THE MECHANISMS OF REACTIVE OXYGEN SPECIES GENERATION IN PHAGOCYTES UNDER ACTION OF LOW STRENGTH ELECTRIC FIELDS

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The endogenous electric fields are found to be generated in organism tissues at damages [1,2]. These are non-uniform fields characterized by low strength values do not exceed 2 V/cm. The stimulating effects of such electric fields on migration and proliferation of the cells which play an important role in reparative regeneration have been demonstrated in vitro [3-6]. However, alterations of other functional properties of the cells under the low strength electric fields have not yet been investigated. Immune cells such as phagocytes participate in reparative processes ingesting and utilizing abnormal materials by the use of reactive oxygen species (ROS). So, the aim of our work is to study the mechanisms of activation of phagocytes to ROS formation under impact of the low strength electric fields.

Methods. Human blood phagocyte suspensions in Earle's solution were placed in glass cuvettes, and stainless steel electrodes located at a distance of 1 cm apart were submerged in the samples. The electrodes were connected to electrical signal generator with shielded twisted pair. The voltage values of 0.1, 0.25, 0.5 or 1 V were applied to electrodes for experimental samples, and value of 0 V was set for control samples. The processes of ROS formation were studied by luminol-amplified chemiluminescence method. Luminol emits visible light after oxidation by ROS such as $\cdot O_2^-$, H_2O_2, $\cdot OH$, OCl^-, $ONOO^-$. The samples with electrodes were located inside completely darkened metal cuvette compartment of biochemiluminometer during the measurements. The values of chemiluminescence integral intensity which characterize the total ROS generation in the experimental and control samples were determined for 20 min. The inhibitors of enzymes were added 30 min prior to the onset of electric stimulation.

Results. It has been established that low strength electric fields induce the increase of ROS generation in phagocytes when the cells are activated by addition of phagocytosis stimulator latex. The most significant effects have been detected at the voltage on the electrodes of 0.25 and 0.5 V. The differences of inhibitory effects on ROS yields ($\Delta I_{inh.}$) in experimental and control phagocyte samples are represented in fig.1. The involvement of redox-enzymes in ROS generation has been studied using DPI (NADPH-oxidase inhibitor), NaN_3 (myeloperoxidase inhibitor), AET (NO-synthase inhibitor) and PTIO (NO scavenger) and illustrated in fig.1, a. Our data indicate contribution of NADPH-oxidase and myeloperoxidase, but not NO-synthase in the increase of ROS formation in phagocytes under electric field exposure.

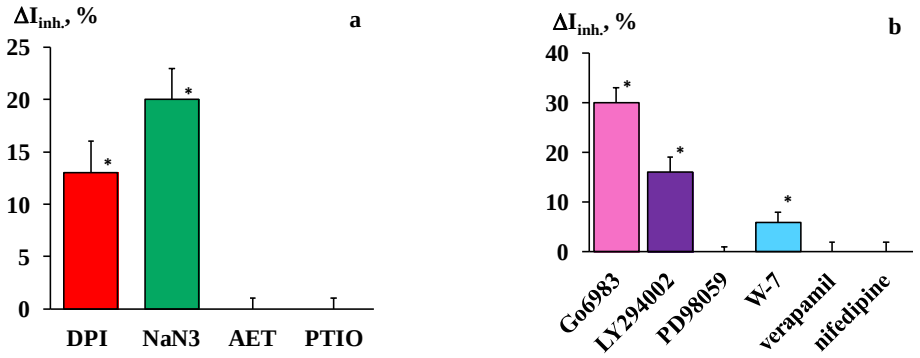

Fig. 1. The alteration of inhibitory effects on ROS generation in phagocytes under exposure to electric field

Activation of phagocytes are known to include signal transduction ways coupled with Ca^{2+}-dependent processes and phosphorylation by kinases which can be protein kinase C (PKC), phosphatidylinositol-3 kinase (PI-3K) or mitogen-activating protein kinases (MAPK) [7]. In the present research the inhibitors Go6983, LY294002, PD98059, W-7, verapamil, nifedipine of PKC, PI-3K, MAPK, calmodulin and L-type of Ca^{2+}-channels have been tested, and the results are shown in fig.1, b. The obtained findings allow to conclude that low strength electric field causes amplification of ROS generation in phagocytes by NADPH-oxidase and myeloperoxidase via modulation of signal transduction associated with PKC, PI-3K and calmodulin.

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