Role of Hemoglobin Affinity for Oxygen in the Activation of Lipid Peroxidation During Fever

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Major parameters of oxygen transport and lipid peroxidation are measured in the blood of rabbits with fever, and it is found that hemoglobin affinity for oxygen is decreased while free-radical processes are activated in this state. The data are subjected to multiple correlation analysis and the matrix of paired correlation coefficients obtained for the measured parameters is presented.

Key Words: *oxygen; hemoglobin affinity for oxygen; lipid peroxidation; pyrogenal; fever*

Lipid peroxidation (LPO) processes are going on in the body all the time. Normally, at least 5% of all electrons transferred via the respiratory chain are used to generate superoxide and hydrogen peroxide [8], whose formation has certain implications for the modification of biomembranes and the synthesis of physiologically active substances [4]. In many pathological conditions, the balance between the oxidase and oxygenase pathways of oxygen utilization is upset by various factors, in particular those that determine the value of tissue Po_2. An important role in the formation of the oxygen flux in tissues is known to be played by hemoglobin affinity for oxygen (HAO) [13,14]. The present experiment was undertaken to gain insight into the role of HAO in the activation of free-radical lipid oxidation in fever.

MATERIALS AND METHODS

The experiment was conducted on 16 random-bred male rabbits (body weight 2.4-3.5 kg) in which fever was induced by intravenous injection of *Salmonella typhimurium*-produced lipopolysaccharide (pyrogenal) in a dose of 0.6 μg/kg body weight. The rectal temperature was recorded with an electric thermometer and blood was sampled from the right atrium, via a catheter introduced through the jugular vein, before pyrogenal injection and at the end of the 2nd, 3rd, and 4th hours postinjection.

Parameters of oxygen transport and acid-base balance were measured with an ABL-330 gas analyzer (Radiometer). The indicator of HAO was P_{50} (i.e., the Po_2 of blood half-saturated with oxygen), which was measured by our modification of a previously described method [1] and the value adjusted using formulas given by Severinghaus [14]. The P_{50} values were then used to calculate the positions of oxyhemoglobin dissociation curves by making use of Hill's equation. The oxygen content and oxygen capacity of the blood were estimated by measuring increments in the Po_2 of blood samples of known volume after displacing the oxygen from the oxyhemoglobin with a 0.33% potassium ferricyanide solution [16]; the degree of blood saturation with oxygen was calculated by a numerical method.

LPO parameters were determined in the plasma and in packed red blood cells. Their chemiluminescence induced by salts of bivalent iron (the final $FeSO_4$ concentration in the cuvette being 0.5 mM) was estimated by recording the amplitude of the "fast" chemiluminescence burst [3] with a bioluminometer. Antioxidant activity was expressed as the percentage ratio of the difference between burst amplitudes in the control and test samples to the burst amplitude in the control

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samples (those collected before pyrogenal injection) [6]. Catalase activity was measured as described by Korolyuk *et al.* [5]. The α-tocopherol concentration was estimated by the intensity of fluorescence recorded for a hexane extract with an F-4010 spectrofluorimeter (Hitachi) at an excitation wavelength of 293 nm and an emission wavelength of 323 nm [7].

The results were subjected to statistical analysis on a PC using Statgraphics software package.

RESULTS

The elevation of body temperature was highest at the end of the 2nd hour after pyrogenal injection (40.56± ±0.13°C *vs.* 38.9±0.16°C before injection; $p<0.05$). The pH of the blood rose from the preinjection (baseline) value of 7.357±0.014 to 7.418±0.017 by the end of the 2nd hour postinjection ($p<0.05$) and fell slightly thereafter (to 7.398±0.013 and 7.399±0.015 by the end of the 3rd and 4th hours, respectively; $p<0.05$). The Pco_2 declined from the baseline value of 38.5±1.1 mm Hg to 30.5±0.6 mm Hg ($p<0.01$) and 31.3±1.8 mm Hg ($p<0.05$) by the end of the 2nd and 3rd hours, respectively. The concentration of hydrocarbonates also declined from the baseline value of 21.34±0.55 mmol/liter to reach 15.93±0.63 mmol/liter by the end of the 3rd hour ($p<0.05$) and to 16.89±0.68 mmol/liter by the end of the 4th ($p<0.05$), and similar changes were recorded for the concentrations of total carbon dioxide and standard bicarbonate. The excesses of standard and actual bases were greatest by the end of the 3rd hour: -8.26±0.91 mmol/liter ($p<0.05$) and -8.48±0.83 mmol/liter ($p<0.0l$), respectively, *vs.* -3.84±0.67 and -3.84±0.57 at baseline. Of the parameters characterizing the oxygen-transporting function of the blood, the greatest changes were noted in Po_2, which fell from the baseline value of 39.7±1.4 mm Hg to 33.2±1.2 mm Hg by the end of the 2nd hour ($p<0.01$), followed by a slight rise. The degree of blood saturation with O_2 decreased from 60.9±3.3% before fever to 50.11±3.6% by the end of the 3rd hour of fever ($p<0.05$). This pattern of variation in the measured parameters reflected the development of compensated alkalosis in the presence of moderate oxygen deficiency.

When standard pH, Pco_2, and body temperature values were considered, the P_{50} value, used as the indicator of HAO, was found to have decreased from 32.2±0.3 mm Hg before pyrogenal injection to 30.2±0.5 and 30.7±0.6 mm Hg by the end of the 2nd and 3rd hours postinjection, respectively, reflecting a shift to the left of the oxyhemoglobin dissociation curve. However, a different pattern of change in HAO was obtained when the actual values of pH, Pco_2, and body temperature were taken into account. Thus, the initial rise in P_{50} from 33.7±0.5 to 37.1±1.2 mm Hg by the end of the 2nd hour ($p<0.05$) was succeeded by a decrease to 35.8±1.2 mm Hg by the end of the 3rd, followed by another rise to 37.1±0.8 mm Hg ($p<0.01$), with a rightward shift of the oxyhemoglobin dissociation curve (Fig. 1). In considering the mechanisms by which HAO is altered, the importance of such factors as Pco_2 and temperature should be emphasized. A rise in temperature increases the P_{50}, whereas hypocapnia decreases it, and the overall effect of these two modulators determines the actual HAO.

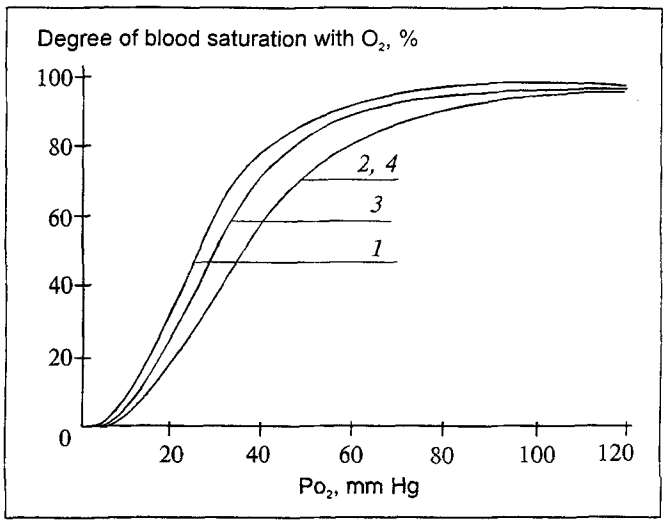

Fig. 1. Oxyhemoglobin dissociation curves obtained with actual pH, Pco_2, and body temperature values before pyrogenal-induced fever (*1*) and by the end of the 2nd (*2*), 3rd (*3*), and 4th (*4*) hours of fever in rabbits.

The intensity of induced chemiluminescence was highest by the end of the 3rd and 4th hours in the plasma (at which times it was 31.4% and 30.8% above baseline, respectively) and by the end of the 2nd and 3rd hours in red cells (54.5% and 33.5% above baseline) (Fig. 2). The antioxidant activity of

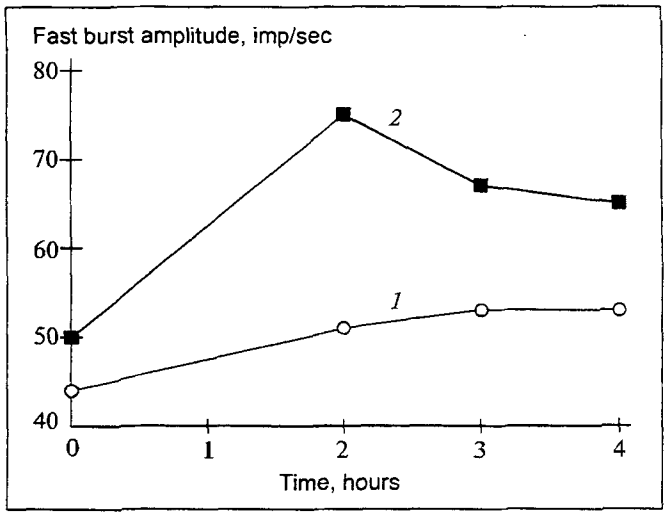

Fig. 2. Variations in the amplitude of the fast chemiluminescence burst in plasma (*1*) and red blood cells (*2*) at different times of pyrogenal-induced fever in rabbits.

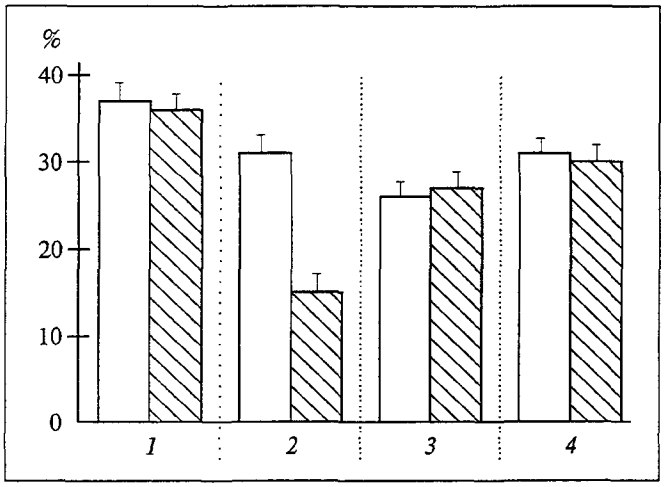

Fig. 3. Antioxidant activity of plasma (white bars) and red blood cells (hatched bars) before (*1*) and by the end of the 2nd (*2*), 3rd (*3*), and 4th (*4*) hours after pyrogenal injection.

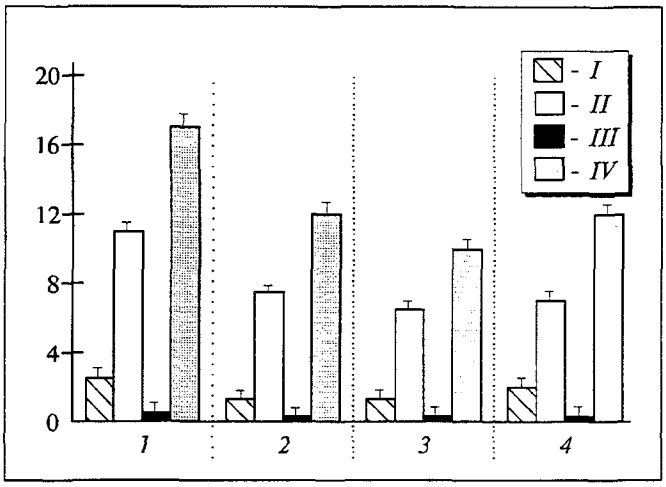

Fig. 4. α-Tocopherol concentration (μmol/ml) and catalase activity (10^4) of plasma (*I* and *III*) and packed red blood cells (*II* and *IV*) before (*1*) and by the end of the 2nd (*2*), 3rd (*3*), and 4th (*4*) hours after pyrogenal injection.

plasma was 16.2%, 28.4%, and 16.1% below baseline by the end of the 2nd, 3rd, and 4th hours, respectively, and decreases in this parameter were also recorded for red cells, particularly by the end of the 2nd hour (by 72.2%) (Fig. 3).

Changes in the parameters of the antioxidant system during fever are depicted in Fig. 4. α-Tocopherol concentrations declined from the baseline value of 2.47±0.26 to 1.19±0.18 μmol/liter in the plasma ($p<0.05$) and from 10.95±1.51 to 6.37±0.92 μmol/liter in red cells by the end of the 3rd hour. Catalase concentrations were also lower - by 25.5, 25.5, and 36.2% in the plasma and by 29.7, 40.2, and 28.9% in red cells at the end of the 2nd, 3rd, and 4th hours, respectively.

Using multiple correlation analysis we examined the body of data characterizing HAO and LPO, and the resulting matrix of coefficients of paired correlation is presented in Table 1. We see a rather high inverse direct correlation between the actual P_{50} and parameters of the antioxidant system (with *r* values ranging from -0.55 to -0.79) and a similarly high direct correlation between the actual P_{50}, on the one hand, and the fast burst amplitudes in the plasma ($r=+0.79$) and red cells ($r=+0.62$), on the other.

The biological significance of changes in HAO has not been fully elucidated. A shift of the oxyhemoglobin dissociation curve to the right has been shown to cause the blood to give off more oxygen to the tissues [12,13]. A decrease in HAO is apparently important as a factor compensating for oxygen deficiency in various pathological states and as a mechanism of adaptation to oxygen deficit. It has also been shown that a shift of this curve to the left

TABLE 1. Matrix of Coefficients of Paired Correlation between Parameters of Hemoglobin Affinity for Oxygen, Chemiluminescence, and Antioxidant Activity in Rabbits with Fever

Parameter	P_{50}, actual	P_{50}, standard	AOA, plasma	AOA, red cells	I_{CL}, plasma	I_{CL}, red cells	α-TOC, plasma	α-TOC, red cells	CAT, plasma	CAT, red cells
P_{50}, actual	1	0.68	-0.69	-0.71	0.79	0.62	-0.79	0.79	-0.65	-0.55
P_{50}, standard		1	-0.93	-0.97	0.54	0.54	-0.81	0.78	-0.72	-0.59
AOA, plasma			1	0.93	-0.54	-0.47	0.82	0.78	0.74	0.55
AOA, red cells				1	-0.57	-0.54	0.84	0.81	0.74	0.58
I_{CL}, plasma					1	0.80	-0.79	-0.83	-0.71	-0.57
I_{CL}, red cells						1	-0.54	-0.58	-0.77	-0.65
α-TOC, plasma							1	0.98	0.75	0.56
α-TOC, red cells								1	0.73	0.50
CAT, plasma									1	0.80
CAT, red cells										1

Note. Underlined figures are statistically significant coefficients of paired correlation reflecting moderate to strong associations between the measured parameters. AOA = antioxidant activity; I_{CL} = amplitude of the fast burst; α-TOC = α-tocopherol concentration; CAT = catalase activity.

renders the body more resistant to severe hypoxic hypoxia [15]. We observed an association between HAO and LPO, namely a fall in the concentrations of major free-radical oxidation products with a rise in HAO and, conversely, their rise with its fall [2]. HAO thus appears to play a role in maintaining a certain prooxidant-antioxidant equilibrium in the body.

LPO may be activated through a change in the oxygen supply to the body - as a result of excess electron donors in hypoxia and of excess electron acceptors (excess oxygen) in hyperoxia [9]. Under conditions of postischemic reoxygenation, for example, a rise in the oxygen concentration boosts superoxide generation [4]. The rates of LPO reactions were found to be significantly altered following a drop in the Pco_2 to below 50-100 mm Hg, probably because oxygen influences not only chain propagation reactions but also those of chain termination [11].

The multistage nature of LPO processes suggests the existence of a multilevel system for their regulation, in which physiological (supracellular) mechanisms possibly predominate over intracellular ones. In our view, one supracellular mechanism may be based on HAO which, by determining the rate of oxyhemoglobin dissociation, produces a capillary-tissue Po_2 gradient and thus sets the conditions for oxygen diffusion to the tissues.

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