Blood Oxygen Transport in Rats under Hypothermia Combined with Modification of the l-Arginine–NO Pathway

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Nitric oxide (NO) has high affinity to heme and by interaction with oxyhemoglobin (HbO₂) is converted into nitrate to form methemoglobin (MetHb) as a side product. In combining with deoxy-Hb NO yields a stable molecule of nitrosyl-hemoglobin (HbFe²⁺NO) that can further be converted into nitrate and hemoglobin (Hb). In addition, Hb was shown to transport NO in a form of S-nitrosohemoglobin (SNO-Hb). These features of the Hb and NO interaction are important for blood oxygen transport including hemoglobin-oxygen affinity (HOA). The present investigation was aimed to study the blood oxygen transport indices (pO₂, pCO₂, pH, HOA, etc.) in rats under hypothermia combined with a modification of the l-arginine–NO pathway. To modify the l-arginine–NO pathway, rats were administered with N⁶-nitro-l-arginine methyl ester (l-NAME), l-arginine, or sodium nitroprusside (SNP) intravenously before cooling. A substantial impairment of oxygen delivery and development of hypoxia, with an important contribution of HOA into the latter accompanied the deep hypothermia in rats. All the experimental groups developed metabolic acidosis, less pronounced in rats treated with l-arginine only. In the experiments with a modification of the l-arginine–NO pathway, an enhanced cold resistance, attenuated oxygen deficiency, and a weaker oxyhemoglobin dissociation curve (ODC) shift leftwards were observed only after the administration of l-arginine. Neither SNP nor l-NAME had not any protective effects.

L-Arginine lowered the value of standard P₅₀ (pO₂, corresponding to 50% Hb saturation with oxygen at 37°C, pH 7.4, and pCO₂ = 40 mmHg). The actual P₅₀ (at actual pH, pCO₂ and temperature) decreased by approximately 15 mmHg and was significantly higher than that under hypothermia without the drug treatment (21.03 ± 0.35 vs 17.45 ± 0.60 mmHg). NO also can contribute to this system through different mechanisms (HOA modification, vascular tone regulation, peroxynitrite formation, and effects).

Key Words: hemoglobin-oxygen affinity; blood; nitric oxide; NO donors; inhibitors of NO synthase; hypothermia; rat.

Nitric oxide has emerged as an important messenger molecule that displays multiple physiologic and pathophysiologic role (1). NO production is catalyzed by the enzyme NO-synthase (NOS) during the conversion of l-arginine to l-citrulline. NO is known to participate in body oxygen delivery (vascular tone regulation, interaction with Hb) and maintenance of thermal balance (2, 3). Electron transfer in nitration and nitrosylation reactions accompanies interactions of NO with its molecular targets in vivo; this mechanism considerably differs from the previously known ways of neurotransmitter and hormone effects (4). NO has high affinity to heme and is converted to a nitrate ion by a reaction with oxyhemoglobin to form MetHb as the side product. NO also generates a stable molecule of HbFe²⁺NO in combi-
ing with deoxy-Hb; then HbFe^+NO is converted into nitrate and MetHb (5). In addition, Hb was shown to transport NO in a form of SNO-Hb from lungs to peripheral tissues, suggesting an involvement of this cycle in regulation of vascular pressure and effective oxygen (O₂) delivery in the capillary circulation both in adult and fetal animals (6). These features of the Hb with NO interaction are important for the determinants of blood oxygen transport including HOA (7). Hypothermia alters the activity of L-arginine–NO pathway, determined by the amount of the final NO metabolic products NO₂ and NO₃ (8). However, many aspects of the NO influence on the blood oxygen transport under these conditions are unclear. The present study was aimed to investigate the blood oxygen transport indices in rats under hypothermia combined with a modification of the L-arginine–NO pathway.

MATERIALS AND METHODS

The experiments were carried out in 58 male laboratory rats (body weight 230-270 g) maintained in a vivarium under standard conditions. Ninety-minute hypothermia was induced by cooling of anesthetized rats (nembutal, 50 mg/kg, i.p.) in a special box with circulating water (17°C). The L-arginine–NO pathway was modified by N⁵-nitro-L-arginine methylester (L-NAME, Sigma, St. Louis, MO), L-arginine (Sigma), or sodium nitroprusside (SNP, Merck). The rats were subdivided into six groups. Group 1 (control) received 1 ml 0.9% NaCl i.v. The animals of the other groups were exposed to cold after the preliminary i.v. administration of the following drugs in 1 ml of saline: group 2, none; group 3, L-NAME (30 mg/kg); group 4, SNP (40 µg/kg/min, 10 min); group 5, L-arginine (300 mg/kg) and L-NAME (30 mg/kg); group 6, only L-arginine (300 mg/kg). The rectal temperature was measured by an electric thermometer. Blood was taken from the right atrium on the 90th min after the onset of cold treatment under anesthesia.

PO₂ and acid–base balance: plasma concentration of hydrocarbontes (HCO₃⁻), the concentration of total carbon dioxide (TCO₂), the actual excess of buffer bases (ABE), the standard excess of buffer bases (SBE), and the standard hydrocarbonate (SBC) were calculated by a program based on the formulas of Severinghaus (9) and the nomograms of Siggaard-Andersen were measured with a micro gas analyzer (ABL-330, Radiometer) at 37°C and then were adjusted to the actual temperature value. HOA was assessed by P₅₀ (blood pO₂ under its 50% saturation by O₂) as determined by a "mixing method" (10) at 37°C, pH 7.4, and pCO₂ = 40 mmHg (P₅₀ stand). The values of P₅₀ at actual pH, pCO₂ and temperature (P₅₀ act) were calculated from P₅₀ stand by Severinghaus's equations (9) and with the temperature coefficient ΔlgP₅₀/ΔT = 0.024; the ODCs were calculated according to Hill's equation with n = 2.8 from the measured P₅₀. The amounts of Hb and MetHb were determined spectrophotometrically.

The data were statistically evaluated by Student's t test with a significance level of P < 0.05. The results are presented as means ± SE. The analyses and graphs were performed using computer software packages.

RESULTS

Figure 1 shows the changes in rat rectal temperature after 90 min of cooling. In group 2 receiving 0.9% NaCl before the cooling the rectal temperature...
The value of $P_{50}$ act (under actual pH, $pCO_2$, and temperature) was significantly decreased in group 2 (0.9% NaCl before cooling) nearly by 15 mmHg: from 35.4 ± 0.94 to 17.5 ± 0.60 mmHg. In l-arginine-treated rats this lowering of $P_{50}$ act was less pronounced (21.03 ± 0.35 mmHg).
P < 0.001) in comparison with group 2 and resulted in a less distinct ODC shift leftwards (Fig. 2). The amount of MetHb (the final product of NO elimination from body) increased by 251.3% in hypothermic rats. L-NAME blunted this increase to near control MetHb level. L-arginine and SNP led to a substantial rise of MetHb by 55 and 26%, respectively.

DISCUSSION

Our results showed that hypothermia (lowering of rat body temperature by more than 15°C) was accompanied by a considerable impairment of oxygen delivery and development of hypoxia with involvement of HOA. In the experiments with a modification of the l-arginine–NO pathway enhanced cold resistance, attenuated oxygen deficiency and weaker ODC shift leftwards were observed only after the l-arginine administration. The injections of SNP or l-NAME did not improve the defense in comparison with the rats pretreated with 0.9% NaCl before the cooling.

Hb plays a very important role in the elimination of NO from the body. In arterial blood NO is inactivated in a reaction with HbO₂ to yield nitrate and MetHb. In addition to these products, HbFe²⁺NO is also generated in venous blood (5); under high pO₂ it may be disintegrated by O₂ to Hb and NO₃. There are also other physiologic mechanisms for inactivation of circulating NO. NO generated by inducible NOS in red blood cells, was shown to convert their Hb into SNO-Hb (11). The balance between SNO-Hb and HbFe²⁺NO depends upon O₂. Fe²⁺-heme is a target for NO in the absence of low molecular weight thiols, such as cysteine. But in the presence of Cys the first stage is a production of Cys-SNO, which is followed by a transfer of the NO moiety to the specific residue Cys-93 of β-globin (12). The nitrosothiols formed in the vascular network from NO-mediated thiol nitrosation play an important role in NO transport, storage and metabolism (13). These different species of HbFe²⁺NO compounds can change HOA differently. NO can modify HOA by the following mechanisms: conformational Hb transition (from the R- to the T-structure); rise of the MetHb level in red cells; generation of supplementary Hb oxidation products; and formation of nitrosothiols (14). MetHb and SNO-Hb increase HOA, whereas HbFe²⁺NO decreases it (12). The high doses of glyceroltrinitrate (NO donor) induce HbFe²⁺NO production, correlating with the P₅₀ and the respective shift of ODC rightwards (15). However, this modulation of blood oxygen-binding properties is expressed only at high concentrations of glyceroltrinitrate (5% or more), but it can be important for the gas exchange processes in capillaries.

The relatively stable vasoactive compounds may serve as a system for NO storage. Hb can perform the depot function in the microcirculatory network (16). The capillary processes of SNO-oxy-Hb deoxygenation induce an allosteric transition of Hb (from R- to T-state), thereby initiating a release of NO (17). SNO-Hb is a vasodilator with the activity being a subject of allosteric modulation by O₂. Its oxygenated structure promotes vasoconstriction, and the deoxygenated structure facilitates a vasorelaxant action (12). By thus sensing the physiological oxygen gradient in tissues, hemoglobin exploits conformation-associated changes in the position of cysteine[SNO] to bring the local blood flow into line with the oxygen requirements (17).

NO can affect tissue oxygenation through its influence on HOA and blood flow regulation. Simultaneously the mechanisms of O₂ transport (including the blood oxygen-binding properties) can modify the activity of the l-arginine–NO pathway. An enzymatic NO synthesis is an oxygen-dependent process
that is reduced at pO2 < 30 mmHg (18). O2 is an important determinant of NO synthase activity in the hypoxic tissues or vascular beds that under normal conditions have the pO2 values of about 40 mmHg, maybe inhibited by a hypoxia because O2 is a necessary substrate for endothelial NOS (19). The HbFeII-NO concentration (as measured by EPR spectroscopy) in arterial and mixed venous blood of normoxic and hypoxic sheep after NO inhalation depended on the O2 and NO levels, and the HbFeII-NO level had a marked negative correlation with the arterial oxygen saturation (20). In our experiments, the administration of L-arginine (NO precursor) can result in positive effects at the later stages of hypothermia and be accompanied by a less marked worsening of blood oxygen transport because of its influence upon the Hb and blood flow.

NO can react with O2 to generate a potent oxidant peroxynitrite (21) that can modify the Hb properties (22). ONOO− causes direct iron oxidation and nitration of thymine residues in a Hb molecule (23). Thus, Hb by sequestration of the significant part of NO released during the transition to the T-structure can save the NO moieties that otherwise might be released too early or with damage (12). Hb may also defend against peroxynitrite, thereby functioning as an intracellular antioxidant.

The analysis of the visible spectra of hemoglobin treated with peroxynitrite revealed that in the presence of CO2 HbO2 was oxidized to ferryl species, suggesting a some way of peroxynitrite-dependent hemoglobin oxidation of dominating importance in CO2-containing biological systems (22). Such a reaction can be of importance in modification of intrinsic Hb properties and its involvement in a formation of O2 flux into tissues and maintenance of the body prooxidant-antioxidant balance (7).

Our experiments indicated different changes of the blood oxygen transport indices in rats receiving SNP and L-arginine. These can be possibly explained by generation of L-citrulline from L-arginine during the natural NO synthesis; then L-citrulline can be reconverted into L-arginine. On the other hand, the SNP administration cause an excessive rise of NO level and therefore its rapid utilization to form nitrite and nitrate anions and to generate the powerful oxidant peroxynitrite in the presence of large amounts of superoxide.

L-arginine administration during hepatic ischemic/reperfusion damage is known to result in tissue pO2 normalization and restoring of the blood flow after ischemia, i.e., to have a protective effect (24). Moreover, in experiments in vitro L-arginine, added to the reperfuase during deep hypothermia (20°C), significantly improved a restoring of cardiac performance and coronary blood flow through a stimulation of NO production (25). In our experiments, rats treated by L-arginine had the most negligible ODC shift leftwards among other animals exposed to hypothermia. This can be explained by a lesser decrease of body temperature in these rats, and also by other mechanisms because of their large P50 stand values (34.18 ± 0.69 vs 30.04 ± 0.69 mmHg in hypothermic animals treated with 0.9% NaCl). Hypothermia is known to be accompanied by a lowering of 2,3-diphosphoglycerate (26). The HOA changes observed are obviously most favorable for tissue oxygenation. The relatively autonomous intraerythrocyte system of HOA regulation provides adaptive changes of blood oxygen-binding properties; in this system 2,3-diphosphoglycerate functions as a trigger for glycolysis and as an instrument for monitoring the relationships between the metabolism and the functional state. However, our data show that NO also can contribute to this system through different mechanisms (HOA modification, vascular tone regulation, peroxynitrite formation and effects). The effect of L-arginine appears to be both a direct result of the NO interaction with Hb and is mediated through an oxygen-dependent mechanism for regulation of NO synthesis.

Thus, the results of the experiments with a modification of the L-arginine–NO pathway under hypothermia suggest a protective effect of L-arginine related to blood oxygen transport changes and attenuation of developing hypoxia.

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