BIOPHYSICS AND BIOCHEMISTRY

Induction of the Monooxygenase System in Rat Blood Neutrophils by Methylcholanthrene and Sovol *in Vivo* and Its Effects on the Properties of These Cells

K. N. Novikov, R. R. Asfaramov, and K. N. Timofeev

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The xenobiotics methylcholanthrene and sovol (the latter being a mixture of polychlorinated biphenyls), which are monooxygenase system inducers, were tested for their effect on the respiratory burst in rat blood neutrophils *in vivo*. The chemiluminescence accompanying this burst was more intensive in the neutrophils of rats treated with methylcholanthrene or sovol than in untreated rats. Observed changes in the $2A_{max}$ parameter of the electron paramagnetic resonance spectrum recorded for the spin probe 5-doxyl stearate in the presence of neutrophils indicated that methylcholanthrene and sovol can exert a direct effect on the viscous properties of neutrophil plasma membranes *in vivo*. These changes were similar in direction to those in the intensity of chemiluminescence during the respiratory burst in neutrophils.

Key Words: *neutrophils; methylcholanthrene; sovol; respiratory burst*

The monooxygenase system plays an important role in determining chemical homeostasis in the body and may be closely associated with another protective system, namely the immune system [3]. The monooxygenase system is predominantly located in the endoplasmic reticulum of hepatocytes, but has also been identified in cells of other types, including immunocompetent cells [1,2].

During the development of inflammation, functionally significant properties in primary cell-mediated immune reactions are manifested by macrophages, leukocytes, and particularly neutrophils. Thanks to these properties, the respiratory (oxidative) burst is activated in cells as a result of their interaction with a foreign antigen or chemical compound (activator) [6,12].

It is significant that the monooxygenase system of leukocytes and macrophages is capable of being induced by polycyclic aromatic hydrocarbons and of expressing aryl hydroxylase activity [2,14]. Effects of polychlorinated biphenyls (PCB) on the generation of superoxide radical and on the degranulation of neutrophils *in vitro* have been described [11].

Very little is known, however, about the relationship between the activity of immunocompetent cells and the induction of a monooxygenase system in these cells by xenobiotics. In this study, therefore, an attempt was made to determine how the induction of rat blood neutrophils with methylcholanthrene and sovol (a mixture of PCB) might alter the ability of these cells to exhibit a respiratory burst.

Laboratory of Physical Chemistry of Biomembranes, Department of Biology, Moscow State University (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

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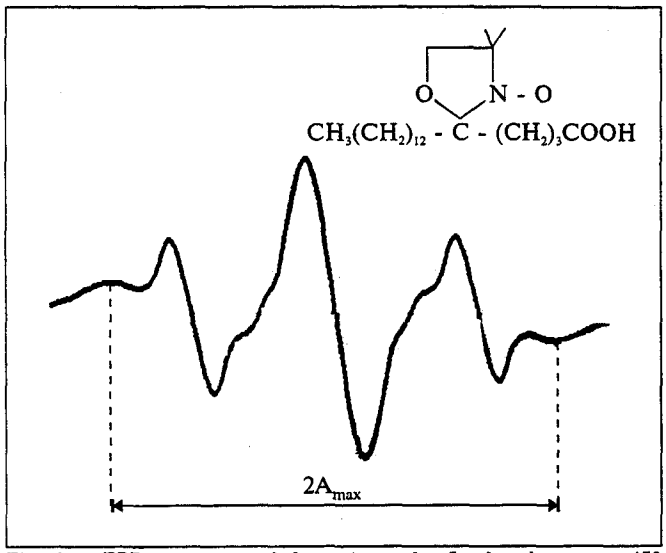

Fig. 1. EPR spectrum of the spin probe 5-doxyl stearate (50 μM) in the presence of a neutrophil suspension (10^6 cells/100 μl of the medium where measurements were performed).

MATERIALS AND METHODS

Random-bred white rats weighing up to 300 g were used. A monooxygenase system was induced in them, after a 24-h fast, with methylcholanthrene or sovol injected in vegetable oil intraperitoneally in a single dose of 200 and 40 mg/kg, respectively. Rats were killed 48 h postinjection, and neutrophils were isolated from heparinized blood of their abdominal aorta by Boyum's method [9] (with some modifications) into a salt medium of the following composition (mM), in which all measurements were also made: 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 1 Na_2HPO_4, 5 glucose, and 10 HEPES+5 Tris buffer (pH 7.4).

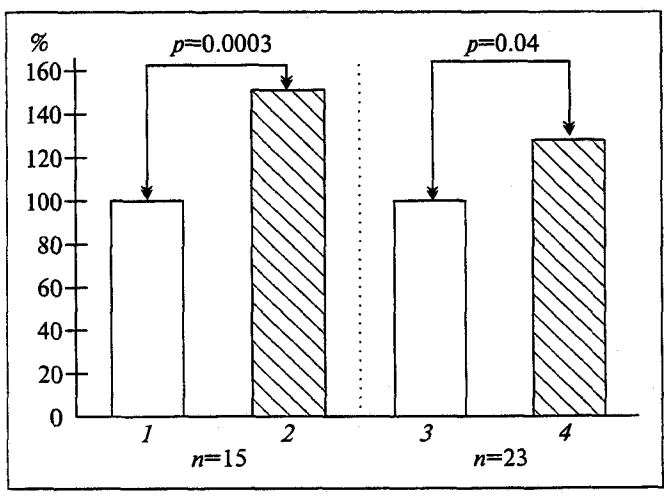

Fig. 2. Intensity of the respiratory bursts shown by neutrophils induced with methylcholanthrene or sovol in relation to that of uninduced neutrophils taken as 100%. *1* and *3*) uninduced (control) neutrophils; *2* and *4*) neutrophils induced by methylcholanthrene and sovol, respectively.

Neutrophils were counted in Goryaev's chamber and their viability, as estimated by the dye exclusion test using 5% trypan blue, was found to be no less than 98%.

The intensity of neutrophil chemiluminescence was measured at 37°C with a PKhL-01 luminometer after adding luminol (10^{-4} M) and the oxidative burst activator phorbol-12-myristate-13-acetate (PMA, 10^{-7} M) to the salt medium of the above composition containing 0.5×10^5 cells/ml. The chemiluminescence intensity at the peak of the chemiluminescence curve was expressed in relative units per 10^6 cells.

The electron paramagnetic resonance (EPR) spectrum of the spin probe 5-doxyl stearate incorporated into the neutrophil plasma membranes (Fig. 1) was recorded with an RE-1307 radiospectrometer of the 3-cm range. For recording the EPR spectrum, 1 μl of the probe (50 μM) was added to 100 μl of the salt medium, which was then vigorously agitated and placed in a special cell in the chamber of the radiospectrometer's resonator.

The results were statistically analyzed on a computer using Statgraphics software.

RESULTS

Neutrophils of the blood are more active than mature bone marrow neutrophils, for the entry of these cells into the circulation causes them to complete their functional maturation. High chemiluminescence of neutrophils accompanies many diseases and appears to reflect the degree to which homeostatic mechanisms are functionally strained. Agents that raise neutrophil activity to a new level are referred to as conditioners [5,6]. Neutrophil-conditioning factors probably include certain xenobiotics.

The respiratory burst in neutrophils was recorded 48 h after the rats had been injected with methylcholanthrene or sovol. By this time, these xenobiotics effectively induce the cytochrome P-450-dependent monooxygenase system of the liver - a process that can be monitored by measuring the cytochrome P-450 concentration [13] and 7-ethoxycoumarin-O-de-ethylase activity [15] in the microsomal fraction of rat liver (data not shown).

The recording of PMA-induced neutrophil chemiluminescence demonstrated an elevated level of the respiratory burst in the cells isolated from the blood of rats injected with methylcholanthrene or sovol, as compared to cells from control animals (Fig. 2). The results were evaluated by the Mann-Whitney test for paired comparisons.

During the course of their 48-h induction of the monooxygenase system accompanied by a shift

in the body's chemical homeostasis, the xenobiotics apparently conditioned the rat neutrophils; that this conditioning activated the neutrophil chemiluminescence is indicated by its heightened level. The difference in chemiluminescence intensity between neutrophils from methylcholanthrene-treated rats and those treated with sovol (cf. *2* and *4* in Fig. 2) may be attributed to the different compositions and properties of these two inducers. The hydrophobic polycyclic aromatic hydrocarbon methylcholanthrene, a potential carcinogen and an immunomodulator, reliably induces certain forms of cytochrome P-450 [2,10], while sovol, representing a mixture of PCB, is a strong inducer of the monooxygenase system [7] and, by virtue of its heterogeneous composition, may be an active modulator of chemical homeostasis.

The shift in chemical homeostasis after the induction of the monooxygenase system by methylcholanthrene probably proceeds via a pathway distinct from that after the induction by sovol because the metabolic pathways of these xenobiotics differ, as do the toxicities of their metabolic products. It appears that the susceptibility of immunocytes to homeostatic alterations may be determined by their intrinsic properties.

The population of neutrophils is heterogeneous and the induction of their monooxygenase system by xenobiotics may affect different differentiation stages of these cells. During the 48-h period of induction by methylcholanthrene or sovol, neutrophils manage to pass through some phases of their life cycle. The time elapsing between the start of their generation in the bone marrow and their exit into the circulation and their death there ranges from 10 to 15 days, of which they circulate in the blood for only 6-7 h [6]. Neutrophils, therefore, may be conditioned at different stages of their formation. Direct induction of their monooxygenase system by xenobiotics in the bone marrow is likely [8], so that neutrophils entering the bloodstream may already show enhanced chemiluminescence. Moreover, a contribution to the conditioning of neutrophils can probably be made by the cytochrome P-450-dependent system which, it is believed, may be located in the plasma membrane of immunocytes and act as a "chemoanalyzer" there [3].

Furthermore, one cannot rule out a direct action on neutrophils *in vivo* by inducers such as xenobiotics or by their metabolic products, which may entail, in particular, alterations in physicochemical properties of neutrophil plasma membranes. Bearing in mind this possibility, we compared the viscosity of neutrophil membranes from rats induced by methylcholanthrene or sovol with

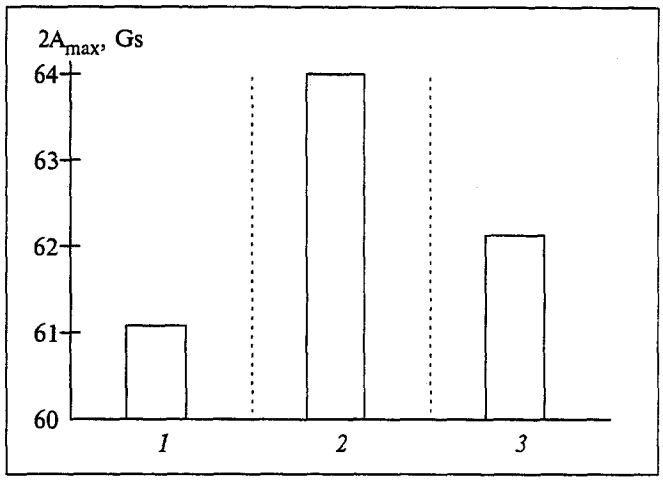

Fig. 3. Parameter $2A_{max}$ of 5-doxyl stearate EPR spectrum in the presence of rat blood neutrophils. *1*) untreated rats; *2*) rats treated with methylcholanthrene (*2*) or sovol (*3*).

that of membranes from intact rats. The indicator of altered membrane viscosity was the $2A_{max}$ parameter of the 5-doxyl stearate EPR spectrum (Fig. 1). The lower the membrane viscosity, the more rapid the movement of this probe in the membrane and the lower the value of $2A_{max}$, and vice versa [4]. The EPR spectrum was recorded at least 3 times in different portions of each neutrophil preparation. Mean $2A_{max}$ values for neutrophils from the three groups of rats are shown in Fig. 3. Owing to the high sensitivity of the spin probe method used, the values measured under identical conditions were very similar. The resolving power of the EPR spectrometer was sufficient to regard differences between $2A_{max}$ values in the range of 1-2 Gs, which were observed in this study, as being significant. The results suggest that different $2A_{max}$ values obtained in the presence of different neutrophils were due to the disordered structure of their membranes after induction with methylcholanthrene or sovol.

As seen in Fig. 3, the $2A_{max}$ values for neutrophils from methylcholanthrene- and sovol-treated rats were higher than for those from control animals, but the induction with methylcholanthrene led to much higher values of this parameter than the induction with sovol. One likely explanation for this difference is that the highly hydrophobic methylcholanthrene can greatly increase the viscosity of neutrophil membranes by interacting with them directly *in vivo* more actively than does sovol, which may in turn be associated with the above-mentioned differences between the properties of these two inducers.

On the basis of the concept that homeostasis has an immunochemical function [3], it may be surmised that such potent xenobiotics as methylcholanthrene and sovol produce, in the course of

their 48-h inducing activity and metabolism, appreciable shifts in the body's homeostasis, with the result that not only the pathway along which neutrophils develop but also their morphological and functional properties become modified. This is suggested by the changes detected in the EPR spectrum of 5 doxyl stearate in the presence of different neutrophils.

It is noteworthy that the direction of the change in $2A_{max}$ was the same as that of the change in the intensity of the respiratory burst in neutrophils. Whether a direct correlation exists between these two effects of xenobiotics, cannot be stated with certainty at present, although such a correlation may prove to be significant.

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