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Analysis of the relationship between hemoglobin-oxygen affinity and lipid peroxidation during fever

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Endogenous hyperthermia was induced in rabbits by i.v. pyrogenal administration. Hemoglobin-oxygen affinity and parameters of free radical lipid oxidation in plasma and red blood cells were measured. The content of diene conjugates, malonic dialdehyde and Schiff bases were determined at a pyrogenal dose of 4 minimal pyrogenic doses/kg, and iron-initiated chemiluminescence, catalase activity and α-tocopherol concentration were determined at 6 minimal pyrogenic doses/kg. A rightward shift of the real oxyhemoglobin dissociation curve and activation of lipid peroxidation were observed. Relationships between the parameters measured were analyzed. Decreased hemoglobin-oxygen affinity is considered to be a possible mechanism of activation of free radicals during fever.

Hemoglobin-oxygen affinity (HOA)[1], reflected by an S-shaped oxyhemoglobin dissociation curve (ODC), plays an important role in lung blood oxygenation and tissue blood deoxygenation. However, HOA has also other and not always elucidated functions [1]. A rightward shift of ODC was shown to reflect enhanced tissue oxygen extraction [2–4]. This change in HOA is obviously important for compensation of oxygen deficiency under different pathological conditions and for adaptation to chronic hypoxia. However, a leftward shift of ODC was shown to reflect enhanced body tolerance to heavy environmental hypoxia. We have previously shown that concentrations of the main lipid peroxidation (LPO) products were lowered when HOA was increased, and were increased when HOA was decreased [5].

This work aimed to explore relationships between oxygen-binding hemoglobin properties and LPO activity during pyrogenal-induced fever leading to marked changes in body energetics and lipid exchange.

METHODS

Experiments were performed on male laboratory rabbits (body weight 2.4–3.1 kg). Fever was induced by intravenous administration of pyrogenal (4 or 6 minimal pyrogenic doses (MPD)/kg; n = 15 and n = 16, respectively). Rectal temperature was measured with an electrothermometer.

Blood samples were withdrawn through a catheter, *via* the jugular vein, from the right

[1]Abbreviations: AOA, antioxidant activity; HOA, hemoglobin-oxygen affinity; Icl, amplitude of "rapid" burst of chemiluminescence; ODC, oxyhemoglobin dissociation curve; LPO, lipid peroxidation; MPD, minimal pyrogenic dose; MDA, malondialdehyde; α-TOC, α-tocopherol concentration; CAT, catalase activity; *t*, temperature. Indices pl and rbc mean plasma and erythrocytes, respectively.

atrium prior to, and 2, 3 and 4 h after, pyrogenal administration and the main parameters of oxygen-transporting blood function and LPO products were determined.

pO_2 and acid-base balance were measured by micro gas analyzer ABL (Radiometer). HOA was assessed by p_{50} (blood pO_2 under its 50% saturation with O_2) determined by the "mixing method" [6] at 37°C, pH 7.4 and pCO_2 (p_{50}st). p_{50} At real pH, pCO_2 and temperature (p_{50}real) were calculated from p_{50}st by Severinghaus' equations [7] and using the temperature coefficient $\Delta \log p_{50}/\Delta T = 0.024$ [8]. ODC was calculated from the measured p_{50} by Hill equation with n = 2.8; small changes of n have little effect on the middle part of ODC, and this assumption would not lead to a large error in calculations [9].

Conjugated diene content was determined by UV absorption at 232–234 nm (characteristic for conjugated double bonds of lipid hydroperoxides). The results were expressed as ΔA_{233}/ml [10].

Concentration of malondialdehyde (MDA) was determined by its reaction with thiobarbituric acid and calculated assuming the molar extinction ratio of 1.56×10^{-5} $M^{-1}cm^{-1}$ [11].

The level of Schiff bases was determined by measuring fluorescence intensity of chloroform extracts at excitation and emission wavelengths of 344 and 440 nm, respectively, with spectrofluorimeter F-4010 (Hitachi). Results were expressed in relative units of intensity per 1 ml of plasma or red blood cells [12].

Chemiluminescence induced by ferrous salts (final concentration of $FeSO_4$ in cuvette 0.5 mM) was determined from the amplitude of "rapid" burst with biochemoluminometer BKhL-06 (Bioavtomatika, Russia) [13].

Antioxidation activity (AOA) was measured as the ratio of difference between the "rapid" burst amplitudes in control and experimental samples to its amplitude in the control [13].

Catalase activity was determined by measuring the rate of decomposition of hydrogen peroxide capable of generating a stable coloured complex with molybdenum salts, and was measured with a spectrophotometer SF-46 (LOM, Russia) at 410 nm [14].

α-Tocopherol concentration was evaluated by fluorescence intensity of heptane extracts at excitation and emission wavelengths of 292 and 325 nm, respectively, with spectrofluorimeter F-4010 (Hitachi) [15] using α-tocopherol (Sigma) as reference.

The data obtained were statistically evaluated on a personal computer by multiple correlation-regression analysis (program "Statgraphics").

RESULTS AND DISCUSSION

Pyrogenal in the intravenous dose of 4 MPD/kg led to a rise of temperature with a maximum at 3 h (from 36.7 ± 0.4°C to 39.6 ± 0.2°C; $P < 0.05$). Rectal temperature at 4 h of pyrogenal fever was equal to 39.0 ± 0.2°C ($P < 0.05$).

p_{50} At standard pH, pCO_2 and temperature conditions (7.4, 40 mm Hg and 37°C, respectively) decreased after 2 h of fever from 32.6 ± 0.5 to 29.7 ± 0.5 mm Hg ($P < 0.05$) and during the next 2 h remained at that level. Hemoglobin oxygenation is an exothermic reaction; therefore oxygen dissociation from oxyhemoglobin leads to heat consumption [7], and a rise in temperature leads to lowering of HOA. Thermodynamics of this process agrees with Van't Hoff equation. This peculiarity of Hb-O_2 interaction thermodynamics was used for induction of conditions of decreased HOA. Rabbit p_{50} under real pH, pCO_2 and temperature conditions increased at the maximum temperature from 29.8 ± 1.4 to 34.2 ± 0.8 mm Hg ($P < 0.05$), which caused a rightward shift of real ODC (Fig. 1A). After 4 h of fever the real p_{50} was again closer to the initial one.

The contents of the main LPO activity markers in plasma and red blood cells had an increasing tendency (Table 1). The critical diene content in plasma was virtually unchanged but in erythrocytes rose at 2 and 3 h after pyrogenal administration. Plasma and red cell malondialdehyde and Schiff bases were most markedly increased at maximal hyperthermia (after 3 h) which pointed to an enhancement of LPO processes.

Multiple correlation analysis of relationships between the main LPO products and p_{50} (real or standard) gave the values of paired correlation coefficients presented in Table 2. Values of r between real p_{50} and critical diene content, malondialdehyde or Schiff bases ranged from +0.43 to +0.55, except for the plasma critical diene content, pointing to a moderate correla-

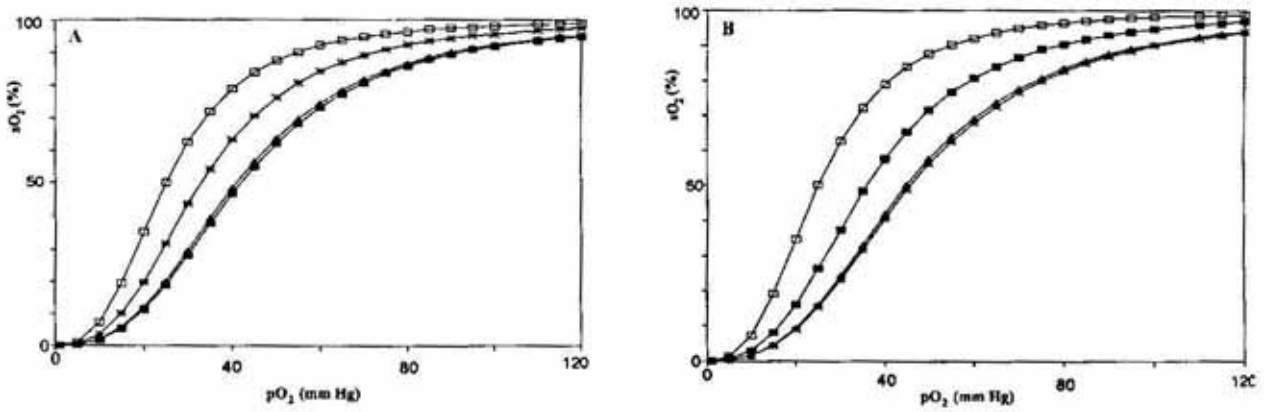

Fig. 1. Rabbit oxyhemoglobin dissociation curves under real values of pH, pCO_2 and temperature before and after pyrogenal-induced fever.
A, 4 MPD/kg; B, 6 MPD/kg; open squares, before pyrogenal administration; filled triangles, at 2 h; filled squares, at 3 h; asterisks, at 4 h, after pyrogenal administration.

Table 1
LPO Activity parameters before and after pyrogenal administration in rabbits.
Given are mean values ± SEM; n = 15.

Parameter	Initial	After pyrogenal (4 MPD/kg)		
		at 2 h	at 3 h	at 4 h
CDC_{pl}	1.82 ± 0.41	1.84 ± 0.41	1.96 ± 0.11	1.34 ± 0.14
CDC_{rbc}	15.30 ± 1.16	19.76 ± 1.08**	18.74 ± 1.13*	15.74 ± 1.48
MDA_{pl}	0.75 ± 0.05	1.34 ± 0.08**	1.47 ± 0.07**	1.16 ± 0.06**
MDA_{rbc}	6.25 ± 0.32	8.74 ± 0.44**	9.31 ± 0.56**	7.31 ± 0.41
SB_{pl}	5.01 ± 0.72	9.81 ± 1.12**	12.8 ± 1.29**	8.61 ± 1.42
SB_{rbc}	30.38 ± 4.44	43.50 ± 6.39	56.24 ± 9.14*	40.73 ± 6.26

Abbreviations: CDC, conjugated diene content; MDA, malondialdehyde; SB, Schiff bases. Indices pl and rbc mean plasma and erythrocytes, respectively.

Units: MDA, μM. CDC, ΔA_{233}/ml, SB, ΔA_{540}/ml.

* $P < 0.05$; ** $P < 0.01$.

tion. p_{50} Real is preferable for analysis of relationships between HOA and LPO because it reflects capillary blood desaturation more objectively than does p_{50}st. A rightward shift of ODC reflects facilitated capillary blood desaturation [1, 5]. For example, perfusion of rabbit heart by blood with p_{50} increased by 13 mm Hg caused an enhancement of oxygen consumption by 30% [2].

In a dose of 6 MPD/kg pyrogenal induced the maximal rise of temperature 2 h after administration (from 38.9 ± 0.2 to 40.6 ± 0.2°C; $P < 0.05$); after 3 h the temperature remained almost the same (40.3 ± 0.2°C). Standard p_{50} decreased after 2 and 3 h from 32.2 ± 0.3 to 30.2 ± 0.5 and 30.7 ± 0.6 mm Hg, respectively, reflecting a leftward ODC shift. However, at real pH, pCO_2 and temperature values the pattern of changes in HOA was different. Real p_{50} initially increased from 33.7 ± 0.5 to 37.1 ± 1.2 mm Hg (2 h; $P < 0.05$), then slightly decreased to 35.8 ± 1.2 (3 h) and again increased to 37.1 ± 0.8 (4 h; $P < 0.01$ comparing with 0 h). The difference between initial real p_{50} values at the pyrogenal doses of 4 and 6 MPD/kg may be explained by the difference in initial body temperatures exceeding 2.0°C (due to different seasons of the year). This difference led to a shift of ODC rightward (Fig. 1B).

Maximal Fe^{2+}-induced chemiluminescence was increased after pyrogenal administration (Fig. 2A); the increase was most significant in plasma after 3 and 4 h (by 31.4 and 30.8%, respectively), and in red cells after 2 and 3 h (by

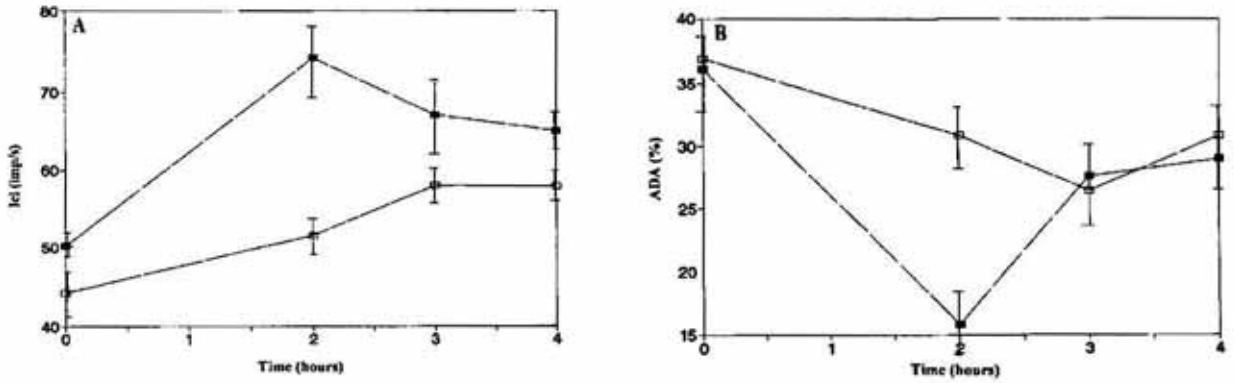

Fig. 2. Changes in: A, the amplitude of iron-induced chemiluminescence (Icl); and B, the antioxidation activity (AOA) in rabbit plasma (open squares) and erythrocytes (filled squares) before and after pyrogenal administration.

Table 2

Matrix of paired correlation coefficients between indices of hemoglobin-oxygen affinity and main parameters of lipid peroxidation during fever induced in rabbits by pyrogenal (4 MPD/kg)

Index	Real p_{50}	Standard p_{50}	CDC_{pl}	CDC_{rbc}	MDA_{pl}	MDA_{rbc}	SB_{pl}	SB_{rbc}
Real p_{50}	1	0.22	0.19	0.51*	0.55*	0.43*	0.48*	0.46*
Standard p_{50}		1	0.13	0.43*	0.034	0.01	0.16	0.43*
CDC_{pl}			1	0.43*	0.29	0.23	0.06	0.43*
CDC_{rbc}				1	0.68*	0.70*	0.73*	0.82*
MDA_{pl}					1	0.88*	0.78*	0.72*
MDA_{rbc}						1	0.78*	0.81*
SB_{pl}							1	0.74*
SB_{rbc}								1

Underlined are paired correlation coefficients which reflect moderate or strong relationships between the two parameters.

* $P < 0.001$.

Abbreviations are the same as in Table 1.

54.5 and 33.5%, respectively). Plasma AOA decreased after 2, 3 and 4 h of fever by 16.2, 28.4 and 16.1%, respectively (Fig. 2B). Erythrocyte AOA was also lowered, most markedly after 2 h (72.2%).

Figure 3 shows the changes in antioxidation parameters. Plasma and erythrocyte α-tocopherol concentration decreased, respectively, from the initial 2.47 ± 0.26 and 10.95 ± 1.51 μM to 1.19 ± 0.18 and 6.37 ± 0.92 μM after 3 h (P 0.01), respectively. Catalase activity was lowered after 2, 3 and 4 h by 25.5, 25.5 and 36.2% in plasma and by 29.7, 40.2 and 28.9% in red cells, respectively.

The data set which characterizes HOA and LPO was subjected to multiple correlation analysis. Table 3 shows the matrix of paired correlation coefficients for these parameters. One can see a sufficiently close reversed correlation between real p_{50} and antioxidation parameters (r from –0.55 to –0.79). There was also a high direct correlation between real p_{50} and the amplitude of iron-induced chemiluminescence burst in plasma and red cells (r = +0.79 and r = +0.62, respectively).

The observed lowering of standard p_{50} after pyrogenal administration suggests some additional mechanism of HOA modulation, differing from the action of temperature, pH or pCO_2. Wood [16] proposed two possible ways of change of temperature effect on HOA *in vivo*: (1) synthesis of thermotolerant hemoglobins, and (2) modulation of the allosteric interaction between hemoglobin and ligands. Change of

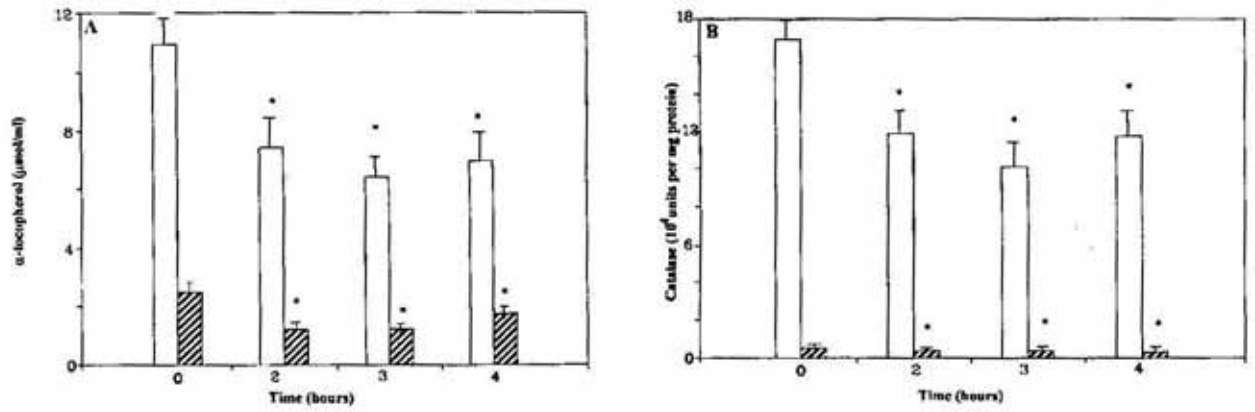

Fig. 3. Time dependence of α-tocopherol concentrations (A) and catalase activities (B) in rabbit plasma (open columns) and erythrocytes (hatched columns) before and after pyrogenal administration.

Table 3

Matrix of paired correlation coefficients between indices of hemoglobin-oxygen affinity, chemiluminescence and antioxidant protection during fever induced in rabbits by pyrogenal (6 MPD/kg)

Index	Real p_{50}	Standard p_{50}	AOApl	AOA_{rbc}	Icl_{pl}	Icl_{rbc}	α-TOC_{pl}	α-TOC_{rbc}	CAT_{pl}	CAT_{rbc}	t
Real p_{50}	1	0.68*	−0.69*	−0.71*	0.79*	0.62*	−0.79*	−0.79*	−0.65*	−0.55*	0.73*
Standard p_{50}		1	−0.93*	−0.97*	0.54*	0.54*	−0.81*	−0.78*	−0.72*	−0.59*	−0.93*
AOA_{pl}			1	0.93*	−0.54*	−0.47*	0.82*	0.78*	0.74*	0.55*	−0.92*
AOA_{rbc}				1	−0.57*	−0.54*	0.84*	0.81*	0.74*	0.58*	−0.95*
Icl_{pl}					1	0.80*	−0.79*	−0.83*	−0.71	−0.57*	0.66*
Icl_{rbc}						1	−0.54*	−0.58*	−0.77*	−0.65*	0.61*
α-TOC_{pl}							1	0.98*	0.75*	0.56*	−0.89*
α-TOC_{rbc}								1	0.73*	0.50*	−0.87*
CAT_{pl}									1	0.80*	−0.74*
CAT_{rbc}										1	−0.60*
T											1

Underlined are paired correlation coefficients which reflect moderate or strong relationships between the two parameters.

* $P < 0.001$.

Abbreviations: AOA, antioxidation activity; Icl, amplitude of "rapid" burst of chemiluminescence; α-TOC, α-tocopherol concentration; CAT, catalase activity; t, temperature. Indices pl and rbc mean plasma and erythrocytes, respectively.

HOA in our experiments with pyrogenal may be explained by lowering of red cell 2,3-diphosphoglycerate concentration, similar to that observed under environmental hyperthermia [17].

Pyrogenal undoubtedly plays a primary role in LPO activation but, under conditions of disturbed tissue O_2 utilization, lowering of HOA can also facilitate free radical reactions *via* a rise of O_2 flux to tissues. It is well known that activation of LPO processes can result from changes in body oxygen supply, due either to an excess of electron donors in hypoxia or to an excess of electron acceptors (oxygen) in hyperoxia [18]. For example, a rise of O_2 concentration during reoxygenation after ischemia enhances superoxide generation [19]. There were also observations of marked LPO velocity changes when pO_2 was below 50–100 mm Hg, which could have been due to oxygen influence on both propagation and termination of chain reactions [20].

Body LPO processes occur continuously. Under normal conditions, 1–2% of electrons

carried by the respiratory chain are utilized for generation of superoxide and H_2O_2 [21]. The latter are important for modifications of biomembranes and for synthesis of different physiologically active compounds. However, pathological conditions are often accompanied by a disturbance of the equilibrium between the oxidase and oxygenase pathways of O_2 utilization, resulting from various factors including tissue pO_2 determinants. HOA plays an important role in determination of oxygen flux to tissues [22].

The data obtained in our experiments on rabbits with pyrogenal-induced fever thus reflect close relationships between the parameters of free radical lipid oxidation and hemoglobin-oxygen affinity. This enables us to assume that hemoglobin is a component of some mechanism activating free radical reactions.

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