils in the process of adaptation to different light regimes.

MATERIALS AND METHODS:

The experimental part of the work was performed in the conditions of a vivarium on laying hens from 3 groups of "Haysex brown" cross at the age of 10 months, 16 animals in each. The 1st group of hens is the control one, they were kept under light conditions for 12 hours. Light (C) - 12 hours, darkness (T); the hens of the 2nd group - under the light regime of 7 h. C - 1 h. T; the hens of the third group - under light conditions with the parameters of 2 hours. C - 3 hours. T - 5 hours. C - 1 hour. T - 4 hours. C - 9 hours. T. The adaptive features of hen body were studied during 30 days [6].

The hens were in the conditions of cellular maintenance with the planting density, the front of feeding and drinking in accordance with zootechnical norms and the technology of this cross growing. The blood for the study of morphofunctional parameters of the cells was taken from the axillary vein of hens, 5 ml on the 6th, the 16th and the 30th day of adaptation. The scanning of the cells (n = 10) was carried out using an atomic force microscope (AFM) "INTEGRA Vita" (NT MDT, Zelenograd) in the research laboratory "The physiology of adaptation processes" NRU BelSU by a semi-contact method. The results were processed using Nova 1.0.26 software, the Build 1397 (NT MDT). The potential of the cell surface was evaluated by the Kelvin probe. The reliability of the differences was assessed by Student's t-criterion.

RESULTS AND DISCUSSION:

Table 1 presents the results of neutrophil certain parameter study during the adaptation to light regimes.

Table 1. The dynamics of neutrophil parameters in hen blood at the adaptation to light modes

Parameters	Adaptation day	Groups		
		1	2	3
Surface area, mcm² (S)	6-th	176.1±27.2	146.3±3.8	110.2±4.1
	16-th	174.1±7.2	132.1±6.9	128.2±1.7
	30-th	172.1±2.2	120.5±0.9*	131.1±1.2
Volume, mcm² (V)	6-th	65.3±10.3	70.9±7.6	73.5±6.6
	16-th	66.2±0.8	65.1±8.4	75.5±9.1
	30-th	66.3±8.3	69.1±1.5	70.3±2.0
Height, mcm (h)	6-th	0.6±0.02	0.8±0.1	0.5±0.4
	16-th	0.7±0.1	0.8±0.1	0.4±0.3
	30-th	0.6±0.01	0.4±0.1	0.4±0.2
Diameter, mcm (d)	6-th	10.9±0.3	11.4±0.01	10.3±0.5
	16-th	10.4±0.2	9.2±0.8	9.6±3.1
	30-th	9.8±0.3	11.5±0.7	13.4±0.6

The width of surface protrusions, nm	6-th	1.9±0.5	1.9±0.5	6.4±1.1*
	16-th	1.7±0.6	1.2±0.5	5.7±1.7*
	30-th	1.8±0.9	1.9±0.01	2.6±0.8
The height of surface protrusions, nm	6-th	53.1±2.5	77.7±1.9*	16.1±1.5**
	16-th	52.2±2.6	10.4±1.7**	20.7±1.9*
	30-th	54.2±1.5	50.7±9.2	16.1±2.3*
The width of plasma membrane ingrowth, nm	6-th	3.3±0.5	3.0±1.2	7.3±0.6*
	16-th	3.2±0.2	2.0±1.1	12.0±1.5*
	30-th	3.8±0.8	1.8±0.3	0.2±0.001*
The depth of plasma membrane ingrowth, nm	6-th	68.3±4.7	165.8±1.4**	145.9±6.9*
	16-th	67.3±2.6	67.8±6.03	299.3±7.1**
	30-th	69.3±2.3	117.5±7.5*	63.8±5.7

Note: the reliability of differences in comparison with the data of the 1st group
* – at P<0.05, **– at P<0.01

In our studies on the 6th day of adaptation to different lighting regimes, the following significant differences were found in the parameters of hen blood neutrophils in experimental groups as compared with the control one: the width of the surface protrusions among the hens of the 3rd group increased by 236 %, in group №2 - by 46 % and the same figure decreased for the hens of group № 3 by 69%. The width of the plasma membrane invagination increased by 121% in the hen blood neutrophils of the group № 3, the depth of plasma membrane invagination significantly increased among the hens of the group № 2 and № 3 by 142% and 113%, respectively.

On the 16th day of adaptation, significant differences in the parameters of hen blood neutrophils were revealed in the experimental groups characterizing the surface folding. Thus, the width of the surface protrusions increased by 235% in hen blood cells of group № 3, the height of the surface protrusions increased among the hens of group № 2 and № 3 by 80% and 60%, respectively.

On the 30th day of adaptation, they revealed significant differences in the parameters of hen neutrophils in experimental groups according to the following parameters: surface area, the height of surface protrusions, the width and the depth of plasma membrane invaginations.

When the functional properties of neutrophils are characterized, their mobility and secretory activity are important. Neutrophils are very sensitive to the appearance of blood factors capable of their function modulation, such as bacterial endotoxins, pro-inflammatory cytokines, etc.

An important consequence of a systemic inflammatory response development in inflammatory reactions is the bonus (the preparation for activation) and the activation of circulating neutrophils. A number of studies have shown that neutrophil activation and bonus provision is accompanied by the inhibition of their motor activity, i.e. the competitive ratios of neutrophil mobility and their secretory activity were revealed. There is the evidence of spontaneous migration, chemokinesis, chemotaxis and phagocytosis inhibition during neutrophil activation. The inhibition of motor activity of neutrophils during activation is associated with partial switching of cytoskeleton structures from the motor apparatus to the secretory one [3, 7, 8]. It was also shown that the oppression or the activation of motor activity of neutrophils can serve as a specific indicator of toxic factor presence in blood that reward or activate neutrophils and affect the intoxication of a body and the state severity during inflammatory processes [3].

It was established that such geometric parameters of neutrophils as diameter, surface area, height and volume have significant changes in inflammatory processes and indicate the degree of their activation. When neutrophils are activated, the granules move to a plasma membrane. The process of exocytosis involves the passage of intracellular granules through the cortical cytoskeleton and their subsequent fusion with a plasma membrane, during which the membranes of the granules become the part of the neutrophil plasma membrane, supplying receptor

proteins, enzymes or the components of enzyme complexes in it. After granule membrane and the cytoplasmic membrane fusion the area of the cell surface increases significantly, contributing to their diameter increase. The magnitude of the phase height depends on the characteristics of nuclear structures and the cell granular device. Thus, the increase of neutrophil surface area, volume and diameter indicates the activation of their secretory activity [2, 6, 7, 8].

In our studies significant and reliable changes in neutrophil parameters were noted, such as the width of the surface protrusions, the height of the surface protrusions, the width of plasma membrane invagination, the depth of plasma membrane invagination, which characterize the presence and the characteristics of pseudopodia. A moving neutrophil releases a lot of pseudopodia, their presence makes it possible to judge the motor activity of a cell. Thus, it can be assumed that when hens are adapted to a change in a light regime, cellular mechanisms are aimed at the mobility of neutrophil activation and their secretory activity reduction.

The studies of the mechanical properties of a neutrophil membrane have shown that there is a definite relationship between the rigidity of a neutrophil membrane and the degree of their activation [5]. Table 2 presents the results of functional properties of hen blood neutrophil membrane during the adaptation to the studied light regimes.

Table 2. Functional properties of hen blood neutrophil membrane during the adaptation to lighting conditions.

Cell parameters	Day	Groups		
		1	2	3
Elasticity modulus, mPa	6-th	9.2±0.8	0.8±0.1*	1.4±0.8*
	16-th	9.4±0.6	9.5±5.1	2.8±0.5*
	30-th	9.6±0.9	3.4±0.7*	2.9±0.2*
Surface potential, mV	6-th	-6.9±1.2	-10.8±1.9	-25.3±2.3*
	16-th	-6.4±0.8	-8.2±2.6	-10.2±3.5
	30-th	-6.7±0.9	-9.7±1.3	-16.8±1.7*

The elastic modulus of the neutrophil membrane characterizes its rigidity. In our studies, throughout the period of adaptation, its value among the hens of the experimental group was significantly lower than among the hens of the control group by 59-79% on average. The decrease of elasticity modulus characterizes the increase of cell membrane elasticity and viscosity, the decrease of its stiffness in hens during the adaptation to the studied light regimes. The studies have shown that the increase of neutrophil membrane rigidity is noted during their activation. The dynamics of surface protrusion parameters among hen blood neutrophils during the adaptation to the lighting regimes is consistent with the decrease in the stiffness of cytoskeleton structures and indicates the activation of their motor capacity [10, 11].

Analyzing the potential of neutrophil surface in the course of adaptation to the illumination modes under study, it can be noted that the increase of the negative charge was detected on the surface of hen blood cells from group № 3. The change in the surface charge of cells is apparently associated with the activation of ion channels as the result of the change in the membrane potential and the influx of Ca^{2+} ions through the potential dependent channels into the cytoplasm of a cell. It can be assumed that the change of the neutrophil surface potential reflects their functional state and is associated with its transformation during adaptation. There is evidence in the literature that the activation of neutrophils occurs as the result of changes in the membrane potential of cells [1].

SUMMARY:

Thus, the result of the conducted studies determined that in the case of adaptation to lighting regime changes, the stiffness of the neutrophil membrane surface is reduced, and the width of the surface protrusion increases with their height decrease. These changes can characterize the decrease of neutrophil secretory activity and the activation of their motor capacity.

CONCLUSIONS:

1. During the adaptation of hens to a lighting regime change, significant and characteristic changes occur

in the morphofunctional parameters of blood neutrophils.

2. During the adaptation of hens to the lighting regime, the width and the height of the surface protrusions, as well as the modulus of elasticity of the neutrophil membrane, change to the greatest extent.

3. The changes in the morphometric parameters of neutrophils during the adaptation of hens to the changes in the lighting regime may indicate a secretory activity decrease and the activation of the motor ability of cells.

REFERENCES:

1. Arvizo, R. R., Miranda, O. R., Thompson M. A. et al., 2010. Effect of nanoparticle surface charge at the plasma membrane and beyond. Nano Lett., 10 (7): 2543-2548.
2. Vasilenko I.A., Nikitin A.A. and N.V. Malichenko, 2008. Cytometry of neutrophils for evaluation of complex treatment efficiency in patients with mandibular osteomyelitis. Almanac of clinical medicine, 18: 63-68. (In Russian).
3. Galkin, A.A., Demidova, V.S., 2011. The role of Activation of neutrophils and their cytotoxic interaction with endothelium. Vishnevsky Institute of Surgery, 131 (1): 62 – 78. (In Russian).
4. Roca-Cusachs P., Almendros I., Sunyer Raimon, 2006. Rheology of Passive and Adhesion-Activated Neutrophils Probed by Atomic Force Microscopy. Biophys. J., 91: 3508–3518.
5. Gainitdinova V.V., Sharafutdinova L.A., Kamaltdinov I.M and M.V. Mavletov, 2015. Atomic and power spectroscopy as the method of research of viscoelastic properties of blood neutrophils of patients with the chronic obstructive pulmonary disease. Bulletin of Bashkir University, 20 (2): 440-443. (in Russian).
6. Buslovskaya, L.K., Belyaeva E.Y. and A.Y. Kovtunenko, 2013. Adaptive Dynamics of Blood Cell Parameters in Hens upon Changes in the Lighting Conditions. Global Veterinaria 11 (4): 441-445.
7. Beste, M.T., Lomakina, E.B., Hammer, D.A. and R.E. Waugh, 2015. Immobilized IL-8 Triggers Phagocytosis and Dynamic Changes in Membrane Microtopology in Human Neutrophils. Annals of Biomedical Engineering, 43 (9): 2207-2219.
8. Kay R.R., Langridge p., Traynor D. and O. Hoeller, 2008. Changing directions in the study of chemotaxis. Nat. Rev. Mol. Cell. Biol., 9: 455-463.
9. Afonso, P.V., Parent, C.A, 2012. Neutrophils under Tension. Developmental Cell, 22 (2): 236-238.
10. Rocheleau, A.D., Sumagin, R., Sarelius, I.H. and M.R. King, 2015. Simulation and analysis of tethering behavior of neutrophils with pseudopods. PLoS ONE, 10 (6), e0128378.
11. Gaspar, D., Freire, J.M., Pacheco, T.R., Barata, J.T. and M.A.R.B. Castanho, 2015. Apoptotic human neutrophil peptide-1 anti-tumor activity revealed by cellular biomechanics. Biochimica et Biophysica Acta - Molecular Cell Research, 1853 (2): 308-316.