ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Morphofunctional Characteristics of Blood Lymphocytes of Chickens under Chronic Stress.

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ABSTRACT

The atomic force microscopy has studied the dynamics of morphometric and functional parameters of blood lymphocytes of chickens under chronic stress which is caused by photojetlag. It has been found out that in the process of chickens' adaptation to this factor the surface area and the volume of lymphocytes are increased at constant height and diameter of the cells. In this case, apparently the plasma membrane ability becomes more intense and forms protuberances that provide for the use of the membrane cells reserve. It has been discovered that under these conditions the membrane lymphocytes elastic modulus decreases by increasing the fluid properties of the membrane, its fluidity and permeability. Presumably all these changes are aimed at the cellular mechanisms adaptation attachment.

Keywords: Lymphocytes: the surface area, volume, diameter, height, elastic modulus, surface potential; chickens, photojetlag, chronic stress.

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ISSN: 0975-8585

INTRODUCTION

It is known that lymphocytes are actively involved in the physiological mechanisms of regulation of vital processes and affect the function of various tissues, organs and systems. They accumulate, store and carry information about all the changes in the body, including its maladaptive states. According to the Gavrilova T. et al., quantitative and qualitative changes in the parameters of the cells are highly sensitive and objective criteria for evaluating disorders of homeostasis and universal components of compensatory-adaptive reactions of a body [1-4]. The method of atomic force microscopy allows us to study morphological changes that occur in all cells and lymphocytes in particular, at a new level [5; 6], which offers the possibility of using these patterns in the analysis of stress states, as well as in the development of diagnostic methods of antistress and adaptive reactions.

METHODS

We carried out the experimental part of the work in a vivarium on the 18 months chickens of Haysex brown cross formed on the analogue principle in the control group (#1) and experimental group (#2), each of 16 animals. The chickens were kept in cages, under cage density and feeding and watering space complying with zootechnical standards and growing technology of this cross. Chronic stress - photojetlag - was achieved by exposing the chickens to permanent light for three days [7]. We scanned the cells (n = 10) with an atomic force microscope (AFM) «INTEGRA Vita» (NT MDT, Zelenograd) in a research laboratory 'Physiology of adaptation processes", SRU BelGU, by semi-contact method, and processed the obtained results using Nova 1.0.26 Build 1397 (NT MDT) software. The potential of cell surface was assessed by Kelvin Probe. The significance of differences was assessed by Student t-test.

MAIN PART

In the course of our studies on the effect of changes of lighting modes on the chickens we found that photojetlag causes chronic stress, which is confirmed by evaluation of the functional state of the birds' organisms, as well as their hematological and biochemical parameters. These findings were confirmed by further studies in the analysis of the dynamics of morphometric and functional properties of the membrane of red blood cells and different groups of white blood cells by atomic force microscopy [7]. Table 1 shows the results of a study of some parameters of lymphocytes that underwent significant changes during adaptation of chickens to photojetlag.

Table 1: The dynamics of the geometric parameters of blood lymphocytes of chickens when adapting to photojetlag

Groups	Day	Cell parameters			
		Surface area, μm^2	Volume, μm^2	Height, μm	Diameter, μm
1	6	109.7±13.6	57.1±13.6	0.7±0.1	9.2±0.8
	16	110.2±2.7	58.6±1.3	0.9±0.2	10.1±1.2
	30	109.2±8.2	56.2±0.8	0.5±0.2	10.6±2.2
2	6	137.9±8.1*	87.2±5.1	0.7±0.1	9.1±1.5
	16	136.8±9.2*	98.8±6.8**	0.4±0.3	7.6±0.6
	30	137.1±8.2*	96.8±3.2**	0.6±0.2	9.6±2.2

Note: significance of differences compared to the data of the group 1
* – P<0.05, ** – P<0.01

Analysis of the results showed that the surface area of the blood lymphocytes of chickens significantly increased on average by 26% on day 6 after photojetlag, by 25% on day 16, and on 26% on day 30, as compared to the values of the control group. The volume of lymphocytes also increased significantly by 73% on day 16 and by 70% on day 30. At the same time no significant changes in height and diameter of lymphocytes were identified. This may be due to the ability of the plasma membrane of lymphocytes to form numerous outgrowths and microvilli, which increased formation leads to an increase in the surface area of the cell and its volume and enhances the use of reserve membrane by cells. All this can be regarded as an indication of the increased functional activity of lymphocytes and activation of mechanisms of cellular adaptation [8; 9].

One of the mechanisms of change in cell volume can be an influence of hormones that causes cell

swelling or contraction due to increased membrane fluidity and permeability of cell membranes to K^+, Na^+, and $Ca2^+$ ions. The data on the dynamics of cell volume can serve as an additional criterion for diagnosing and evaluating the stressful states [8; 10].

Changes in stiffness of the lymphocyte membrane identified in our study can lend evidence to this fact. Table 2 shows the results of a study of some functional parameters of lymphocyte membrane during adaptation of chickens to photojetlag.

Table 2: Functional properties of membrane of blood lymphocytes of chickens when adapting to photojetlag

Groups	Day	Cell parameters	
		Elastic modulus, mPa	Surface potential, mV
1	6	11.5±0.9	-6.9±1.2
	16	10.1±1.4	-7.2±1.1
	30	12.6±3.6	-8.2±3.2
2	6	2.1±0.8**	-7.7±1.8
	16	3.1±0.8**	-7.8±2.0
	30	2.6±0.8*	-6.9±2.6

We noted that the elastic modulus of the lymphocyte membrane was significantly reducing in chickens during their adaptation to photojetlag, and it was 82% lower than the initial value on day 6, 69% lower on day 16, and 79% lower on day 30. Surface potential of lymphocytes underwent no significant changes at this period.

A reduced modulus of elasticity reflects structural changes in the phospholipid layer of the lymphocyte membrane. It causes the increase in the amount of unsaturated fatty acids and reduction in the amount of saturated ones, at the same time the microviscosity of lipid phase decreases and fluid properties of the membrane grow. A significant reduction in the elasticity modulus of the membrane in our study may be due to a decrease in the cholesterol content in the membrane. Gilmetdinov R.R. et al. showed that there is a decrease of cholesterol content in the plasma membranes of cells in certain diseases, which primary function is to provide a certain rigidity of the membrane and maintain its stability, while changes in the content of cholesterol can lead to poor physical condition of the lymphocyte membrane. Changes in morphological and functional parameters of lymphocyte membrane may indicate poor adaptability to the effects of the factor studied, because the sufficient adaptation may activate adaptive-compensatory reactions such as the accumulation of certain forms of easily oxidized lipids aimed at maintaining the structural integrity and functional activity of membrane [8].

On the other hand, according to some findings, the reduction in the stiffness of the membrane indicates increased metabolism and activity of lymphocytes, and, as a result, their recognition of their own or foreign cell receptors, which ultimately leads to the formation of an adequate immune response [10].

CONCLUSION

Thus, as a result of studies, we found that the chronic stress in chickens induced by photojetlag leads to the increase in surface area and the volume of lymphocytes at constant height and diameter of the cells. This is presumably due to the ability of the plasma membrane to form numerous outgrowths and microvilli, which causes cells to use the membrane reserve more completely. This, in turn, decreases the elastic modulus of lymphocyte membrane, increases both fluid properties and permeability of the membrane, and activity and immune properties of the cells, thereby ensuring the cell adaptation mechanisms.

SUMMARY

- Chronic stress in chickens caused by photojetlag leads to significant and characteristic changes in morphofunctional parameters of blood lymphocytes.
- During the adaptation of chickens to photojetlag the surface area and the volume of lymphocytes are increased, which makes cells use the membrane reserve.

- This decreases the elastic modulus of lymphocyte membrane, increases its fluidity and permeability, as well as activity and immune properties of the cells, thereby ensuring the cell adaptation mechanisms.

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