# PHYSIOLOGY

# Prooxidant-Antioxidant State after Administration of Lipopolysaccharide during Correction of the L-Arginine-NO System and Affinity of Hemoglobin for Oxygen

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 1, pp. 40-43, January, 2001
Original article submitted April 20, 2000

We studied the effect of correction of the L-arginine-NO system on fever reaction and prooxidant-antioxidant state in rats with increased hemoglobin oxygen affinity caused by sodium cyanate after intramuscular injection of lipopolysaccharide. The imbalance between parameters of the prooxidant-antioxidant state in rats with increased hemoglobin oxygen affinity was least pronounced after lipopolysaccharide administration. Correction of the L-arginine-NO system in rats with increased hemoglobin oxygen affinity had no effect on the contents of Schiff bases and antioxidants after lipopolysaccharide administration.

**Key Words:** *hemoglobin oxygen affinity; lipid peroxidation; lipopolysaccharide; nitric oxide*

Molecular oxygen is necessary for the formation of its reactive intermediates and initiation and progression of lipid peroxidation (LPO) [1]. The decrease in intracellular oxygen concentration is the major mechanism of antioxidant protection [5]. The oxygen-binding capacity of the blood determines tissue $Po_2$ and plays an important role in the antioxidant system [5]. Since LPO is an oxygen-dependent process, it would be interesting to evaluate the role of oxygen transport functions of the blood and regulatory factors in the maintenance of prooxidant-antioxidant equilibrium. In our previous experiments, we studied correction of the L-arginine-NO system and hemoglobin oxygen affinity (HOA) during hyperthermia [3,4]. Here we evaluated the prooxidant-antioxidant state during correction of HOA and L-arginine-NO system in rats injected with lipopolysaccharide (LPS).

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## MATERIALS AND METHODS

Experiments were performed on male rats weighing 200-260 g and kept in a vivarium. The animals were divided into 7 groups. Group 5-7 rats were given water solutions of 0.5% sodium cyanate for 8 weeks to increase HOA. HOA was measured in some animals. Group 1 rats were intraperitoneally injected with 10 ml/kg isotonic NaCl. Group 3 and 5 rats were intraperitoneally injected with 30 mg/kg nitroglycerin (NG, Isis-Chemie). Group 4 and 7 rats received daily intraperitoneal injections of 25 mg/kg $N^G$-nitro-L-arginine methyl ester (L-NAME, Sigma) for 3 days. Group 2, 5, 6, and 7 rats were intramuscularly injected with 0.1 mg/kg *Salmonella typhi* LPS (N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences). Fever reaction was evaluated by the rise of rectal temperature. Blood from the right atrium and liver, kidney, and heart specimens were taken 120 min after administration of these substances.

The parameter of HOA $P_{50}$ ($Po_2$ corresponding to 50% oxygen saturation of hemoglobin) was measured

0007-4888/01/1311-0029$25.00 

by the mix method with modifications [2] at 37°C, pH 7.4, and $Pco_2$ 40 mm Hg. $P_{50}$ corresponding to actual pH, $Pco_2$, and temperature ($p50_a$) was calculated by formulas described elsewhere [10]. $Po_2$, $Pco_2$, and pH in blood samples (0.13 ml) were measured at 37°C on an ABL-330 gas analyzer (Radiometer). The actual base excess and plasma concentrations of total $CO_2$ and carbohydrates ($HCO_3^-$) were estimated by Siggaaard-Andersen nomographs.

The content of Schiff bases was determined by fluorescence of the chloroform extract on an F-4010 spectrofluorometer (Hitachi) at excitation and emission wavelengths of 344 and 440 nm, respectively [8]. Catalase activity was determined by the amount of $H_2O_2$ interacting with molybdenum salts with the formation of colored complexes (410 nm) on a SF-46 spectrophotometer [6]. The content of α-tocopherol was estimated by fluorescence of heptane extract on an F-4010 spectrofluorometer (Hitachi) at excitation wavelength of 292 nm and emission wavelength of 325 nm [8]. The results were analyzed using Statgraphics software.

## RESULTS

LPS administration during correction of HOA and L-arginine-NO system modified oxygen transport functions of the blood, LPO, antioxidant content, and fever response in rats. The maximum rise of temperature (by 1.60±0.05°C) was observed in animals receiving only LPS. Fever response to LPS was least pronounced in rats with increased HOA and did not differ from that in animals injected with NG or L-NAME.

LPS caused moderate metabolic acidosis (Table 1) and deteriorated oxygen supply to tissues. The LPS-induced impairment of oxygen transport functions and acid-base equilibrium was least pronounced in rats with increased HOA and after correction of the L-arginine-NO system. In rats injected with sodium cyanate and LPS, $P_{50}$ was much lower than in controls ($p<0.001$, Table 1), which contributed to a leftward shift in the oxyhemoglobin dissociation curve (Fig. 1). $P_{50}$ values in rats with increased HOA and injected with modulators of the L-arginine-NO system, were similar at the standard and actual pH, $Pco_2$, and temperature. In these animals, LPS only slightly changed $P_{50}$. Methemoglobin content was minimum in rats injected with NG and LPS ($p<0.05$ compared to the control, Table 1).

LPS markedly activated LPO, which was determined by the content of Schiff bases (Table 2). LPO intensification was least pronounced in animals with increased HOA. The content of Schiff bases in erythrocytes increased by 250, 159, 142, and 154% in rats injected with LPS, displaying increased HOA, pretreated with L-NAME, and receiving NG, respectively. Thus, correction of the L-arginine-NO system had no effect on the content of Schiff bases in rats treated with LPS. LPS inhibited the antioxidant system in rats with increased HOA (Table 2). Catalase activity and α-tocopherol content in erythrocytes, kidney, liver, and heart decreased after LPS administration, but remained unchanged after injection of the NO synthase inhibitor or NO donor to rats with increased HOA. The antioxidant system and LPO in tissues of rats with increased HOA receiving only NG or L-NAME did not differ from the control.

**TABLE 1.** Oxygen Transport Functions of the Blood in Rats Injected with LPS during Simultaneous Correction of HOA and L-Arginine-NO System (*M*±*m*)

| Parameter | | Control (*n*=6) | LPS (*n*=7) | NG (*n*=5) | L-NAME (*n*=5) | Increased HOA | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | LPS (*n*=5) | LPS+NG (*n*=6) | LPS+ L-NAME (*n*=6) |
| pH | standard | 7.312±0.006 | 7.220±0.033* | 7.299±0.008 | 7.325±0.015 | 7.269±0.013* | 7.275±0.013* | 7.266±0.013* |
| | actual | 7.294±0.006 | 7.191±0.033* | 7.298±0.006 | 7.309±0.014 | 7.254±0.015* | 7.256±0.110* | 7.242±0.014* |
| $Pco_2$, mm Hg | standard | 50.33±1.84 | 51.89±1.47 | 50.17±0.61 | 50.88±1.89 | 52.82±0.80 | 52.03±2.64 | 51.6±2.06 |
| | actual | 51.45±1.87 | 56.53±1.59 | 50.39±0.74 | 51.83±1.61 | 55.09±0.85 | 55.15±2.73 | 53.87±2.11 |
| $Po_2$ | standard | 28.30±1.21 | 23.71±1.32* | 27.37±0.55 | 27.77±1.17 | 24.18±0.73 | 24.10±0.40* | 25.76±0.41 |
| | actual | 29.37±1.43 | 27.28±1.55 | 27.57±0.72 | 28.61±1.12 | 26.20±1.05 | 26.50±0.11 | 27.61±0.65 |
| Methemoglobin, % | | 0.76±0.18 | 2.41±0.08 | 3.78±0.37*+ | 0.74±0.12 | 3.71±0.54*+ | 6.30±0.48*+ | 1.78±0.22 |
| $HCO_3^-$, mol/liter | | 22.32±1.23 | 24.27±0.71 | 24.37±1.04 | 25.14±1.17 | 25.14±1.17 | 24.42±1.27 | 23.37±1.44 |
| Base excess, mmol/liter | | -3.08±0.87 | -2.86±1.12 | -1.53±0.66 | -0.63±0.55 | -0.90±0.55 | -2.10±0.91 | -2.84±0.48 |

**Note.** Here and in Table 2: $p<0.05$: *compared to the control, +compared to LPS.

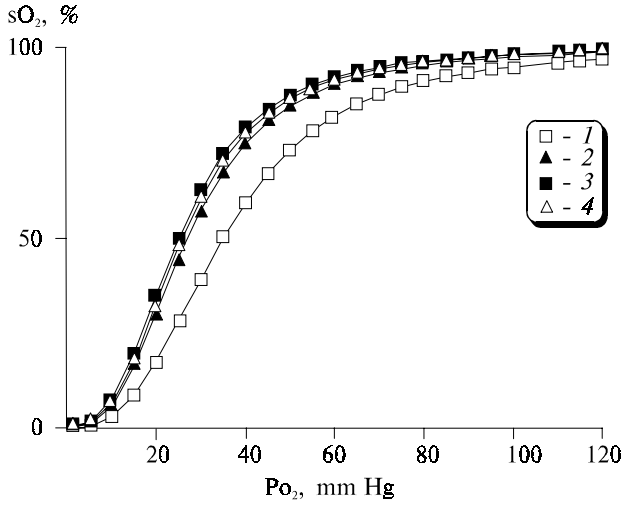


**Fig. 1.** Oxyhemoglobin dissociation curve at actual pH, $Pco_2$, and temperature in rats with normal (*1*) and increased hemoglobin oxygen affinity (*2-4*) injected with 0.9% NaCl (*1*), lipopolysaccharide (*3*), and lipopolysaccharide+L-NAME (*4*).

Our previous studies showed that the shift in the oxyhemoglobin dissociation curve under conditions of LPS administration, increased HOA, or NO synthase inhibition correlates with LPO parameters [3,13]. Therefore, HOA is an important physiological factor maintaining the prooxidant-antioxidant equilibrium. In our experiments, attenuation of LPS-induced fever reaction and LPO was not more pronounced in rats with increased HOA after administration of the NO synthase inhibitor or NO donor. The leftward shift in the oxyhemoglobin dissociation curve should be analyzed from the viewpoint of the oxygen-dependent nature of LPO. It is accompanied by functional imbalance between oxygen consumption and demands, alteration of proton pumps, inhibition of mitochondrial respiration, conformational changes in membrane-bound protein-enzyme complexes, low energy production, accumulation of partially oxidized metabolites, and destructive processes at the cellular and molecular levels.

Hemoglobin plays an important role in NO elimination from the body. In the arterial blood, NO is inactivated in the reaction with oxyhemoglobin yielding nitrate and methemoglobin, while in the venous blood NO is transformed into nitrosohemoglobin [9]. Its concentration in the arterial and mixed venous blood of normoxic and hypoxic sheep inhaling NO depends on $O_2$ and NO levels. There is a negative correlation between NO content and blood $O_2$ saturation [12]. NO can enhance HOA by interacting with hemic groups with the formation of nitrosohemoglobin and methemoglobin or by changing blood viscosity [9]. Nitrosohemoglobin modulates the tone of microvessels and probably serves as the source of NO [11]. Spectral characteristics of peroxynitrite-treated hemoglobin suggest the existence of another hemoglobin oxidation

**TABLE 2.** Antioxidant System in Rats Injected with LPS during Simultaneous Correction of HOA and L-Arginine-NO System (*M*±*m*)

| Parameter | Control (*n*=6) | LPS (*n*=7) | NG (*n*=5) | L-NAME (*n*=5) | Increased HOA | | |
|---|---|---|---|---|---|---|---|
| | | | | | LPS (*n*=5) | LPS+NG (*n*=6) | LPS+ L-NAME (*n*=6) |
| Catalase, U/mg protein | | | | | | | |
| erythrocytes | 579.9±30.4 | 226.9±4.3* | 503.4±9.9 | 422.6±34.7 | 300.7±9.7*+ | 306.6±16.6*+ | 311.6±5.6*+ |
| kidneys | 216.3±2.8 | 141.6±3.4* | 194.6±5.7* | 210.9±2.1 | 173.7±5.1*+ | 170.5±5.2*+ | 165.9±2.1*+ |
| liver | 406.2±9.9 | 314.1±5.4* | 424.6±16.5 | 377.5±6.9 | 342.6±9.9*+ | 358.5±11.8*+ | 355.9±6.3*+ |
| heart | 36.5±0.2 | 21.7±0.4* | 35.9±1.4* | 34.4±2.7 | 26.6±1.2*+ | 26.4±0.9*+ | 26.5±1.4*+ |
| α-Tocopherol, μmol/ml | | | | | | | |
| erythrocytes | 36.9±1.5 | 20.3±0.3* | 34.9±2.1 | 32.3±1.12 | 26.2±1.4*+ | 26.9±1.3*+ | 28.6±0.9*+ |
| kidneys | 80.9±1.0 | 61.2±0.4* | 79.1±1.1 | 79.2±1.9 | 71.7±1.5*+ | 70.2±1.8*+ | 74.4±1.8*+ |
| liver | 78.5±2.1 | 63.4±0.4* | 74.5±1.3* | 73.3±1.8 | 71.4±1.5*+ | 69.6±3.6*+ | 70.5±1.2*+ |
| heart | 80.6±2.2 | 62.5±0.6* | 80.4±1.5 | 79.1±1.5 | 70.4±1.9*+ | 69.2±2.6*+ | 69.1±1.5*+ |
| Schiff base concentration, fluorescence units | | | | | | | |
| erythrocytes | 9.97±2.64 | 36.80±6.17* | 10.31±1.05 | 14.19±2.32 | 30.37±3.35* | 24.88±1.14 | 21.19±2.95 |
| kidneys | 55.4±7.5 | 102.90±5.08* | 62.07±4.44 | 59.30±3.68 | 73.14±5.61* | 75.03±4.68 | 83.07±4.21* |
| liver | 159.28±13.75 | 321.31±6.91* | 157.20±3.95 | 153.10±12.97 | 235.70±11.84* | 243.87±6.97 | 218.25±9.16* |
| heart | 81.87±6.72 | 136.01±5.61* | 78.9±6.3 | 69.93±5.06 | 110.02±6.48* | 112.03±6.51 | 116.40±5.33* |

pathway in biological systems [7]. This pathway is important for modification of functional properties of hemoglobin, its involvement in the formation of $O_2$ influx into tissues, and maintenance of the prooxidant-antioxidant equilibrium in the body. The protective effect of simultaneous correction of HOA and L-arginine-NO system did not surpassed those observed after their individual correction [13], which probably reflects exhaustion of adaptive antioxidant reserves realized via oxygen-binding properties of hemoglobin.

These data indicate that the L-arginine-NO system and oxygen-binding capacity of the blood maintain the prooxidant-antioxidant equilibrium and enhance antioxidant activity by the same mechanisms.

This study was supported by the Russian Foundation for Basic Research (grant No. 1993292).

## REFERENCES

1. M. V. Bilenko, *Ischemic and Reperfusion Damages to Organs* [in Russian], Moscow (1989).
2. M. V. Borisyuk, M. A. Dobrodei, I. K. Dremza, and V. V. Zinchuk, *Methods for Studying Mass Transfer in Microcirculation* [in Russian], Novosibirsk (1991), pp. 156-162.
3. V. V. Zinchuk and M. V. Borisyuk, *Byull. Eksp. Biol. Med.*, **127**, No. 6, 616-619 (1999).
4. V. V. Zinchuk and M. V. Borisyuk, *Usp. Fiziol. Nauk*, **30**, No. 3, 38-48 (1999).
5. V. P. Skulachev, *Mol. Biol.*, **29**, No. 6, 1199-1209 (1995).
6. O. I. Aruoma and S. L. Cuppett, *Antioxidant Methodology: in Vivo and in Vitro Concepts*, New York (1997).
7. M. Minetti, G. Scorza, D. Pietraforte, *Biochemistry*, **38**, No. 7, 2078-2087 (1999).
8. C. A. Rice-Evans, A. T. Diplock, and M. C. R. Symons, *Laboratory Techniques in Biochemistry and Molecular Biology: Techniques in Free Radical Research*, London (1991).
9. A. Sakinis and A. Wennmalm, *Biochem. J.*, **330**, No. 1, 527-532 (1998).
10. J. W. Severinghaus, *J. Appl. Physiol.*, **21**, No. 5, 1108-1116 (1966).
11. J. S. Stamler, L. Jia, J. P. Eu, *et al.*, *Science*, **276**, 2034-2766 (1997).
12. Y. Takahashi, H. Kobayashi, N. Tanaka, *et al.*, *Am. J. Physiol.*, **43**, No. 1, H349-H357 (1998).
13. V. Zinchuk, *Respiration*, **66**, No. 5, 448-454 (1999).