

Morphofunctional Parameters of Blood Lymphocytes, in the Process of Adaptation of Chickens to Lighting Regimes

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Abstract

The dynamics of morphometric and functional parameters of blood lymphocytes of chickens, in the process of adaptation to lighting regimes, was studied using the atomic force microscopy. It was established, that in the process of adaptation, the initial in duration of cells was replaced by an increase in their volume and surface area, the modulus of elasticity and the surface potential of lymphocyte membrane under these conditions had decreased, increasing the liquid properties of membrane, thereby providing cellular mechanisms of adaptation.

Keywords: Adaptation, Lymphocytes: Surface Area, Volume, Diameter, Height, Modulus of Elasticity, Surface Potential; Chickens, Lighting Regime.

Introduction

Lymphocytes are the heterogeneous population, whose elements differ from each other in origin, structure, functions and morphological characteristics. These cells accumulate, store and transfer information about adaptive and disadaptive processes in the body, participating in the physiological mechanisms of life-sustaining activity. It is known, that the morphometric parameters of blood cells are highly sensitive and objective criteria for assessing homeostatic disorders [1-4]. The method of atomic force microscopy makes it possible to study morphofunctional changes, occurring at the level of lymphocytes [5, 6]. This allows their use when analyzing the adaptive reactions of the organism and assessing the stress reaction.

Methodology

The experimental part of the work was performed in vivarium conditions, on 10-month laying hens of the cross «Haysex brown», combined in 3 groups, 16 animals each. The 1st group of hens was control. Chickens were kept in the following lighting regime: 12 hours in light (L) –and 12 hours in darkness (D). Hens of the 2nd group were kept during 7 hours in light, and during 1 hour in darkness. The following lighting regime was used for the 3rd group of hens: 2 hours in light – 3 hours in darkness, 5 hours in light – 1 hour in darkness, 4 hours in light –9 hours in darkness. Adaptive features of hens were studied during the 30 days [7].

Blood was drawn in chickens from the axillary vein, on the 6th, 16th and 30th days of adaptation. Scanning of cells (n = 10) was carried out, using the atomic force microscope INTEGRA Vita (NT MDT, Zelenograd) in the research laboratory "Physiology of Adaptation Processes" of the Belgorod State National Research University, at the tapping mode. The results were processed using software Nova 1.0.26 Build 1397 (NT MDT). The potential of the cell surface was evaluated by the Kelvin probe. The significance of differences was assessed by Student's t-test.

Result and Discussion

The analysis of morphological parameters of lymphocytes allows to evaluate the functional activity of cells [2]. Table 1 shows the dynamics of some parameters of hens' blood lymphocytes when adapting to the studied lighting regimes.

Table 1: Dynamics of Some Parameters of Blood Lymphocytes of Hens, in the Process of Adaptation to Lighting Regimes

Parameters	Days	Groups		
		1	2	3
Surface area, μm^2	6 th	109.7±13.6	109.6±6.0	109.7±5.9
	16 th	110.2±2.7	85.9±2.8*	56.0±2.3*
	30 th	109.2±8.2	157.3±1.6*	145.2±2.3*
Volume, μm^3	6 th	57.1±13.6	60.1±5.6	66.2±6.5
	16 th	58.6±1.3	53.8±5.6	49.8±7.9
	30 th	56.2±0.8	92.3±0.5*	96.4±2.5*

Height, μm	6 th	0.7±0.1	0.6±0.6	0.6±0.7
	16 th	0.9±0.2	0.4±0.7	0.6±0.6
	30 th	0.5±0.2	0.5±0.01	0.6±0.1
Diameter, μm	6 th	9.2±0.8	9.2±0.8	9.3±0.7
	16 th	10.1±1.2	7.2±0.9*	6.9±1.2*
	30 th	10.6±2.2	7.9±0.8	9.1±2.4

Note: the significance of differences is compared to the data of the 1stgroup
 * - for P <0.05, ** - for P <0.01

In the process of hens' adaptation to lighting regimes, a significant decrease in the surface area of blood lymphocytes by 22% and 49% was observed on the 16th day of adaptation, in the experimental groups #2 and #3. At the same time, a significant decrease in lymphocyte diameters in chickens, belonging to the same groups, was revealed. On the 30th day of adaptation, the surface areas of lymphocytes significantly increased by 44% and 33% in chickens of groups #2 and #3, respectively, that was accompanied by a 64% and 72% increase in the volume of white blood cells in these groups. There were no significant changes in the height of lymphocytes during the experiment.

It is known, that the plasma membrane of lymphocyte can form outgrowths and microvilli, that leads to an increase in the surface area and, according to some data, it is a sign of increased functional activity of lymphocytes [8, 9]. In the studies of Zubareva E.V. et al., 2011, it was shown, that in the process of evaluation of compensatory reactions of the body,

when adapting to stressors, the use of membrane reserve by lymphocytes had an important diagnostic value. The decrease in the volume of lymphocytes, found in our experiments at the first stages of adaptation, can mean the economical use of the plasma membrane reserve, and can be interpreted, apparently, as a manifestation of the "saving" type of response of white blood cells to the exposure [10].

By the end of the experimental period, an increase in the volume of lymphocytes has been detected. Gilmetdinov R.R. et al., 2013 explained this effect by an increase in the intracellular concentration of Na⁺, H⁺, Ca⁺ ions, due to an increase in membrane fluidity and permeability [8, 10].

As was established by Sladkova E.A., 2015, the transformation of lymphocytes was directly related to the changes in structure and functions of their plasmalemma, primarily its electrical and mechanical properties [11]. Table 2 presents the functional properties of blood lymphocyte membrane of hens, when adapting to lighting regimes.

Table 2: Functional Properties of the Blood Lymphocyte Membrane of Hens, when Adapting to Light Conditions

Cell Parameters	Days	Groups		
		1	2	3
Modulus of elasticity, mPa	6 th	11.5±0.9	10.5±0.8	1.9±0.5**
	16 th	10.1±1.4	16.6±3.3	2.9±0.4**
	30 th	12.6±3.6	4.9±0.5*	3.5±0.8**
Potential of the surface, mV	6 th	-6.9±1.2	-8.0±1.1	-25.3±2.3**
	16 th	-7.2±1.1	-8.2±2.6	-25.8±2.6**
	30 th	-8.2±3.2	-8.2±2.6	25.2±3.1**

The analysis of morphofunctional parameters of lymphocyte membranes showed, that in the process of adaptation to new lighting regimes, the modulus of elasticity and the potential of cell surface significantly changed. Thus, in chickens, belonging to group #2, a significant decrease in the modulus of elasticity of the lymphocyte membrane by 61% was noted on the 30th day of adaptation, compared to the control group. In the chickens of group #3, the

modulus of elasticity of the lymphocyte membrane during the entire adaptation period was significantly lower than in the control group: on the 6th day of adaptation by 83%, on the 16th day - by 71%, on the 30th day - by 72%. According to Gilmetdinov, the change in the modulus of elasticity of lymphocytes reflects the state of phospholipid layer of membrane, and it is due to the ratio of saturated and unsaturated fatty acids in the membrane. When the modulus of

elasticity in the phospholipid layer of the membrane decreases, the content of unsaturated fatty acids increases and the content of saturated fatty acids decreases, that reflects by a reduce in the microviscosity of the lipid phase and an increase in the membrane's liquid properties [8].

In 2011, Fedorova M.Z. showed that the elastic properties of membrane could characterize the adaptive abilities of the organism to the impact of the studied factor. Since, with sufficient adaptation, compensatory reactions are activated to maintain the structural integrity and functional activity of the membrane. According to some reports, the decrease in the induration of membrane indicates an intensification of metabolism and activity of lymphocytes, and as a result, the improvement of the processes of recognizing their own or foreign cellular receptors, that ultimately leads to the formation of an adequate immune response [10].

In the blood of chickens from the third group, significant changes in the potential of lymphocyte surfaces were detected. Namely, the total negative charge of the blood cells surfaces during the adaptation period was significantly higher, than the values, revealed in the control group of chickens. The surface charge of cells –is the most important biophysical parameter, which depends on the composition of cytoplasmic membrane and the physiological state of cells. In a number of experimental studies, it has been shown, that the potential of cell surface is directly involved in maintaining the necessary balance between proliferation, differentiation and apoptosis. In particular, there are some evidences, that an increase in the total negative charge of the surface of blood cells is a marker of reduced viability of cells and early apoptosis [11, 12].

Summary

Unidirectional changes in the geometric parameters of lymphocytes were noted in the blood of chickens from experimental groups, during the process of adaptation to the studied light regimes. So on the 6th day of adaptation, the tendency was observed for the increase in the compactness of cells, that was primarily expressed in decreasing the surface area of cells, their volume and diameter. Such a dynamics, apparently, reflects the manifestation of a "saving" type of cells response to the presented exposure. On the 30th day of adaptation, the reverse dynamics was noted, namely the cell had increased in volume, making maximum use of the membrane reserve, with an increase in the surface area. These features can characterize the increased functional activity of immune cells. The assessment of the functional properties of lymphocyte membrane during the experiment showed, that the intermittent lighting regime had the greatest impact on the bird's organism with the following parameters: 2 hours in light – 3 hours in darkness, 5 hours in light – 1 hour in darkness, 4 hours in light – 9 hours in darkness. The hens of the experimental group #3 were kept in this regime. Significant decrease in the induration and the

potential of lymphocyte membrane surface of chickens' blood in this group, apparently, indicate their activation and some tension of adaptive processes at the cellular level.

Conclusions

1. In the process of chickens' adaptation to different lighting regimes, there are significant changes in the morphofunctional parameters of blood lymphocytes.

2. During the adaptation of chickens to lighting regimes, the staging is observed in the change of morphometric parameters of blood lymphocytes, the compaction is replaced by an increase in their volume and surface area that indicates the change in the functional state of cells.

3. Surface potential and modulus of elasticity of the chicken blood lymphocyte membrane, in the process of adaptation to lighting regimes decrease, increasing its fluidity and permeability, thereby ensuring the activity, immune properties of cells and cellular mechanisms of adaptation.

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