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Fe^{2+}-INITIATED CHEMILUMINESCENCE IN RATS WITH HIGH HEMOGLOBIN-OXYGEN AFFINITY DURING FEVER

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The effect of high hemoglobin-oxygen affinity (HOA) on chemiluminescence initiated by Fe^{2+} was studied in rat plasma and red cell ghosts during fever. The high HOA was induced by daily ingestion of sodium cyanate with drinking water for 8 weeks. Rats with high or normal HOA received i.p. lipopolysaccharide *Salmonella typhi* (LPS). The half-saturation oxygen pressure (p50) after 240 min of fever was 23.3 ± 0.7 in cyanate-treated rats comparing with 30.4 ± 0.4 Torr in the group received placebo. The maximal value of initiated chemiluminescence rose in plasma and red cell ghosts of rats with normal HOA by 26.5 and 27.5%, respectively, and in rats with modified HOA by 17.1 and 23.8%, respectively. The antioxidant activity of plasma and red cell ghosts decreased under high HOA to a less extent. These investigations show that the lowering of oxygen flux to tissues due to hemoglobin carbamylation may decrease the production of free radicals in rats during fever.

Key words: *chemiluminescence, hemoglobin-oxygen affinity, sodium cyanate, fever.*

INTRODUCTION

Administration of gram-negative bacterial lipopolysaccharide (LPS) to mammals leads to development of fever. Such condition is known to be accompanied by substantial changes in energy metabolism and heat production: with a rise of temperature by 1 °C metabolism accelerated by 10% or more (1). Several investigators had shown that fever changed the oxygen consumption and the mechanisms of its blood transport and led to uncoupling of oxidative phosphorilation with tissue respiration (2, 3). The previous investigation (4) has shown the increase of hemoglobin-oxygen affinity (HOA) during fever that correlated with the increased activity of lipid peroxidation (LPO). The shift of oxyhemoglobin dissociation curve (ODC) position leftwards may substantially influence the body tolerance to hypoxia or heat treatment (5—7). The present work was aimed to investigate the HOA effect

during LPS-induced fever on the activity of initiated chemiluminescence, that is known to be one of the most reliable and sensitive markers of free radical activities (8).

MATERIAL AND METHODS

The experiments were performed on 48 male rats (body weight 200—240 g). The animals were maintained under thermoneutral conditions (26 ± 1.5°C) and fed ad libitum. The rats were pre-adapted to the conditions of the experiment for avoiding a stress. Fever was induced by intraperitoneal administration of *Salmonella typhi* LPS (5.0 μg kg^{-1}). The fever response was monitored for 240 min by an electric thermometer with a sensor in the rectum at a depth of 5 cm. HOA was increased by including of 0.5% water solution of sodium cyanate in the diet for eight weeks (9); control animals received saline. Blood sampling was performed from the right atrium cavity to a heparinized (50 IU ml^{-1}) syringe under ether anesthesia.

Blood gas parameters were measured immediately after LPS administration by micro gas analyzer (ABL 330, Radiometer). HOA was assessed by p50 (blood oxygen pressure (pO_2) under its 50% saturation by O_2) determined with a 'mixing method' (10) at 37°C, pH 7.4 and $pCO_2 = 40$ Torr ($p50_{st}$). p50 at actual pH, pCO_2 and temperature ($p50_{act}$) were calculated from $p50_{st}$ by Severinghaus' equations (11) and with a temperature coefficient $\Delta/gp50/\Delta T = 0.024$ (12). ODC were calculated from measured p50 Hill's equation with $n = 2.8$.

Another blood portion was centrifuged, and plasma and red blood cells were separated; red cell fraction was disintegrated by ultrasound to receive red cell membranes (13). Plasma and red blood cell ghosts were frozen and kept at −20°C. Chemiluminescence initiated by ferrous salts (final concentration of $FeSO_4$ in cuvette 0.5 mM, volume of sample 0.1 ml; red cell ghosts were diluted 1:10 by Tris buffer, pH 7.4) was determined by the amplitude of 'rapid' burst with biochemiluminometer BKL-06 (8). The antioxidant activity (AOA) was measured by the ratio of a difference between 'rapid' burst amplitudes in the control (with addition of Tris buffer, pH 7.4) and the experiment (with addition of plasma or red cell ghosts) to its amplitude in the control (8).

Rats of group I were used as controls. In group II rats were treated by LPS, and blood sampling was performed after 240 min of fever. In rats of group III HOA was preliminary increased by the abovementioned method, and then LPS was injected, and 240 min later HOA and chemilumenescence values were analyzed. The data were statistically evaluated by Student's t-test with significance level at $p < 0.05$. The results are presented as means ± SE. The analyses and graphs were performed using computer software packages (version 2.1, Statgraphics; version 4.0, Quatropro).

RESULTS

Fig. 1. shows the data on rectal temperature changes in the rats of groups II and III. In cyanate-treated animals the fever response was less prominent; 180 min after LPS administration their rise of rectal temperature was 0.79 ± 0.2°C ($p < 0.01$) comparing with 1.38 ± 0.1°C in rats fed by a standard diet.

The value of $p50_{st}$ decreased during fever from 34.5 ± 0.6 (control) to 32.7 ± 0.6 Torr ($p < 0.05$). In the rats with modified HOA $p50_{st}$ was equal

to 24.8 ± 0.7 Torr ($p < 0.01$). $p50_{act}$ in the animals of these groups (I, II, III) were equal to 31.6 ± 0.7, 30.4 ± 0.4 ($p < 0.05$), and 23.3 ± 0.7 ($p < 0.001$) Torr, respectively. The ODC had a substantial shift leftwards in rats fed with cyanate and much lesser shift leftwards in the intact animals that received only LPS (*Fig. 2*).

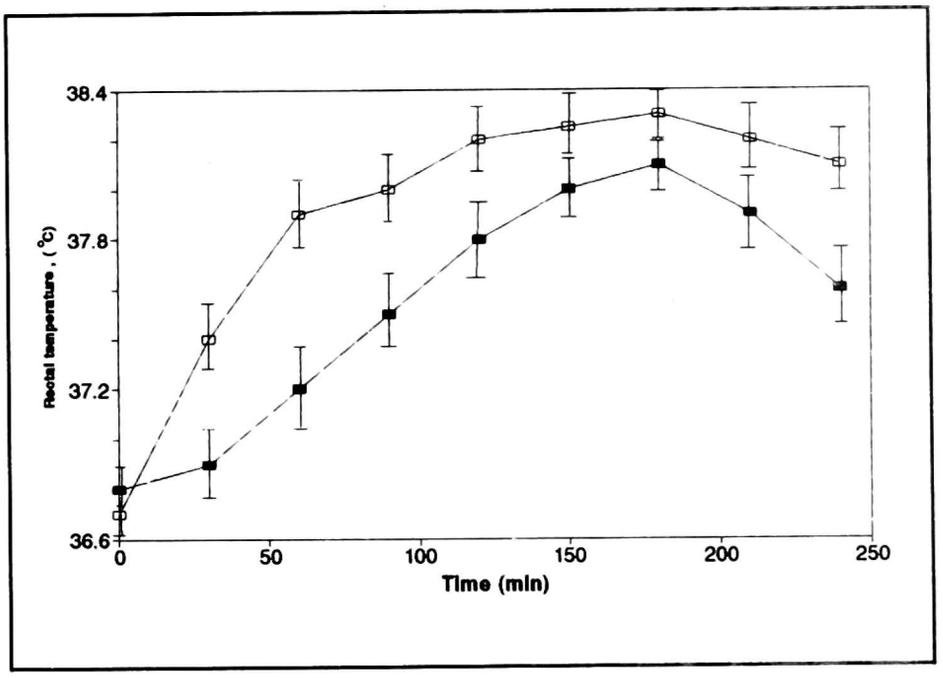

Fig. 1. Mean change in rectal temperature after intraperitoneal injection of LPS in rats with normal (open square) and increased (filled square) HOA.

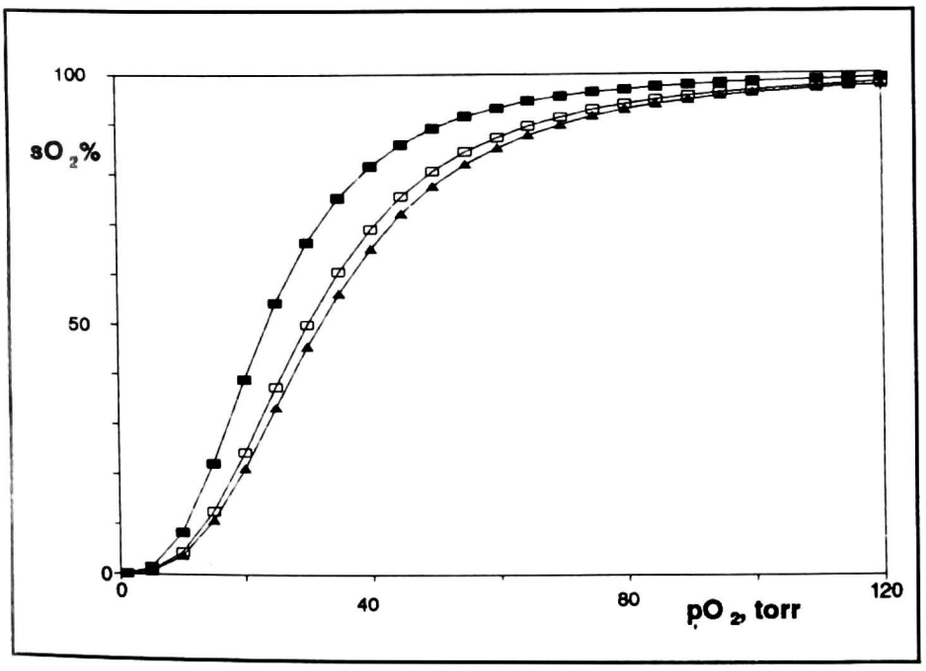

Fig. 2. Oxyhemoglobin dissociation curves in intact rats (triangle), and during LPS administration to rats with normal (open square) and increased (filled square) HOA.

Fig. 3. shows the main parameters of chemiluminescence in plasma and red cell ghosts from animals of these groups. During fever the maximal value of initiated chemiluminescence increased in plasma and red cell ghosts of rats with normal HOA by 26.5 and 27.5%, respectively, and in animals with high HOA by 17.1 and 23.8%, respectively. Plasma AOA decreased during fever from 28.62 ± 1.78 to $22.32 \pm 0.83\%$ ($p < 0.01$), and AOA of red cell ghosts changed from 42.53 ± 3.08 to $31.95 \pm 2.49\%$ ($p < 0.05$). In rats with modified HOA these changes during fever were even larger (to $23.43 \pm 1.59\%$ ($p < 0.05$) in plasma and $33.98 \pm 1.84\%$ ($p < 0.05$) in ghosts).

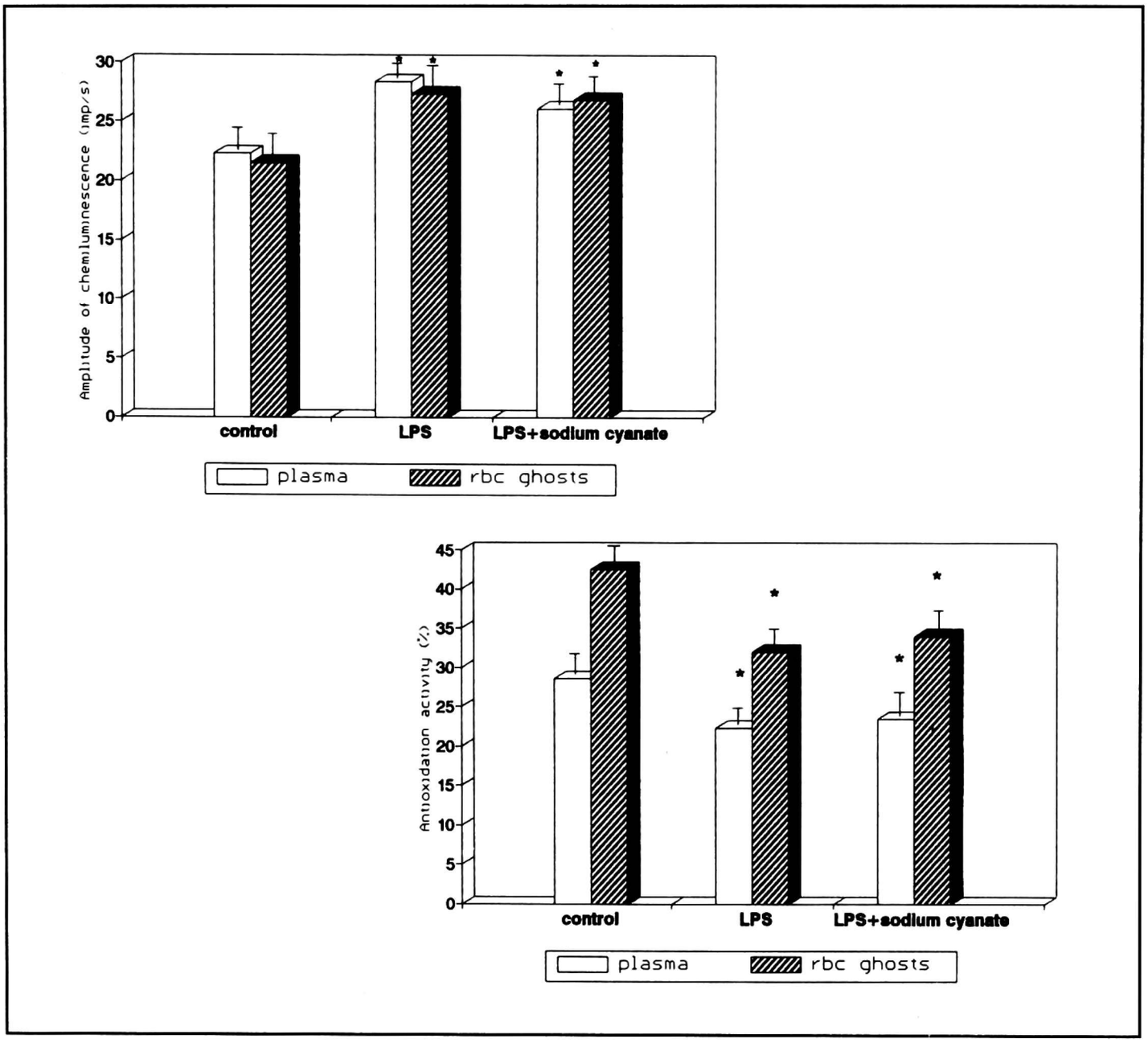

Fig. 3. Maximal amplitude of chemiluminescence induced by Fe^{2+} (A) and antioxidant activity (B) of rat plasma and red blood cell ghosts during fever.

DISCUSSION

The present study investigated the effects of HOA on the activity of free radical processes during fever. For such purposes ODC was preliminary shifted leftwards. The activity of LPO processes was evaluated using the measurement of initiated chemiluminescence and determination of antioxidant activity in plasma and red cells. Rats with high HOA had weakened fever response and diminished LPO activity.

The shift of ODC rightwards was considered traditionally as favourable for tissue oxygen supply and thus for the body as a whole, and the shift leftwards seemed to be negative (14). Administration of inositolhexaphosphate to piglets increased p50 by 20% and oxygen consumption — from 4.3 to 6.02 ml min^{-1}

(15). In rats the increase of p50 by 25% led to an elevation of tissue pO_2, in average, by 78% (16). The left shift of ODC due to carbon monoxide binding (level of carboxyhemoglobin = 12%) decreased the oxygen transport to tissues from 70 to 52%, CO breathing for 60 min lowered p50 by 22% and in combination with low perfusion decreased a tissue oxygen delivery by 38% from the basal level (17). The perfusion of isolated rabbit heart by hemoglobin solution with p50 = 12 Torr decreased the oxygen consumption by 36% comparing with p50 = 30 Torr (18). Tissue oxygen supply during heart surgery at p50 = 20.3 Torr was diminished by 30% comparing with p50 = 31.1 Torr (19).

However meaning of ODC shift remains unclear in many aspects (20). Eaton (5) had shown that animals with higher HOA were more resistant to severe hypoxia. In hamsters with high HOA (p50 = 15.7 Torr) substantial hemodilution (lowering of hematocrit by 40%) and hypoxic ventilation resulted in milder worsening of oxygen delivery than in hamsters with normal HOA (p50 = 26.1 Torr) (6). The same phenomenon was observed during systemic hypoxia that decreased oxygen flux through capillary network by 13.3% under normal HOA and by 11.0% under high HOA (21). These workers believed that higher HOA was not obviously advantageous for oxygen transport in the capillary network of striated muscles but such advantage might become clear under increased metabolic rate or more severe hypoxia. The perfusion of isolated canine m.gracilis by blood with high oxygen affinity under submaximal working loads resulted in a lower tissue oxygen supply comparing with normal blood, and enhanced blood flow did not compensate this (22). Gonzales *et al.* (23) had shown that under high altitude hypoxia (barometric pressure 380 Torr) increase of HOA caused by bicarbonate improved oxygen loading in rat lungs without worsening of its unloading in tissues, and this had been favourable for hypoxic exercises. In rats with high HOA the heat resistance during environmental hyperthermia increased by 24% (7).

We believe that the shift of HOA may change the levels of LPO products (4). The increase of blood oxygen content can undoubtedly result in activation of non-enzymatic LPO reactions that is known to occur in the body always (at least, at a low speed) because of the presence of LPO activators. Thus, the value of pO_2 may influence on tissue generation of LPO products. In vascular endotelial cells the increase of oxygen fraction in air from 0 to 10% resulted in acceleration of H_2O_2 release by a factor of 3 (24). The LPO velocity in microsomes during tissue incubation increased with oxygen fraction (the latter being from 3 to 20%; (25)). This was due to the influence of oxygen on the production of lipid and lipid dioxyl radicals not only during propagation reactions but also during chain breaks (26). The higher pO2 increased total tissue oxygen delivery and its fraction used for generation of partly reduced forms: superoxide, hydrogen peroxide or hydroxyl radicals (27). Under the

conditions of ineffective body utilization of oxygen the fraction of oxygen incomplete reduction dramatically grows thus worsening the body prooxidant-antioxidant balance. The limitation of oxygen flux to tissues due to increased HOA may possibly be more favourable. The increase of HOA lowers blood O_2 content and therefore creates a possibility for weakening of LPO processes in conditions of disbalanced oxygen utilization. Many of the physiological protective mechanisms for temperature homeostasis are energy-dependent, but there are also some mechanisms that decrease body oxygen demands (28). The ODC shift leftwards may take part in lowering of energy metabolism in fever patients with artificial hypothermia (29). During fever, HOA modification decreases the oxygen flux to tissues and hence results in antioxidant action. This mechanism allows weakening of chemical thermoregulation, and we consider it as a part of compensatory adaptive body responses for temperature maintenance during fever.

In conclusion, the results of the present investigation support a hypothesis about the important role of HOA in the modulation of LPO activity during fever. Therefore, HOA correction may be used for the maintenance of temperature homeostasis under such condition.

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