Biochemistry (Moscow), Vol. 68, No. 9, 2003, pp. 1036-1039. Translated from Biokhimiya, Vol. 68, No. 9, 2003, pp. 1266-1270.

Effect of High-Pressure Helium on Latex-Induced Activated Chemiluminescence of Human Blood Leucocytes

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Received September 11, 2002
Revision received January 28, 2003

Abstract—High-pressure helium reduces the latex-induced activated chemiluminescence of diluted human blood. This effect is more noticeable, when lucigenin rather than luminol is used as the activator of chemiluminescence. The effect lessens in the presence of Mg^{2+} but not Ca^{2+}. The data suggest the association of this effect with actin polymerization in leucocytes phagocytosing the latex particles.

Key words: pressure, hyperbaria, chemiluminescence, leucocytes, blood, luminol, lucigenin, actin, phagocytosis, latex

The full exploration of the ocean depths is only possible by immediate human activity in depth, that is, in a hyperbaric environment. The hyperbaric environment implies some factors that are dangerous to human health but can be overcome merely technically via changes in gas composition, temperature, and compression/decompression regimes. The only compelling factor is the hydrostatic pressure.

People and some other animals whose bodies are not built to remain in great depths meet an imbalance of vital functions already at hydrostatic pressure of 5-10 MPa, whereas in most cases significant changes on the cellular and subcellular levels become detectable only at pressures exceeding 40-50 MPa [1]. This fact poses a problem concerning the existence and search for "primary" effect manifesting already at low pressure and triggering the complete chain of impairments in the whole organism. Our studies on biophysical parameters of biological membranes and a series of cell models yielded the same threshold of several hundred atmospheres that is necessary for important changes in the parameters [2-5].

A decrease in the resistance to infections is indicative of some body functions, along with blood circulation and nervous system processes, most suffering from hyperbaria [6]. This is partly due to the activation of conditionally pathogenic microflora [7] and the impairment of immune status [8]. One of the early steps of the impact of a foreign agent on the body is its interaction with leukocytes accompanied by formation of active oxygen forms (AOF) which can be observed by via chemiluminescence (CL) [9]. The production of AOF is known to change during the simulation of dives [10]. The present study was aimed to investigate this function in leukocytes exposed to pressure *in vitro* with the purpose to search for the primary effects of the pressure and to evaluate their importance in the genesis of hyperbaric disturbances.

MATERIALS AND METHODS

Blood was taken from a finger into a heparinized 75-μl glass capillary, mixed, the ends of the capillary were sealed with wax or mastic D553 (Radiometer, Denmark), and the sample stored in strictly horizontal position at 4°C for one day. To prepare the sample for experiment, a part of the capillary containing 12 μl of blood was cut off, and the blood was sampled into the working cell using a micropipette, then the capillary was rinsed several times with the content of the cell.

The following media were used as the incubation medium: 1) 0.140 M NaCl, 5.6 mM glucose, 0.1 mM adenine, and 10 mM Na-K-phosphate buffer, pH 7.4;

Abbreviations: AOF) active oxygen forms; CL) chemiluminescence; Lum-CL) luminol-activated chemiluminescence; Luc-CL) lucigenin-activated chemiluminescence.

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and 2) Hanks' balanced salt solution with glucose and without phenol red (NPP PanEko, Russia).

To activate chemiluminescence, 1 mM luminol solution (3.5 mg luminol (Koch Light, UK) was dissolved in 10 ml 0.1 M NaOH and then 8 ml 0.1 M NaH_2PO_4 was added to adjust pH to 7.4) was added to the sample to the final concentration of 10^{-4} M, or 4 mM lucigenin solution (2.4 mg lucigenin (Sigma, USA) dissolved in 1 ml water) was added to the sample to the final concentration of $8 \cdot 10^{-5}$ M.

A suspension of natural latex was used to trigger phagocytosis. The suspension was diluted with distilled water to produce the stock-solution which being diluted 1 : 1000 gave OD_{540} of 0.15. The suspension prepared in this way and added (50 µl) to the sample gave 10^6 particles/µl. An additional portion of latex did not result in additional CL response.

Then a 1-cm quartz cell containing 1.35 ml incubation medium, 12 µl blood, and chemiluminescence activator was placed into a pressure chamber (0.4 liter) with quartz illuminators equipped with an intra-chamber thermostat and dispenser. The reaction mixture was agitated with an external magnet mixer. The temperature was monitored with a thermistor inserted into the sample and adjusted to 37.5 ± 0.2°C. Pressure was applied with helium during 2-6 min incubation with the rate of 3 MPa/min. To maintain constant partial pressure in the chamber, the gas supply into the preliminarily ventilated chamber was achieved simultaneously through "input" and "output" tubes.

After 10-min incubation, 50 µl latex suspension was added with the intra chamber dispenser and the chemiluminescence was recorded for 20-30 min. The monitoring was carried out from the side wall of the cell using a KhLM1Ts-01 chemiluminometer (USSR), the photoelectron multiplier of which was attached to the illuminator of the pressure chamber. The kinetic curves were recorded and processed with a computer.

RESULTS AND DISCUSSION

In preliminary studies we varied the incubation conditions, such as the temperature and osmolarity, to search for the optimum conditions for maximum effect of the pressure and to verify the stability of the effect. The maximum effect was observed under standard physiological conditions: mesobaric pressure and temperature range 37-38°C. The upper temperature limit of the CL-response (42.8 ± 0.2°C) was unchanged under the pressure of 10 MPa. The basic study was carried out under the standard physiological conditions described in the previous section.

The maximum intensity of luminol-activated chemiluminescence (Lum-CL) was decreased by the elevated helium pressure by 30% at 10 MPa in minimal medium and by 15% at 10 MPa in Hanks' solution (Figs. 1 and 2). The decrease of the lucigenin-activated chemiluminescence (Luc-CL) under the pressure was more prominent, being 27% at 10 MPa in Hanks' solution, whereas in minimal medium it was distinctly nonlinear, being 62% at 10 MPa (Figs. 3 and 4).

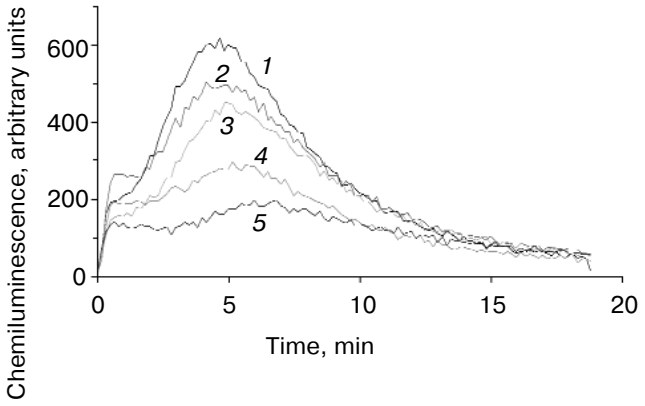

Fig. 1. Effect of pressure on the shape of Lum-CL curves in minimal medium at 5 (*2*), 10 (*3*), 15 (*4*), and 20 MPa (*5*); *1*) control.

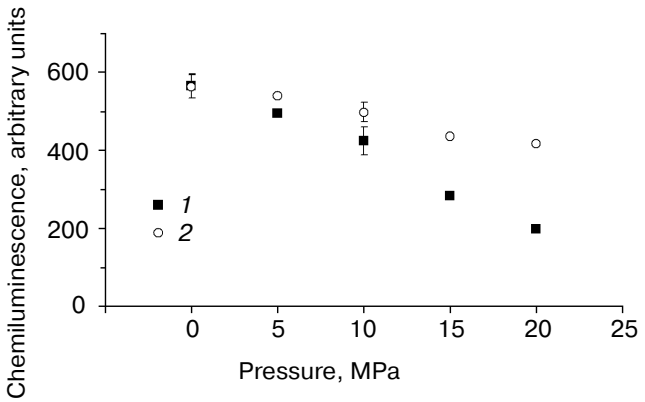

Fig. 2. Effect of pressure on the Lum-CL intensity in minimal medium ($n = 10$) (*1*) and in Hanks' solution ($n = 9$) (*2*).

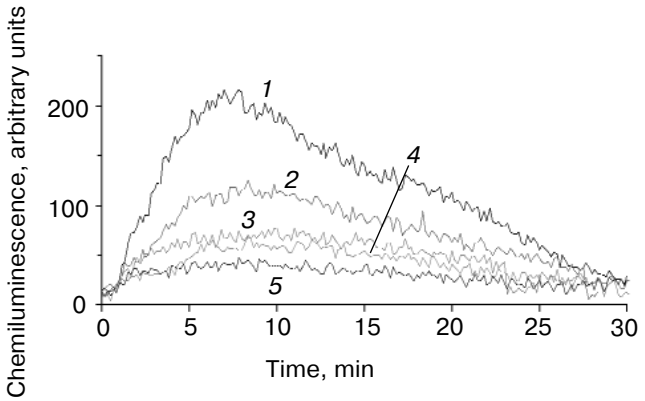

Fig. 3. Effect of pressure on the shape of Luc-CL curves in minimal medium at 5 (*2*), 10 (*3*), 15 (*4*), and 20 MPa (*5*); *1*) control.

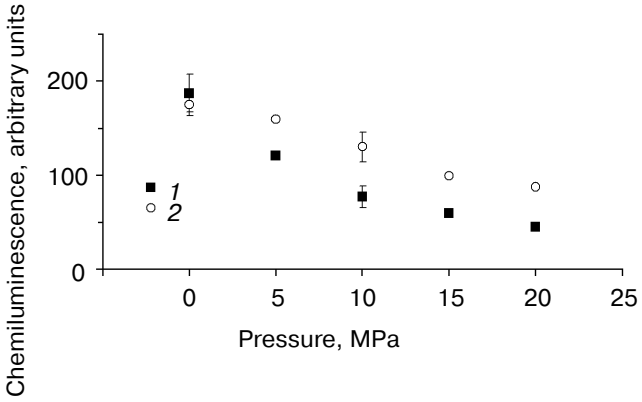

Fig. 4. Effect of pressure on the Luc-CL intensity in minimal medium ($n = 10$) (*1*) and in Hanks' solution ($n = 9$) (*2*).

Magnesium (II) is the active component providing the discrepancy between the pressure effects in minimal medium and Hanks' solution. The presence of Ca^{2+} and SO_4^{2-} in the concentrations equal to those in Hanks' solution does not influence the shape of the curve and sensitivity of CL-response to the pressure (Fig. 5), whereas the chemiluminescence intensity grows with increase in Mg^{2+} concentration and flattens out in the presence of 1.5-2 mM Mg^{2+}. The concentration-dependent increase in the chemiluminescence was observed under pressure as well, but the Mg^{2+} concentration corresponding to the CL plateau was increased. The refinement of quantitative evaluation of the effect requires further studies.

Polymorphic granulocytes and monocytes give the maximum contribution to the formation of AOF in blood under stimulation. The mechanism of AOF formation depends substantially on the type of stimulus. Two various types of cell response are described for insoluble stimuli [11]. When the interaction of the cell with the stimulus is mediated by a receptor, a major portion of AOF is formed outside the cell due to granule efflux from granulocytes and via the myeloperoxidase reaction. This response depends on the intracellular Ca^{2+} content and increases in the presence of cytochalasin B. The second type of response is not related to the receptor recognition of the stimulus. This response results in phagocytosis of foreign particles and intracellular AOF formation. This response type is independent on Ca^{2+} and is deteriorated by cytochalasin B. The chemiluminescence stimulated by non-opsonized latex studied here is this type of response.

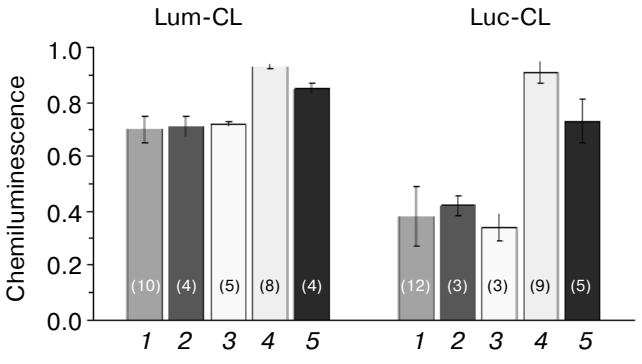

Fig. 5. Effects of ions on the CL decrease under the pressure of 10 MPa. The vertical scale shows chemiluminescence intensity in proportion to the control level at normal pressure. The horizontal scale shows the effect in minimal medium in the absence (*1*) or in the presence of 1.2 mM $CaCl_2$ (*2*), 0.4 mM Na_2SO_4 (*3*), or 0.9 mM $MgCl_2$ (*4*); Hanks' solution (*5*) (the number of experiments is given parentheses on the background of the columns).

The chemiluminescence dynamics represents an initial rapid burst followed by slow elevation and decrease in the intensity. The "rapid" and "slow" bursts often fuse together and one can detect the presence of two processes only by measuring the slope of the curve of chemiluminescence increase. However, as the temperature increases to the upper temperature limit (42.8°C), as well as at the moderate hypertension of the medium (210 mM NaCl) the separation of chemiluminescence peaks takes place due to the restraining of the "slow" burst. An analogous, although less prominent, change in the shape of chemiluminescence curve occurs under elevated pressure of the medium. This effect manifests as the decrease in both bursts, increase in slow burst duration, and more prolonged rise of the slow burst up to its maximum; however, the moment of the initiation of the rapid burst remains unchanged and corresponds to the moment of latex addition (with an accuracy of 1 sec).

The incubation of blood cells with an activator with their subsequent transfer into the medium without any activator resulted in the complete disappearance of Luc-CL, or, in the case of Lum-CL, only slow burst remained, although its amplitude was substantially decreased. Hence, the rapid burst may be associated with extracellular AOF formation and the slow burst with the intracellular one, which occurs inside phagosomes, as well as in other cell compartments, by analogy to the processes considered for the receptor-mediated Lum-CL [12].

The difference between the levels of the pressure effects on Lum-CL and Luc-CL is of interest. Lucigenin is known to remain continuously in ionic form under physiological conditions and, hence, it does not penetrate into the cell, whereas luminol can be converted into uncharged form and does penetrate into the cell [13]. Thus, Lum-CL reflects AOF formation in various cell compartments and outside the cell, whereas Luc-CL does this only in phagosomes and outside the cell. Besides, chemical specificity of the activators exists: luminol is luminous in the presence of most AOF, whereas lucigenin is luminous only in the presence of superoxide anion-radicals.

It is generally agreed that helium, among a number of the gas fillers employed in hyperbaria (such as hydro-

gen, nitrogen, and argon), alters physical properties of objects to a lesser degree [14]. The available evaluations of its anesthetic activity enable it to be ignored up to the pressure of 13-15 MPa. This suggests that the effects of helium under the pressures employed in our work are mainly due to the pressure effect *per se*.

It is known from the thermodynamic relations that the curve describing the dependence of parameters coupled with volume changes on the pressure must be exponential:

$$\ln(K_1/K_2) = (P_2 - P_1)\cdot\Delta V/(R\cdot T),$$

where K_1 and K_2 are the equilibrium constants at the pressures P_1 and P_2, respectively, ΔV is the change in volume in the course of the process, R is the universal gas constant, and T is absolute temperature. One can obtain, with the collection of processes resulting in chemiluminescence taken conventionally as depending on some equilibrium process, the increase in volume in the course of this process is 193 ml/mole for Luc-CL and 126 ml/mole for Lum-CL in the medium without Mg^{2+}.

Among Mg^{2+}-dependent processes that may be related to the observed effect of pressure on chemiluminescence, the process of actin polymerization–depolymerization seems to be the most probable. As judged from the sensitivity of the process to cytochalasin B, actin filaments participate in the response of leukocytes to latex stimulation [11]. Actin polymerization is associated with an increase in volume [15], resulting in its sensitivity to pressure, as was repeatedly demonstrated in cell-free systems [16]. Reversible decomposition of actin filaments in cells under pressures above 40 MPa was immediately observed by electron microscopy earlier [17]. Reorganization of cytoskeleton including actin filaments already after the exposure of cells to the hydrostatic pressure of 4 MPa was observed by the immunofluorescence method [18]; however, in the absence of any indication for the rate of the pressure increase and decrease it is not possible to be sure that the pressure magnitude, but not the rate of its change, is the virtual agent.

The formation of actin filaments is a complex process, the course of which may be influenced by various ions in the medium, but, in most cases, it was Mg^{2+} that induced the acceleration of polymerization and formation of more enduring filaments, in comparison with monovalent cations or Ca^{2+} [19].

All these facts indicate that the processes of actin filament formation associated with the latex phagocytosis by leukocytes may be responsible for the observed effects of the decrease in latex-stimulated chemiluminescence.

Thus, the effect of the decrease in latex-induced activated chemiluminescence under helium pressure has been revealed in the present work. This effect is found to be more prominent for Luc-CL; the role of Mg^{2+} in the effect is demonstrated. These phenomena may be explained by the effect of pressure on actin polymerization involved in the chain of processes resulting in chemiluminescence. The described phenomenon, in particular its connection with Mg^{2+} deficit, may be important for hyperbaric medicine.

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