The effect of NO synthase inhibition on blood oxygen-carrying function during hyperthermia in rats

V. Zinchuk [a],*, M. Borisiuk [b]

[a] *Department of Physiology*, *Grodno Medical Institute*, *230015 Grodno*, *Belarus*
[b] *Department of Biophysic*, *Grodno Medical Institute*, *230015 Grodno*, *Belarus*

Abstract

Hyperthermia is known to be accompanied by considerable worsening of body oxygen delivery. Nitric oxide (NO) is a messenger that contributes to the regulation of oxygen transport (vasodilation, formation of nitrosohemoglobin, erythrocyte deformability), but also has cytotoxic effects (when abundantly generated by inducible NO synthase and through a formation of peroxynitrite). The effects of NO synthesis inhibition on the blood oxygen transport (hemoglobin-oxygen affinity and erythrocyte deformability) were investigated in rats with hyperthermia. The most considerable changes in blood oxygen transport indices and the most pronounced hypoxia were observed in rats that received the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) i.p. Its administration before heating significantly impaired body oxygen delivery, with a shift of the oxyhemoglobin dissociation curves rightwards and lowering of erythrocyte deformability. The changes in the blood oxygen transport in animals receiving L-arginine and L-NAME to prevent NO synthase inhibition were similar to those in rats treated with isotonic NaCl before heating. © 1998 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Hyperthermia was shown to be accompanied by considerable worsening of body oxygen delivery developing with involvement of mechanisms of blood oxygen transport (Borisyuk and Zinchuk,

1995). Hemoglobin-oxygen affinity (HOA) is one of important determinants of oxygen flux to tissues (Khandelwal et al., 1993).

Nitric oxide (NO) has recently emerged as an important messenger molecule which displays multiple physiologic and pathophysiologic roles (Moncada and Higgs, 1993). NO production is catalyzed by the enzyme NO-synthase (NOS) during the conversion of L-arginine to L-citrulline.

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* Corresponding author. Tel.: +375 0152 449324; fax: +375 0152 335341; e-mail: zinchuk@ggmi.belpak.grodno.by

The substrate for NO synthesis is L-arginine, and some of its analogues have been used as NOS blockers for the investigation of NO role, for example, N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Moncada and Higgs, 1993). NO is a free radical produced by a variety of cell types by the action of inducible and constitutive NOS. NO is a potent vasodilator, and also possesses a wide range of other actions, including antimicrobial, antiproliferative and anticoagulant effects. The increased production of NO, however, has cytotoxic effects that appear to be partially mediated by peroxynitrite, produced by the reaction between NO and superoxide (Wink et al., 1995). Peroxynitrite reacts with proteins to form the stable product nitrotyrosine.

During hyperthermia the plasma levels of NO metabolites increase (Carter et al., 1994; Hall et al., 1994). Hemoglobin (Hb) plays an important role in NO elimination from the body (Wennmalm et al., 1993). The binding of oxygen to heme irons in hemoglobin promotes the binding of NO to cysteine[b93] with formation of S-nitrosohemoglobin (a regulator of blood flow) (Stamler et al., 1997). The effects of NO synthesis inhibition at high temperature and the associated changes of blood oxygen transport have been unclear. Therefore, it seemed important to investigate the effect of NO synthesis inhibition on blood oxygen-carrying function during hyperthermia.

2. Methods

2.1. Experimental protocol

The experiment was carried out in male laboratory rats (body weight 190–230 g) maintained in a vivarium at 20°C. The animals were divided into five groups ($n = 6$ in each): group 1, control (receiving isotonic solution of NaCl i.p.); group 2 received NOS inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) i.p. (Sigma, St. Louis, MO). The other three groups were exposed to heat immediately after being treated with: group 3, isotonic solution of NaCl i.p.; group 4, L-NAME i.p.; group 5, L-arginine (Sigma) and L-

NAME i.p. to prevent the inhibition of NOS. The drugs were administered as a single dose in 1 ml of the solution (L-NAME 25 mg/kg, L-arginine 600 mg/kg). The animals were exposed to heating by hot air (40°C) in a thermal chamber for 60 min. The rectal temperature was measured by an electric thermometer. Blood was taken from the right atrium on the 60th min after the start of heat treatment under ether anesthesia.

2.2. Measurement of hemoglobin-oxygen affinity

P_{O_2} and acid–base balance were measured by a micro gas analyser (ABL-330, Radiometer) under 37°C and then were corrected to the actual temperature value. Blood oxygen content (Cv_{O_2}) was evaluated by a rise of P_{O_2} in a blood aliquot after the oxygen release from a complex with Hb by 0.33% potassium ferrocyanide (Volter and Herigault, 1972); the percent of hemoglobin saturation by oxygen (S_{O_2}) was calculated. HOA was assessed by P_{50} (blood P_{O_2} under its 50% saturation by O_2) as determined by a 'mixing method' (Scheid and Meyer, 1978) at 37°C, pH 7.4 and $P_{CO_2} = 40$ Torr (P_{50}st). The values of P_{50} at actual pH, P_{CO_2} and temperature (P_{50}act) were calculated from P_{50}st by Severinghaus equations (Severinghaus, 1966) and with the temperature coefficient $\Delta LgP_{50}/\Delta T = 0.024$ (Willford et al., 1982); the oxyhemoglobin dissociation curves (ODC) were calculated according to Hill's equation with $n = 2.8$ from the measured P_{50}.

2.3. Measurement of red blood cell deformability

The erythrocyte deformability (ED) was measured by ektacytometry, based on a determination of deformability index from the diffraction pattern after the transmission of monochromatic coherent light ($\lambda = 850$ nm) through a laminary flow of a red cell suspension under thermostate conditions (Hochmuth, 1993). The decrease of deformability index reflects an impairment of red cells deformability.

Table 1
Effect of L-NAME on hematological and blood gas parameters during hyperthermia in rats

Parameter	Control	L-NAME	Hyperthermia	Hyperthermia + L-NAME	Hyperthermia + L-arginine + L-NAME
pH	7.326 ± 0.007	7.316 ± 0.016	$7.295 \pm 0.007^*$	$7.280 \pm 0.11^*$	$7.292 \pm 0.011^*$
pHact	7.309 ± 0.007	7.295 ± 0.015	$7.256 \pm 0.008^*$	$7.232 \pm 0.012^*$	$7.248 \pm 0.010^*$
P_{CO_2} (Torr)	47.65 ± 4.47	47.15 ± 6.31	41.2 ± 0.95	$37.15 \pm 2.08^*$	39.77 ± 2.11
P_{CO_2}act (Torr)	50.12 ± 4.65	50.03 ± 6.68	46.27 ± 1.08	42.74 ± 2.41	45.19 ± 2.30
P_{O_2} (Torr)	29.02 ± 1.72	27.53 ± 1.53	$23.67 \pm 1.41^*$	$19.83 \pm 1.54^*$	$20.02 \pm 1.64^*$
P_{O_2}act (Torr)	31.55 ± 1.92	30.36 ± 1.72	28.60 ± 1.73	$24.95 \pm 24.95^*$	$24.67 \pm 2.01^*$
P_{50}stand (Torr)	35.66 ± 0.65	37.9 ± 0.50	$33.26 \pm 0.61^*$	$40.70 \pm 0.93^*$	$33.71 \pm 0.34^*$
P_{50}act (Torr)	34.94 ± 0.73	37.37 ± 1.18	34.03 ± 0.44	$42.32 \pm 1.19^*$	35.25 ± 0.65
Hb (g/dl)	12.63 ± 0.17	12.2 ± 0.19	12.1 ± 0.25	12.55 ± 0.28	12.87 ± 0.23
Cv_{O_2} (ml/dl)	8.41 ± 0.29	3.71 ± 1.28	5.93 ± 0.27	5.52 ± 0.50	6.32 ± 0.29
S_{O_2} (%)	49.00 ± 1.44	43.2 ± 1.56	$36.68 \pm 1.16^*$	$32.48 \pm 3.07^*$	$37.94 \pm 2.70^*$
HCO_3^- (mmol/l)	25.28 ± 0.52	23.89 ± 0.42	$19.73 \pm 0.77^*$	$17.05 \pm 0.24^*$	$18.98 \pm 0.92^*$
ABE (mmol/l)	-1.43 ± 0.53	-2.3 ± 0.59	$-5.53 \pm 0.96^*$	$-8.78 \pm 0.50^*$	$-6.08 \pm 1.07^*$
SBC (mmol/l)	23.73 ± 0.99	22.78 ± 0.58	$19.41 \pm 0.76^*$	$18.47 \pm 0.46^*$	$19.52 \pm 1.26^*$
t_{CO_2} (mmol/l)	26.14 ± 0.57	25.08 ± 0.43	$20.69 \pm 0.77^*$	$19.1 \pm 0.27^*$	$20.78 \pm 1.25^*$
T_{rect} (0 min) (°C)	38.03 ± 0.12	38.05 ± 0.13	38.08 ± 0.08	38.15 ± 0.08	38.28 ± 0.05
T_{rect} (60 min) (°C)	38.17 ± 0.10	$38.37 + 0.12$	$39.65 + 0.14^*$	$40.2 \pm 0.20^*$	39.93 ± 0.12 *

Values are mean $\pm$ SD. $n = 6$ for each group.

* Statistically significant difference from control ($P < 0.05$).

2.4. Statistic methods

The data were statistically evaluated by Student's t-test with a significance level of $P < 0.05$. The correlations were obtained from the least-squares linear regression analysis. The results are presented as means $\pm$ SE. The analysis and graphs were performed using computer software packages (version 3.0, Statgraphics; version 4.0, QUATROPRO).

3. Results

The blood oxygen-carrying function had some changes in the investigated rats (Table 1). The high temperature brought about a development of metabolic acidosis accompanied by moderate hypercapnia (7.295 ± 0.007, $P < 0.05$ vs. 7.326 ± 0.07 in control); the actual and standard base excesses were significantly lower. The oxygen delivery had also worsened (P_{O_2} decreased from 29.02 ± 1.72 to 23.67 ($P < 0.05$) and S_{O_2} from 49.00 ± 1.44 to 36.7 ± 1.16 ($P < 0.01$). The administration of L-NAME alone to control animals did not significantly affect the indices of acid–base balance and blood oxygen transport, despite somewhat lowered P_{O_2}, S_{O_2}, and Cv_{O_2}. The metabolic acidosis was most severe in L-NAME pretreated hyperthermic rats (pH $= 7.280 \pm 0.11$; after the correction for temperature the lowering of pH was even more significant (to 7.232 ± 0.012 ($P < 0.01$) vs. 7.309 ± 0.07 in control rats). The hypoxic signs were more marked ($P_{O_2} = 19.83 \pm 1.54$, $P < 0.01$; $S_{O_2} = 32.48 \pm 3.07$, $P < 0.01$). The heating after the L-arginine and L-NAME pretreatment was accompanied by a change in the acid–base balance and oxygen state towards their values in group 2. The heated animals pretreated with L-NAME had the highest temperature rise (by 2.05 ± 0.19°C).

In rats which received L-NAME before heating, the value of P_{50}act was 42.32 ± 1.2 Torr vs. 34.9 ± 0.73 in control ($P < 0.01$), indicating a shift
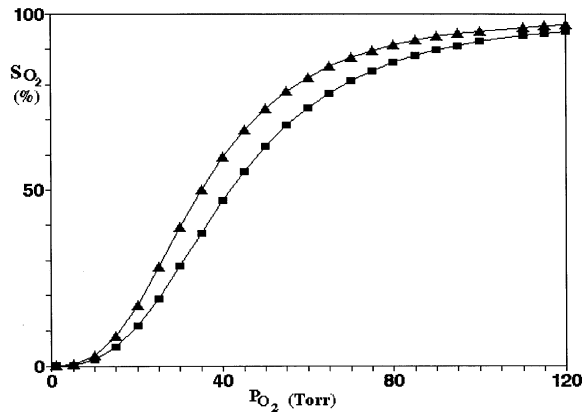
Fig. 1. Actual oxyhemoglobin dissociation curves in control (triangle) and heated rats with inhibition of NO synthase (filled square).

of actual ODC rightwards (Fig. 1). The corresponding values of $P_{50}st$ were 40.70 ± 0.93 vs. 33.91 ± 1.10 Torr in control ($P < 0.01$). The values of $P_{50}st$ and $P_{50}act$ during hyperthermia in rats of group 3 (33.26 ± 0.61 and 34.03 ± 0.44 Torr) and group 5 (33.71 ± 0.34 and 35.25 ± 0.65 Torr) were very similar, but the administration of L-NAME alone without heating resulted in a rise of $P_{50}st$ to 37.9 ± 0.50 Torr.
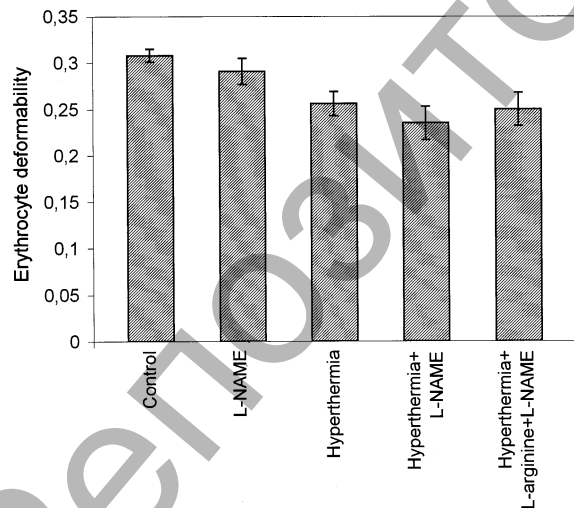

Fig. 2. Index of erythrocyte deformability in rats under NO synthase inhibition during hyperthermia. Mean values $\pm$ SE are shown; significant differences from the control group are marked with asterisks (*).

The ED worsened during heat exposure (Fig. 2). Its decrease was most marked under NOS inhibition (0.235 ± 0.018 vs. 0.308 ± 0.07 in control; $P < 0.01$), while the heating of intact rats resulted in ED $= 0.256 \pm 0.013$ ($P < 0.01$).

4. Discussion

The results indicate that administration of NOS inhibitor L-NAME before the heating significantly worsened body oxygen delivery, with a shift of ODC rightwards and lowering of ED. The changes in blood oxygen transport in the animals, receiving L-arginine and L-NAME to prevent NOS inhibition, were similar to those in rats receiving isotonic NaCl before the heating.

The exposure to high temperature caused an enhancement of the NO production by the body, as determined by the urinary excretion of nitrate (Carter et al., 1994) and the NO-heme electronic paramagnetic resonance signal (Hall et al., 1994); this is undoubtedly important for enhancement of body heat losses; but the excessive NO formation may cause toxic effects. Recent investigations indicated an important role of NO and peroxynitrite (the product of its interaction with superoxide anion) in cellular damage (Wink et al., 1995). In the present study the effect of NO on the thermoregulatory vasodilation was negligible because the environment had a higher temperature than the body, but its effect on the vaporative heat loss might be considerable. The NOS inhibition decreased the thermolytic salivation in rats during heating (Damas, 1994). Therefore, the restriction of NO synthesis by a constitutive isoenzyme of NOS is important for maintaining of temperature homeostasis and vascular tone.

NO produced by endothelial cells maintains a vasodilation and hence the blood flow at the optimal level for tissue oxygenation. The constitutive isoform of NOS is sensitive to low P_{O_2}, and hypoxia increases the expression of mRNA of this isoform (Xu et al., 1995). The inhibition of NO synthesis in intact dogs lowered the oxygen delivery (Cobb et al., 1995) and simultaneously increased the mean arterial pressure and systemic and pulmonary vascular resistance. The reduction

of the blood flow during the blockade of NO synthesis resulted in a decrease of skeletal muscle tissue P_{O_2} from 29 to 11 Torr after 30 min (Pohl et al., 1993). The ability of endothelium to active synthesis of NO was suggested to play a key role in the optimization of oxygen delivery and oxygen demands both at rest and during a load (Wagner et al., 1992). Impairment of NO synthesis by the endothelium largely explains a loss of vasoconstrictory regulation by means of a lower adaptation of the blood flow to the tissue demands, which was observed during the functional and reactive hyperemia after NO synthesis inhibition (Kostic and Schrader, 1992).

The shift of ODC rightwards is generally considered to rise an oxygen unloading to tissues. The decrease of HOA is both an important compensatory factor for oxygen insufficiency under different pathologic conditions and a mechanism of adaptation to the oxygen insufficiency. In experiments in rats, the rise of P_{50} by 25% was accompanied by an average increase of tissue P_{O_2} by 78% (Khandelwal et al., 1993). The higher than normal value of P_{50} appears to be favourable for optimal oxygen transport to tissues under normoxia or moderate hypoxia (Willford et al., 1982).

During body hyperthermia, the ODC position is largely determined by temperature and pH and to a lesser extent by P_{CO_2} (Borisyuk and Zinchuk, 1995). The actual position of ODC is a result of cumulative action of these non-unidirectional factors. The temperature is the most significant factor influencing on HOA. The absence of correction by 1°C during the measurements of P_{O_2} and P_{CO_2} can lead to an 11% error in calculated fraction of arteriovenous shunt (Siggaard-Andersen and Siggaard-Andersen, 1995). Wood (1980) indicated two possible ways to change the temperature effect on HOA in the whole body— through a switching of the heterogenous system of Hb synthesis or through the mechanism of allosteric interaction. The first way may be based on the synthesis of completely thermotolerant Hbs or Hbs with a weaker temperature-dependent co-operative interaction with oxygen. However, such a way with a morphogenetic change in the synthesis of different Hbs cannot be manifested during

relatively short adaptation to temperature. The adaptive change of blood oxygen-binding properties is essentially caused by a relatively autonomous intraerythrocyte system of HOA regulation. Red cell 2,3-diphosphoglycerate functions as a trigger of allosteric regulation of glycolysis and as a specific reference device for an agreement between the metabolic and functional states. This organic phosphate is known to exert a profound effect on HOA, which can increase the blood P_{50} by 50% (Benesch and Benesch, 1970). The investigation of the 2,3-diphosphoglycerate content in mixed venous red blood cells of heated rabbits had shown its drop by 40% at the end of the heating and during the first 4 h after it (Borisyuk and Zinchuk, 1995). The changes in HOA during hyperthermia combined with the inhibition of NO synthesis do not contribute to the elimination of oxygen deficiency.

Hb plays a very important role in the elimination of NO from the body. In arterial blood NO is inactivated in a reaction with oxyhemoglobin to yield nitrate and methemoglobin, and in addition to these products, nitrosohemoglobin is also generated in venous blood (Wennmalm et al., 1993); under high P_{O_2} it may be disintegrated by O_2 to Hb and NO_3^-. High doses of the NO donor nitroglycerine cause a formation of nitrosohemoglobin, with the concentration correlating with the P_{50} value and the corresponding ODC shift rightwards (Kosaka and Seiyama, 1996). By thus sensing the physiological oxygen gradient in tissues, hemoglobin exploits conformation-associated changes in the position of cysteine[b93]-S-NO to bring the local blood flow into line with the oxygen requirements (Stamler et al., 1997). In these experiments, the shift of ODC rightwards cannot compensate for the insufficiency of blood flow under hyperthermia combined with NOS inhibition. At earlier stages of heating L-NAME firstly inhibits endothelial rather than inducible NOS, as in the case of fever (Redford et al., 1995), and this inhibition plays a key role in a genesis of developing hypoxia. The limitation of NO synthesis under conditions of lowered endothelial and increase inducible NOS activities may cause a positive effect at later stages of hyperthermia and be accompanied by less marked disturbances in blood oxygen transport.

When the NO synthesis was inhibited, the erythrocyte rigidity enhanced most markedly. The treatment of red blood cells by competitive L-arginine analogues lowered the hypotonic hemolysis, increased the input of K^+, and the spinal changes of erythrocyte morphology (Caramelo et al., 1994). The reduction of ED is not adaptive and reflects a failure in the compensatory abilities of oxygen transport mechanisms. The worsening of deformability is the highest under conditions of the most marked hypoxia (judged by the indices of acid–base balance and blood oxygen transport). The observed worsening of ED restricts the oxygen transport to tissues via different mechanisms: lowering of intraerythrocyte convection, changes in Fahreus–Lindqvist effect, increases in the arteriovenous shunt and in the whole blood viscosity.

Thus, the results have shown that the inhibition of NOS during hyperthermia results in a more significant impairment of body oxygen supply, with important roles of HOA and ED in its mechanisms. Pretreatment with excessive L-arginine prevented the inhibition of NO synthesis and was accompanied by development of a typical response to high temperature and less marked alterations in blood oxygen transport.

References

Benesch, R.E., Benesch, R., 1970. The reaction between diphosphoglycerate and hemoglobin. Fed. Proc. 29, 1101–1104.

Borisyuk, M.V., Zinchuk, V.V., 1995. Hemoglobin-oxygen affinity and the Bohr effect during heat hypoxia. Hypoxia Med. J. 2, 11–13.

Caramelo, C., Riesco, A., Outeirino, G., Millas, I., Blum, G., Monzu, B., Casado, V., Sanchez, L., Mosquera, J.R., Casado, S., 1994. Effects of NO on red blood cells: Changes in erythrocyte resistance to hypotonic hemolysis and potassium efflux by experimental maneuvers that decrease NO. Biochem. Biophys. Res. Commun. 199, 447–454.

Carter, E.A., Derojas-Walker, T., Tamir, S., Tannenbaum, S.R., Yu, Y.M., Tompkins, R.G., 1994. Nitric oxide production is intensely and persistently increased in tissue by thermal injury. Biochem. J. 304, 201–204.

Cobb, J.P., Natanson, C., Quezado, Z.M., Hoffman, W.D., Koev, C.A., Banks, S., Correa, R., Levi, R., Elin, R.J., Hosseini, J.M., Danner, R.L., 1995. Differential hemodynamic effects of L-NMMA in endotoxemic and normal dogs. Am. J. Physiol. 268, H1634–H1642.

Damas, J., 1994. Kallikrein, nitric oxide and the vascular responses of the submaxillary glands in rats exposed to heat. Arch. Int. Physiol. Biochim. Biophys. 102 (2), 139–146.

Hall, D.M., Buettner, G.R., Matthes, R.D., Gisolfi, C.V., 1994. Hyperthermia stimulates nitric oxide formation: Electron paramagnetic resonance detection of NO-heme in blood. J. Appl. Physiol. 77, 548–553.

Hochmuth, R.M., 1993. Measuring the mechanical properties of individual human blood cells. Science 115, 515–519.

Khandelwal, S.R., Randad, R.S., Lin, P.S., Meng, H., Pittman, R.N., Kontos, H.A., Choi, S.C., Abraham, D.J., Schmidt-Ullrich, R., 1993. Enhanced oxygenation in vivo by allosteric inhibitors of hemoglobin saturation. Am. J. Physiol. 265, H1450–H1453.

Kosaka, H., Seiyama, A., 1996. Physiological role of nitric oxide as an enhancer of oxygen transfer from erythrocytes to tissues. Biochem. Biophys. Res. Commun. 218, 749–752.

Kostic, M.M., Schrader, J., 1992. Role of nitric oxide in reactive hyperemia of the guinea pig heart. Circ. Res. 70, 208–212.

Moncada, S., Higgs, E.A., 1993. The L-arginine-nitric oxide pathway. New Engl. J. Med. 329, 2002–2012.

Pohl, U., Wagner, K., de Wit, C., 1993. Endothelium-derived nitric oxide in the control of tissue perfusion and oxygen supply: Physiological and pathophysological implications. Heart J. 14, 93–98.

Redford, J., Bishai, I., Coceani, F., 1995. Pyrogen-prostaglandin coupling in the pathogenesis of fever: Evidence against a role for nitric oxide. Can. J. Physiol. Pharmacol. 73, 146–147.

Scheid, P., Meyer, M., 1978. Mixing technique for study of oxygen-hemoglobin equilibrium: A critical evaluation. J. Appl. Physiol. 45, 616–622.

Severinghaus, J.W., 1966. Blood gas calculator. J. Appl. Physiol. 21, 1108–1116.

Siggaard-Andersen, M., Siggaard-Andersen, O., 1995. Oxygen status algorithm, version 3, with some applications. Acta Anaesthesiol. Scand. 107, 13–20.

Stamler, J.S., Jia, L., Eu, J.P., McMahon, T.J., Demchenko, I.T., Bonaventura, J., Gernert, K., Piantadosi, C.A., 1997. Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. Science 276 (5321), 2034–2037.

Volter, F., Herigault, R., 1972. Methode polarographique de ditermination contenu en oxygene sur microechantillons. J. Physiol. (Paris) 65, 174A.

Wagner, K.F., Krumm, R., Pohl, U., 1992. Inhibition of EDRF formation impairs skeletal muscle oxygen pressure distribution and blood flow in rats. J. Vasc. Res. 29, 223.

Wennmalm, A., Benthin, G., Edlund, A., Jungersten, L., Kieler-Jensen, N., Lundin, S., Westfelt, U.N., Petersson, A.S., Waagstein, F., 1993. Metabolism and excretion of nitric oxide in humans. An experimental and clinical study. Circ. Res. 73, 1121–1127.

Willford, D.C., Hill, E.P., Moores, W.Y., 1982. Theoretical analysis of optimal P_{50}. J. Appl. Physiol. 52, 1043–1048.

Wink, D.A., Cook, J.A., Pacelli, R., Liebman, J., Krishna, M.C., Mitchell, J.B., 1995. Nitric oxide (NO) protects against cellular damage by reactive oxygen species. Toxicol. Lett. 82/83, 221–226.

Wood, S.C., 1980. Adaptation of red blood cell function to hypoxia and temperature in ectothermic vertebrates. Am. Zool. 20, 163–172.

Xu, X.P., Pollock, J.S., Tanner, M.A., Myers, P.R., 1995. Hypoxia activates nitric oxide synthase and stimulates nitric oxide production in porcine coronary resistance arteriolar endothelial cells. Cardiovasc. Res. 30, 841–847.