CHEMILUMINESCENCE OF PERITONEAL EXUDATE MACROPHAGES INDUCED BY EXPOSURE TO AN ELECTRIC FIELD

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In response to chemical stimulation or to contact with a foreign surface macrophages secrete several biologically active substances and generate active forms of oxygen [1, 6, 9]. The mechanism of the primary responses preceding secretion have not yet been explained [5]. It has recently been shown that activation of macrophages by various agents is accompanied by a change in ionic conductivity and membrane potential [4, 5]. One method of changing the membrane potential of vesicular particles is by applying a high-intensity electric field to a suspension [7, 11]. When the potential difference reaches a certain critical value, electrical breakdown of the membranes takes place, leading to pore formation [3, 7, 11].

The aim of this investigation was to study the effect of an external electric field on macrophage function. Macrophage function was studied by the method of chemiluminescence (ChL), by means of which the kinetics of ChL of active forms of oxygen can be recorded during electrical stimulation of macrophages.

EXPERIMENTAL METHOD

A suspension of peritoneal exudate macrophages from noninbred male albino rats weighing 180-200 g was obtained by the method described previously [2]. The viability of the macrophages was determined on the basis of their staining properties with trypan blue [2]. Cell suspensions whose viability was not below 90-92% were used in the experiments. To record ChL in the presence of luminol an apparatus based on the FEU-100 photoelectric multiplier was used. Flat gold-plated electrodes were fixed to the opposite walls of a constant-temperature rectangular cuvette made of transparent plastic so that the total volume of the cuvette (2 ml) was in a uniform electric field during application of the pulse (distance between electrodes 1.7 cm).

A pulse generator, the circuit of which provided for partial discharge of the cumulative capacity (the output cascade of the modulator consisted of three GMI-7 tubes) generated single square pulses with a duration of 1-20 μsec and a voltage of up to 10 kV. The pulse duration in the experiment was 10 μsec. The duration of the leading edge of the pulse was 0.1 μsec, with decay of the flat part by not more than 5% of maximal amplitude.

Light-transmission of the cell suspension was measured by means of a recording photoelectric turbidimeter, based on the FEK-56M photoelectric colorimeter. The transparent plastic cuvette had a volume of 2 ml and was fitted with electrodes on the side walls and a magnetic mixer for continuous mixing (length of the optical path 1.65 cm).

EXPERIMENTAL RESULTS

Activation of macrophages by various inducing agents was accompanied by a flash of ChL as a result of generation of active forms of oxygen and subsequent chemiluminescence reactions [8, 10]. In the present experiments intensification of ChL, i.e., activation of phagocytes, was observed on the addition of OSCh-7-4 amorphous silica powder to the cell suspension (Fig. 1). Activation of ChL also was found to take place as a result of pulses of the electric field with an intensity of about 2 kV/cm (Fig. 1a). In amplitude and duration the chemilum-

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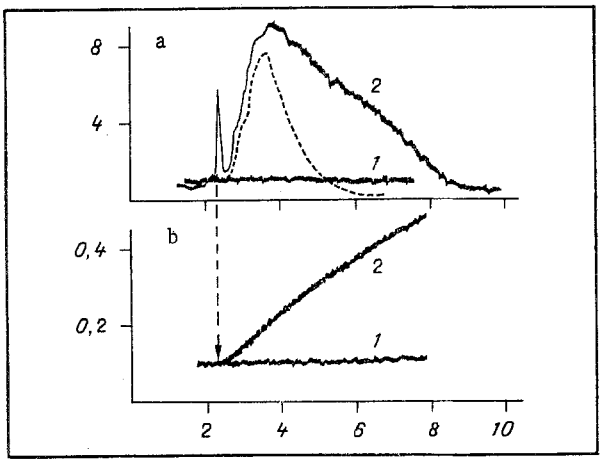

Fig. 1. Effect of pulse of electric field on ChL (a) and scatter of light (b) in suspension of peritoneal macrophages in Hanks' medium. Abscissa, time (in min); ordinate: a) intensity of ChL (in relative units); b) light transmission (in %). 1) Control; 2) pulse of electric field (2.6 kV/cm). Broken line indicates silica (20 mg). Cell concentration 10^6/ml.

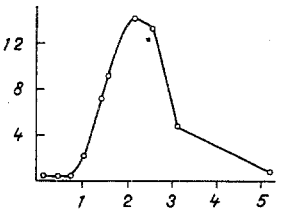

Fig. 2

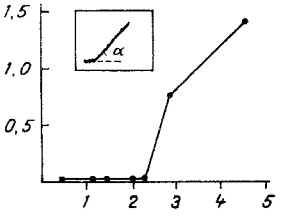

Fig. 3

Fig. 2 Dependence of amplitude of ChL of peritoneal macrophage suspension on intensity of single pulse. Abscissa, intensity of electric field (in kV/cm); ordinate, intensity of ChL (in relative units).

Fig. 3. Dependence of rate of change of light transmission by peritoneal macrophage suspension on intensity of applied pulse. Abscissa, intensity of electric field (in kV/cm); ordinate, tangent of angle of slope (α) of initial part of light transmission curve.

inescence response to the electric field was similar to the response to addition of silica. Incidentally, the quick flash during the action of the pulse was due to electroluminescence of the suspension.

In parallel experiments to measure the scatter of light by the suspension, an increase in light transmission was found after exposure to a single pulse (Fig. 1b). It can be postulated that this effect is due to adhesion of the cells to the walls of the cuvette and to the electrodes. However, counting the cells and visual observation in the light microscope showed that the increase in light transmission was due to swelling of the cells and to the formation of aggregates in the suspension. The presence of Ca^{++} in the medium appreciably inhibited swelling of the cells.

The amplitude of the slow flash of ChL depended on the intensity of the electric field in the pulse (Fig. 2). Activation of ChL was observed with a field intensity of 1 kV/cm, it increased with an increase of intensity, but at 2.2 kV/cm it began to decrease, and was completely absent at 5 kV/cm or more.

Dependence of the rate of change of light transmission of the macrophage suspension on the intensity of electric field shows that the threshold intensity of the field starting from which changes in light transmission began to be found, was 2.5 kV/cm, i.e., it was above the threshold value required for a chemiluminescent response (Fig. 3). Comparison of the data in Figs. 2 and 3 suggests that swelling of the cells is the result of their injury, which also causes a decrease in the chemiluminescent response.

It might be supposed that activation of luminescence after exposure to a pulse with intensity of 1-3 kV/cm was due to the fact that pulses of this kind will damage some cells (for example, the largest) anyhow, and the destroyed cells will serve as activators for intact macrophages. However, on the addition of cells "broken down" by the electric field to the control cells, the intensity of their ChL was unchanged.

The experimental data described above are evidence that pulses of an electric field with an intensity of about 2 kV/cm cause activation of macrophages: they trigger a response which is accompanied by a stereotyped response of ChL. It can be tentatively suggested that this effect is due to electrical breakdown of the plasma membranes of the macrophages. We know that an electric field with an intensity of several kilovolts per centimeter, applied to a cell suspension, will disturb the barrier function of the cell membranes on account of electrical breakdown: pores capable of passing ions are formed in them, and in the case of more powerful agents they may pass large molecules also [3, 7]. Depending on the intensity of the electric field, the breakdown may be reversible, and the defects formed may be healed [3, 7]. It can be postulated that in the present experiments activation of ChL was connected with a reversible increase of ionic permeability, but with higher intensities the decay of ChL was connected with destruction of the macrophage membranes by the electric field. Swelling of the cells was the result of an irreversible increase of membrane permeability and also was observed in the presence of high field intensities and led to lysis of the cells.

LITERATURE CITED

1. E. D. Prishchepov, A. N. Erin, V. V. Perelygin, and A. A. Vorob'ev, Zh. Mikrobiol., No. 6, 3 (1984).
2. I. Ya. Uchitel', Macrophages and Immunity [in Russian], Moscow (1978).
3. Yu. A. Chizmadzhev, L. V. Chernomordik, V. F. Pastushenko, et al., Progress in Science and Technology. Series: Biophysics of Membranes [in Russian], Vol. 2, Moscow (1981), p. 161.
4. E. K. Gallin and J. I. Gallin, J. Cell. Biol. 75, 277 (1977).
5. E. K. Gallin, Fed. Proc., 43, 2385 (1984).
6. R. B. Jonston, C. A. Godzik, and Z. A. Cohn, J. Exp. Med., 148, 115 (1978).
7. K. Kinosita and T.-Y. Tsong, Proc. Natl. Acad. Sci. USA, 74, 1923 (1977).
8 M. Okolow-Zubkovska and A. Hill, Biological and Chemical Aspects of Superoxide and Superoxide Dismutase, New York (1980), p. 201.
9. R. Takemura and L. Werb, Am. J. Physiol., 246, 61 (1984).
10. T. Tono-Oka, H. Wiekerle, and H. Fisher, Immunology, 157, 292 (1980).
11. U. Zimmermann, G. Pilwat, F. Beckers, and F. Riemann, Bioelectrochem. Bioenerg., 3, 58 (1976).