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High hemoglobin affinity to oxygen and its relationships with lipid peroxidation during fever

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The effects of high hemoglobin-oxygen affinity (HOA) on rectal temperature and lipid free radical oxidation were investigated in red blood cells, heart, liver and kidneys of male rats during fever. Fever was induced by intraperitoneal injection of *Salmonella typhi* lipopolysaccharide (LPS; 5.0 mg kg^{-1}). HOA was increased by addition of 0.5% sodium cyanate to drinking water for eight weeks. HOA modification (actual half-saturation oxygen pressure, $P_{50}act$, decreased to 23.3 ± 0.7 vs. 31.6 ± 0.7 Torr in control; $p < 0.001$) weakened a febrile response: rise of temperature after 4 hours was 0.79 ± 0.2 °C vs. 1.38 ± 0.1 °C in rats with normal HOA ($p < 0.05$). In red cells and tissues of rats with normal HOA, concentrations of conjugated dienes and Schiff bases increased during fever, and α-tocopherol level and catalase activity decreased. Rats with increased HOA had an inverse pattern of such changes. Changes in rectal temperature and markers of free radical oxidation correlated with a shift of oxyhemoglobin dissociation curve leftwards. The present results indicate that the intentional increment of HOA may substantially diminish lipid peroxidation activity, increase the body antioxidant content during fever and decrease the febrile response on LPS.

Keywords: Oxygen hemoglobin affinity, Lipid peroxidation, Fever, Lipopolysaccharide, Sodium cyanate.

Fever is accompanied by substantial changes in thermoregulation and heat production mechanisms and in body energy metabolism as a whole (11). The rise of temperature lowers hemoglobin-oxygen affinity (HOA) due to the exothermic nature of hemoglobin oxygenation (3); HOA is known to determine tissue oxygen delivery to a significant degree (12, 23). During lipopolysaccharide (LPS)-

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induced fever in rabbits there was observed a decrease in HOA and changes in several HOA modulators that eventually led to the increase of oxygen flux to tissues, such as temperature rise and acidosis (2). Change in respiratory mode parameters is known to influence heat production and fever reaction (20). Sodium cyanate is a widespread modulator of HOA; its HOA-rising action is mediated by the carbamylation of hemoglobin (1, 19), and permits the creation of animal models for the investigation of the physiological role of HOA in its different aspects.

The effect of intracellular prooxidant formation on free radical levels is counterbalanced by a regular scavenging of free radicals by intra- and intercellular antioxidants, thus generating the definite level of prooxidant-antioxidant equilibrium. We observed a marked correlation between HOA and activity of lipid peroxidation (LPO) processes during LPS-induced fever (2) indicating that high HOA plays an important role in the formation of reactive oxygen intermediates. The present work aims to explore the effects of HOA modification by sodium cyanate on LPO activity and the course of LPS-induced fever.

Materials and Methods

Experimental protocol.– Experiments were carried out in male rats weighing from 180 to 240 g, fed *ad libitum* and maintained under 26 °C. They received 0.5% sodium cyanate in drinking water during 8 weeks for chronic (left) shift of oxygen dissociation curve (ODC), or 0.5% NaCl in control (1). Fever was induced by intraperitoneal (i.p.) injection of LPS of *Salmonella typhi* (5.0 mg kg^{-1} in 1 ml of saline). Rectal temperature was measured with electric thermometer. Rats received saline i.p. (group 1), only LPS (groups 2 and 3), or LPS after rise of HOA (groups 4 and 5). Each group consisted of 16 animals.

Blood and tissue preparation.– Blood was taken by punction from right atrium under ether anesthesia at 120 (groups 2 and 4) and 240 min (groups 3 and 5) after LPS administration and was heparinized (10 U/ml). After blood sampling, liver heart and kidneys were dissected out for analysis. Blood gase measurements were performed immediately after sampling. Three ml of blood were centrifuged (10 min, 3000 rpm). Erythrocyte pellet was washed three times by 0.9% NaCl and then hemolyzed by bidistillated water (1:5 v/v; 10 min). Tissues were homogenized (1:10 v/v) in 10 mM phosphate buffer (pH 7.4) with 0.3 M sucrose and 1 mM EDTA by homogenizer WPW-300. Homogenates were kept frozen at –70 °C.

Measurement of hemoglobin-oxygen affinity.– pO_2 and acid-base balance were measured by micro gas analyzer ABL-330 (Radiometer). HOA was assessed by P_{50} (blood pO_2 under its 50% saturation by O_2) determined with 'mixing method' (21) at 37 °C, pH 7.4 and pCO_2 40 Torr (P_{50}st).P_{50} at actual pH, pCO_2 and temperature (P_{50}act) were calculated from P_{50}st by Severinghaus' equations (22) and with temperature coefficient $\Delta lg\ /P_{50}DT = 0.024$ (3).

Lipid peroxidation.– Conjugated diene content was determined by UV absorption at 232-234 nm (characteristic for conjugated double bonds of lipid hydroperoxides). Results of measurement were expressed in relative units of optical density in 1 ml (ΔA_{233}/ml) (4). Level of Schiff bases was determined by 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 in 1 ml of plasma or red blood cells (6).

Antioxidant system.– Catalase activity was determined by measurement of the decomposition rate of hydrogen peroxide capable of generating the stable coloured complex with molybdenum salts which was measured with spectrophotometer SF-46 at 410 nm (8). α-tocopherol concentration was evaluated by fluorescence intensity of heptane extraction at excitation and emission wavelengths of 292 and 325 nm with spectrofluorimeter F-4010 (Hitachi) using α-tocopherol (Sigma Chemical Co., St. Louis) as reference (7). Protein content was measured by Layne method (13).

Statistic methods.– Data were statistically evaluated by Student's *t*-test with significance level at $p < 0.05$. Correlations were obtained from least-squares linear regression analysis. Results are presented as means ± SE. Analysis and graphs were performed using computer software packages (version 3.0, STATGRAPHICS; version 4.0, QUATROPRO).

Results

I.p. administration of LPS resulted in a rise of rat rectal temperature under thermoneutral conditions. The rise of temperature 2 hours after LPS administration was 1.47 ± 0.1 °C in rats not treated with sodium cyanate ($p < 0.05$) and 1.14 ± 0.1 °C in treated rats ($p < 0.05$). The rise of temperature after 4 hours of fever was 1.38 ± 0.1 ($p < 0.01$) and 0.79 ± 0.2 °C ($p < 0.05$) respectively.

Table I shows the values of hematologic and blood gas parameters in rats with normal and high HOA under LPS-induced fever. LPS administration to rats not treated with sodium cyanate resulted in a significant decrease of $P_{50}st$ from 34.5 ± 0.6 to 32.7 ± 0.6 Torr after 4 hours ($p < 0.05$). In rats with high HOA, $P_{50}st$ at 2 and 4 hours after LPS administration were 22.5 ± 0.7 ($p < 0.001$) and 21.8 ± 0.7 ($p < 0.001$) Torr, respectively. $P_{50}act$ after 2 and 4 hours of fever was not significantly changed in untreated rats and significantly fell in rats treated by sodium cyanate (24.3 ± 0.9, $p < 0.001$ and 23.3 ± 0.7, $p < 0.001$ Torr after 2 and 4 hours, respectively, vs. 31.6 ± 0.7 Torr in control). This indicated a significant shift of ODC leftwards under real conditions despite the rise in body temperature.

Red cell and tissue content of main LPO markers in rats with normal HOA tended to increase after 2 hours of fever and substantially increased after 4 hours (fig. 1). The level of conjugated dienes increased in red cells, liver, kidney and heart by 36.7, 21.6, 27.2 and 22.5%, respectively. Content of Schiff bases also rose, especially in red cells and kidneys (24.8 and 30%). In rats with modified HOA these values decreased, especially after 4 hours of fever (conjugated dienes in red cells, liver and heart - by 24.5, 16.1 and 17.3%, respectively; Shiff bases in red cells, liver and kidneys - by 19.3, 18.1 and 30%, respectively). These changes reflected an enhancement of lipid free radical oxidation in rats with normal HOA and its depression under increased HOA.

Multiple regression analysis of dependence between the measured LPO products and P_{50} (actual or standard) showed a moderate correlation between them (table II). The value of r for a relationship between P_{50} act and conjugated dienes or

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Table I. *Hematological and blood gas parameters in rats with normal and high hemoglobin-oxygen affinity during pyrogenal-induced fever* (mean ± SEM).

Parameter	Control animals (16)	Animals with high HOA (6)	Fever (LPS, 5.0 mg kg^{-1} i.p.)			
			Normal HOA (16)		High HOA (16)	
			120 min	240 min	120 min	240 min
pH, units	7.318 ± 0.009	7.305 ± 0.014	7.319 ± 0.011	7.331 ± 0.016	7.310 ± 0.011	7.324 ± 0.010
pCO_2, Torr	47.5 ± 2.4	47.6 ± 3.7	45.7 ± 2.5	44.0 ± 2.4	44.9 ± 1.6	42.6 ± 0.9
pO_2, Torr	21.5 ± 1.3	18.0 ± 0.7	20.1 ± 1.4	19.2 ± 0.8	18.1 ± 0.7*	16.5 ± 0.75*
P_{50}st, Torr	34.5 ± 0.6	23.7 ± 0.9*	33.5 ± 0.8	32.7 ± 0.6*	22.5 ± 0.7	21.8 ± 0.7*
P_{50}act, Torr	31.6 ± 0.7	21.0 ± 0.8*	35.9 ± 0.9*	35.1 ± 0.7*	24.3 ± 0.9*	23.3 ± 0.7*
HCO_3^-, mM	24.12 ± 1.06	23.76 ± 1.71	23.04 ± 1.14	21.58 ± 1.25	21.59 ± 1.0	19.87 ± 1.03*
TCO_2, mM	25.46 ± 1.22	29.25 ± 1.0	24.13 ± 1.24	20.37 ± 0.51*	22.79 ± 1.11	20.37 ± 1.18*
ABE, mM	–2.3 ± 0.62	–2.9 ± 0.37	–3.56 ± 1.08	–5.01 ± 0.96*	–5.61 ± 0.63*	–5.32 ± 0.44*
SBE, mM	–2.8 ± 0.62	–2.6 ± 0.84	–3.11 ± 1.10	–4.76 ± 1.05	–4.47 ± 0.69	–5.32 ± 0.44*
SBC, mM	18.73 ± 1.38	19.8 ± 1.78	19.71 ± 0.81	20.90 ± 0.85	19.97 ± 0.79	17.68 ± 0.64

* $p < 0.05$ vs. control. In parenthesis, number of animals.

Table II. *Matrix of paired correlation coefficients between parameters of hemoglobin-oxygen affinity, lipid peroxidation and antioxidant system in blood and tissues during fever.*

Param.	P_{50}st	CD_{rbc}	CD_{liv}	CD_{kid}	CD_{hea}	SB_{rbc}	SB_{liv}	SB_{kid}	αT_{rbc}	αT_{liv}	αT_{kid}	αT_{hea}	CAT_{rbc}	CAT_{liv}	CAT_{kid}
P_{50}act	0.93	–0.42	0.18	0.14	0.56	0.52	0.56	0.40	–0.49	–0.52	–0.55	–0.44	–0.60	–0.36	–0.36
P_{50}st		0.32	0.27	0.18	0.52	0.50	0.53	0.40	–0.46	–0.49	–0.52	–0.44	–0.59	–0.35	–0.38
CD_{rbc}			0.21	0.16	0.85	0.67	0.80	0.60	–0.69	–0.44	–0.61	–0.59	–0.73	–0.78	–0.58
CD_{liv}				0.23	0.84	0.30	0.05	0.25	–0.11	0.17	–0.11	0.11	0.01	0.15	0.28
CD_{kid}					0.08	0.01	0.09	0.08	–0.14	–0.24	–0.08	0.04	–0.09	–0.01	0.01
CD_{hea}						0.81	0.94	0.76	–0.77	–0.56	–0.66	–0.74	–0.85	–0.86	–0.79
SB_{rbc}							0.75	0.74	–0.63	–0.51	–0.54	–0.70	–0.73	–0.82	–0.81
SB_{liv}								0.68	–0.82	–0.56	–0.66	–0.73	–0.84	–0.79	–0.71
SB_{kid}									0.48	–0.31	–0.36	–0.62	–0.61	–0.74	–0.89
αT_{rbc}										0.57	0.83	0.57	0.81	0.60	0.55
αT_{liv}											0.50	0.54	0.58	0.40	0.41
αT_{kid}												0.44	0.77	0.43	0.39
αT_{hea}													0.70	0.65	0.71
CAT_{rbc}														0.76	0.66
CAT_{liv}															0.76

Except underlined, values are statistically significant ($p < 0.001$) coefficients of paired correlation which reflects moderate or strong relationship between parameters. Abbreviations used: P_{50}act - half-saturation oxygen pressure under real conditions of temperature, pH and pCO_2, P_{50}st - half-saturation oxygen pressure under standard conditions (37 °C, pH = 7.4, pCO_2 = 40 Torr); CD - conjugated diene content (ΔA_{233}/ml); SB - Schiff base content (units/ml); αT - α-tocopherol content (mM); CAT - catalase activity (10^4 units/mg of protein). Subscripts rbs, liv, kid, hea relate to erythrocytes, liver, kidney and heart, respectively.

Schiff bases ranged from +0.40 to +0.56, except for conjugated dienes in liver and kidneys.

Fig. 2 shows the changes in antioxidant system indices in red cells and tissues. In rats with normal HOA, α-tocopherol concentrations in red cells and tissues most significantly decreased after 4 hours of fever (by 6.0% in red cells, 16.5% in liver, 16.4% in kidneys, and 13.3% in heart). Catalase activity significantly fell in red cells (by 28.3%) and liver (by 12.3%). In rats with modified HOA these values rose (α-tocopherol of red cells, liver and kidneys - by 10.6, 30.5, and 49.7%, respectively; catalase of red cells, liver and kidneys - by 37.6, 6.1, and 8.9%, respectively).

Data about HOA and antioxidant system were treated by multiple regression analysis that had shown a moderate negative correlation between P_{50}act and parameters of antioxidant system (r from –0.36 to –0.60) (table II).

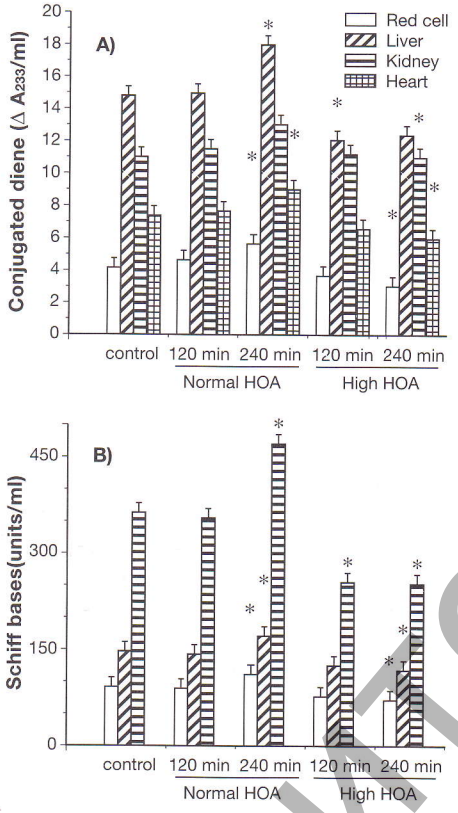

Figure 1. *Concentration of conjugated dienes (A) and Schiff bases (B) in red blood cells and tissues of rats with normal and increased hemoglobin-oxygen affinity after 120 or 240 min LPS administration.* Significant differences ($p < 0.05$) with control group are marked with asterisks (*).

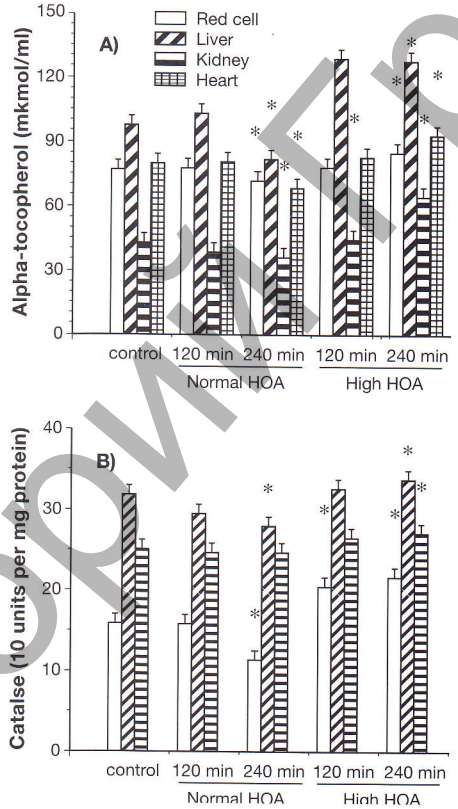

Figure 2. *Concentration of α-tocopherol (A) and activity of catalase (B) in red blood cells and tissues of rats with different hemoglobin-oxygen affinity after LPS administration.*
Mean values ± SE are shown; significant differences with control group are marked with asterisks (*).

Discussion

The present work reports the data concerning the relationship between HOA and the activity of free radical processes during LPS-induced fever. The experimental approach included a preliminary shift of ODC leftwards by sodium cyanate. A weakening of fever response and lowered LPO activity in rats with increased HOA was observed.

HOA is important for oxygen transport to tissues (19, 24). But oxygen transport is not the sole function of HOA, and this item deserves further investigations (12). Particularly, the physiological role of the HOA rise, or the shift of ODC leftwards, has not been sufficiently investigated. The shift of ODC may take part in a change of oxygen consumption and body temperature during the cooling of febrile, critically ill patients (14). The shift of ODC leftwards may deteriorate a tissue oxygen supply. In dogs with carbamylated hemoglobin (P_{50} = 23 comparing with 32 Torr in control) myocardial tissue extracted significantly less oxygen, despite insignificant differences in the convective oxygen transport (1). An increase of HOA during the marked hypoxia plays a favourable role, leading to the improvement of animal survival (5). In animals with increased HOA, oxygen delivery during system hypoxia was impaired less markedly than under normal HOA: oxygen flux through the capillary network fell by 13.3% under normal HOA and by 11.0% under increased HOA (23). According to these workers, these data indicated that an increased oxygen affinity did not provide an obvious advantage for oxygen transport during hypoxia at the level of the capillary network in resting striated muscle; however, such an advantage might become apparent in the presence of an increased metabolic rate or a more severe hypoxic challenge.

About 5% of O_2 delivered to tissue mitochondria is converted to reactive oxygen metabolites (15). Usually these metabolites are eliminated by different antioxidant systems. However under some conditions such as postischemic reperfusion their generation may greatly increase not being balanced by antioxidant defence mechanisms. The value of pO_2 can influence tissue generation of LPO products (17). The rate of H_2O_2 release by vascular endothelial cells rose 3-fold after increase of air oxygen content from 0 to 10% (10). Rate of microsomal LPO during tissue incubation elevated with oxygen content ranged from 3 to 20% (18). Rise of pO_2 enhanced tissue oxygen delivery and fraction of oxygen expended for generation of partly reduced species: O_2^-, H_2O_2, $^{\bullet}OH$.

In conditions of ineffective body use of oxygen its expended fraction in oxygenase reactions dramatically increases and thus, the body prooxidant-antioxidant balance becomes impaired. The elevation of oxygen flux to tissues under low HOA results in a rise of pO_2 which sometimes may cause a disbalance between different ways of body oxygen utilization, including a strengthening of one-electron transfer processes with a formation of such reactive intermediates as singlet oxygen, superoxide radical or hydroxyl radical. The synthesis of these intermediates initiates a chain oxidative reactions which result in the burst growth of lipid oxidative destruction. Limitation of oxygen flux to tissues due to increase of HOA may be more favourable in such conditions.

Rise of body temperature can result in tissue reoxygenation (16) and therefore, fever may be similar to reperfusion in this

respect. Therefore, a rise in HOA (by cyanate treatment) may diminish oxygen flux to tissues and thereby decrease the formation of LPO products and contribution of LPO in thermogenesis (9). Endothermic vertebrates have the antipyretic system; its action is mediated by different (central or peripheral) mechanisms, for example, non-shivering thermogenesis (11). HOA may also be one of such mechanisms, because in the present study its rise led to the decrease of febrile response.

In conclusion, the results of experiments with HOA modification during fever showed a close relationships between HOA and some indexes of lipid free radical oxidation, that allow to consider hemoglobin-oxygen affinity as a factor participating in the maintenance of body prooxidant-antioxidant balance and having an influence on the febrile response.

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V. ZINCHUK. *Hemoglobina de alta afinidad por el O_2 y peroxidación lipídica en situación de fiebre.* J. Physiol. Biochem., **55** (4), 301-308, 1999.

Se estudian los efectos de la hemoglobina de alta afinidad por el oxígeno sobre la temperatura rectal y la peroxidación lipídica en eritrocitos, corazón, hígado y riñón de rata macho en situación de fiebre. La fiebre se induce por inyección intraperitoneal de lipopolisacárido de *Salmonella typhi* (LPS; 5,0 mg Kg^{-1}). La afinidad de la hemoglobina por el O_2 (AHO) se aumenta por adición de cianato sódico 0,5 % al agua de bebida durante 8 semanas. La modificación de la afinidad (el valor real de la P_{50} disminuye a 23,3 ± 0,7 vs. 31,6 ± 0,7 Torr en el control; $p < 0,001$) debilita la respuesta febril: el aumento térmico tras 4 horas es de 0,79 ± 0,2 °C vs. 1,38 ± 0,1 °C en rata con AHO normal ($p < 0,05$). En eritrocitos y los tejidos estudiados de rata con AHO normal, las concentraciones de dienos conjugados y bases de Schiff aumentan durante la fiebre, mientras que el nivel de α-tocoferol y la actividad catalasa disminuyen. En las ratas con AHO aumentada se observa un patrón de cambios inverso. Las modificaciones de la temperatura rectal y de los marcadores de oxidación de radicales libres se correlacionan con un desplazamiento de la curva de disociación de la oxihemoglobina hacia la izquierda. Los resultados indican que la elevación de la afinidad de la hemoglobina por el O_2 puede disminuir la peroxidación lipídica, aumentar la capacidad antioxidante corporal durante la fiebre y disminuir la respuesta febril al LPS.

Palabras clave: Oxihemoglobina, Peroxidación lipídica, Fiebre, Lipopolisacárido, Cianato sódico.

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