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Motion Activity and Energetic of Hemocytes of Representatives of Dictyoptera Order

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ABSTRACT

The article is dedicated to results of the study, in the course of which motility of insects' hemocytes, representatives of Dictyoptera order was investigated, while cells were incubated in various conditions. Morphological changes in blood plasma's formed elements were noticed in the course of fagocytal functions' performance. It was shown that intensiveness of hemocytes' fluorescence increases when cells with Rhodamine B colourant are incubated. Intensification of hemocytes' fluorescence allows mentioning high energy needs for recognition of foreign objects and participation in phagocytosis.

Key words: hemocytes, motility, phagocytosis, rhodamine B, fluorescence, mitochondrial potential.

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INTRODUCTION

The complex of fluorescence colourants is used as optical indicator of membrane potential's dynamics in cells and isolated organelles, which are too small to be tested via microelectrode potential technique. Insinuating cation fluorescent probes are selectively accumulated in live cells' mitochondria. Mitochondrial-specific interaction of such molecules seems to depend on high transmembrane potential, supported by functioning mitochondria [1]. The usage of such potential-dependent probes is observed in the cells, participating in active movement. Such approach to analysis of mitochondrial membrane potential is important in the studies of energetic metabolism's control and energetic requirements of certain biological functions at the cellular level [3].

Over the last years fluorescent colourants for measuring mitochondrial membrane potential ($\Delta\psi m$) have been frequently used for monitoring of changes of this important physiologic parameter, as well as for assessment of its correlation with cells' ability to generate ATP by means of oxidative phosphorylation. Increase of fluorescence intensiveness directly depends on fluorescence elevation of mitochondrial potential [4]. Thus, $\Delta\psi m$ is a key indicator of normal or damaged cell state and indicator of their physiologic activity [5].

Hemocytes form the basis of insects' cell-mediated immunity. Plasmocytes, postulated homologues of mammals' macrophages, as well as granulocytes of representatives of Dictyoptera order form 70% of locomotory subpopulation of circulating hemocytes [6]. They are responsible for tissue apoptosis occurring during metamorphosis, as well as for phagocytosis of bacteria. Up to date the mechanism of activation of plasma's formed elements as a reaction to infection or developmental signals is still under-investigated. Hemocytes of representatives of Dictyoptera order are motile ones; phagocytes are represented at all stages of their life circle and form cellular component of insects' congenital immune system at postembryonic stage of life [7, 8, 9]. Granulocytes and plasmocytes are the most wide-spread types of cells. Thus, the main function of postembryonic hemocytes is elimination of intruding microorganisms and fragments of apoptotic cells. The objective of this work was to study motion activity and level of energetic metabolism of hemocytes of representatives of Dictyoptera order.

METHODS

For meeting the objective set, studies of hemocytes of representatives of Dictyoptera order were implemented: Periplaneta americana, Shelfordella tartara, Gromphadorhina portentosa, Blaberus craniifer, Nauphoeta cinerea. The animals were kept in harden holding cages with coconut and sawdust soil. For successful animals' breeding high level of humidity was kept. Animals were fed twice a week. Blood plasma was obtained per standard method [10]. Blood plasma obtained from the insect was incubated separately in physiological salt solution, as well as with addition of Saccharomyces cerevisiae cells in hypo- and hypertonic mediums. For assessment of energetic metabolism level in hemocytes, rhodamine B was used as a colourant. For preparation of working solution rhodamine B was diluted with 0,15M Phosphate buffered saline in the ratio 1:1500, and then diluted 10 times with 0.9 % NaCl solution. The cells that were incubated in various mediums were coloured and then were being observed for changes in fluorescence intenseness with the help of focal laser scanning microscope микроскопа Nikon Digital Eclipse Ti-E.

MAIN PART

The most commonly encountered morphology of native hemocytes, incubated in physiological salt solution, which was denoted as "normal spreading" (NS), is a fully adherent cell with cellular membrane, having proportional bulging formations and cavities. This type is characterized by motionless sedentary behavior of these hemocytes. Two morphological classes, "non-polarized" (n/P) and "polarized" (P) are typical for hemocytes incubated in medium with addition of yeast cells. Both classes are fully adherent cells, demonstrating an extremely active cellular membrane, which forms bulges and cavities at various angles towards cellular midpoint area, forming ruffles. Significant difference between these two classes lies in the fact that polarized cells produce lamellipodia. Analysis of data, obtained from representatives of Dictyoptera order showed the absence of significant differences in peculiarities of motion activity and fluorescence intensiveness. With a view of data unification, in the course of discussion of the results the focus was set on hemocytes' indices of Gromphadorhina portentosa.

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In natural conditions (incubation in physiological salt solution) 100 % of phagocytes were characterized by normal spreading state. At this, on the surface of granulocytes rhizopodia were distributed homogeneously and their length didn't exceed 1.5 µm. Fluorescence intensiveness equaled 249±51 for granulocytes and 212±51 for plasmocytes. In hypotonic medium activity of both granulocytes and plasmocytes dropped. Upon termination of incubation period plasmocytes were found to be significantly spread. However, energy expenditures for keeping a membrane integral in conditions of reduced osmotic pressure were facilitated the increase of fluorescence intensiveness, which equaled 314±28 for plasmocytes and 387±40 for granulocytes.

In conditions of increased osmotic pressure rhizopodium's length reduced to 0.9 µm. The speed of plasmocydes' spreading became significantly lower. Intensiveness of fluorescence was maximum, and it reached 554±48 for granulocytes and 420±36 for plasmocytes. No changes in motility compared with hemocytes incubated in physiological salt solution, was observed. On addition of yeast cells to hemocytes that had been kept in natural conditions, the percent of granulocytes with normal spreading equaled 34%, the number of polarized and non-polarized hemocytes reached 40% and 26%, respectively. There were no polarized hemocytes among plasmocytes, 92% of non-polarized hemocytes and 8% of hemocytes with normal spreading were observed. On the surface of granulocytes beside rhizopodia (the branchiness of which increased to 1.9 µm), long philopodia with a length of 3.5 µm appeared. Both types of pseudopodia were placed non-uniformly, often on one of the cellular poles, sideward to foreign agents.

Intensiveness of phagocytes'' fluorescence is similarly influenced by increased osmotic pressure and appearance of foreign objects in the medium. Besides, fluorescence intensiveness of phagocytes with normal spreading is higher in the medium, where yeast cells were added to, and is maximum for non-polar and polarized hemocytes (Table 1).

Table 1:Fluorescence intensiveness of hemocytes of various morphology

Species of insect	Granulocytes			Plasmocytes	
	NP	n/P	P	NP	n/P
Periplaneta americana	354±42	597±45	603±68	258±34	439±46
Shelfordella tartara.	351±39	588±51	599±47	251±42	431±39
Gromphadorhina portentosa	346±60	581±67	596±82	245±48	425±54
Blaberus craniifer	342±69	573±60	591±77	240±30	419±25
Nauphoeta cinerea	337±43	569±37	587±46	238±26	411±38

Increasing of hemocytes' fluorescence allows mentioning high energy needs for recognition of foreign objects and participation in phagocytosis.

SUMMARY

Thus, it was stated that incubation of plasma formed elements in mediums different from physiological solution leads to increasing intensiveness of hemocytes' fluorescence, which is evidence of increase of their energetic metabolism's level.

CONCLUSION

Study of hemocytes' motion activity and level of energetic metabolism of representatives of Dictyoptera order allowed to elucidate that cellular reactions of studied five species of insects do not have proved generic difference. In the course of the study three types of hemocytes' morphological forms were determined, whose pseudopodia varied in type, character of location and motion activity. Two active morphological types of hemocytes – "non-polarized" and "polarized" were formed after foreign biological objects were placed to the medium. Incubation of plasma formed elements in mediums that contained foreign objects, as well as incubation of elements which differed from physiological salt solution, leads to increasing intensiveness of hemocytes' fluorescence, which is evidence of increase of their energetic metabolism's level.

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