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SOME BLOOD PARAMETERS AND ANTIOXIDANT DEFENSE ENZYME ACTIVITIES IN THE LIVER OF CARPS (*Cyprinus carpio* L.) UNDER ACUTE HYPOXIC CONDITIONS

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ABSTRACT. Carps (*Cyprinus carpio* L.) were acclimated to oxygen concentration of 7.31 mg/L water (100%)-controls (C). Two experimental groups were exposed to a progressive reduction of O_2 to 4.10 mg/L (56±1%) during 1 hour and 15 minutes (H1) and 1.95 mg/L (26±1%) during 2 hours (H2). In all examined groups haematological values: red blood cells (RBCs) count, haematocrite value (Htc), haemoglobin (Hb), glucose and lipid peroxide (LP) concentrations were studied. The antioxidant defense enzyme activities such as: superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were also determined in the liver of carps.

The acute hypoxia (H1) significantly increased the number of RBCs, Hb concentration and Htc value as well as LP concentration, whereas a significant decrease of glucose concentration was observed when compared with control fish.

The activities of SOD, CAT, GSH-Px, and GST in the liver were significantly increased in both experimental groups of fish (H1 and H2) in respect to the controls. Liver GR activity was significantly increased only in H1 group of carps.

INTRODUCTION

In hypoxic conditions fish always confront their need to satisfy metabolic requirements under reduced oxygen availability [1, 2]. Swimming movements followed by changed oxygen diffusing capacity [3] and metabolic activities [4] are the simplest way to avoid the reduction of oxygen concentration. However, when escape is not possible, haematological response [5], biochemical and physiological alterations [6, 7, 8, 9, 10] must be used to compensate the changes in the oxygen requirements. Under hypoxic circumstances, the number of circulating erythrocytes in the blood of fish [11, 12] increased, as well as the cell volume [13]. Another haematological response includes changes in haemoglobin concentration and haematocrit value [5] which may be in conjunction with

changes in haemoglobin affinity [14]. At a biochemical level the decrease in the rainbow trout (*Oncorhynchus mykiss*) blood glucose concentration was adequate to support oxygen consumption [15] and imply that glucose serves as a primary metabolic fuel for the blood, which indicates anaerobic metabolism of carp (*Cyprinus carpio* L.) [16]. In teleost fish during hypoxia, catecholamines are released into circulation [17] and initiate physiological changes which enhance blood oxygen transport and increase the rate of erythropoeses [18, 19, 20, 21].

In conditions of changed water temperature an oxidative stress may be expressed in lipid peroxidation in gills of freshwater teleosts [22]. Reductions of oxygen concentration in water have resulted in significant changes in antioxidant defense system (AOS) in teleosts [23, 24], as well as in mammalian tissues [25]. Under hypoxic conditions the relatively high rate of generation of reactive oxidative species (ROS) [26] is followed by relatively high superoxide dismutase and catalase activities in the blood, liver and red muscle of freshwater teleosts. Most teleosts respond to hypoxia with a substantional increase in glutathione-S-transferase activity in the liver and gills [27, 28]. An increase in GST activity in some tissues of carp (*Cyprinus carpio* L.) [29] and in the liver, kidneys, gills and olfactory epithelium of rainbow trout (*Oncorhynchus mykiss*) [30, 31] exposed to varied environmental stress factors was also found [32].

Hence, the effects of acute hypoxia are less known in literature data, especially in different tissues of teleosts, we decided to investigate the changes in some haematological parameters in the blood and the activity of antioxidant defense enzymes in the liver of common carps (*Cyprinus carpio* L.). We determined the number of erythrocytes (RBCs), haemoglobin concentration (Hb), haematocrit value (Htc), glucose and lipid peroxide (LP) concentrations. We also measured the activities of superoxide dismutase (SOD, EC 1.15.1.1.), catalase (CAT, EC 1.11.1.6), glutathione-S-transferase (GST, EC 2.5.1.18), glutathione peroxidase (GSH-Px, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2) in the liver.

MATERIAL AND METHODS

In our experiments common carps (*Cyprinus carpio* L.) weighing 544 ± 43.41 g and length 31.01 ± 0.97 cm were used. The fish were acclimated to the aquarium conditions with water temperature of 14 ± 0.5^{o}C, pH 6.6 and concentration of dissolved oxygen of 7.31 mg O_2/L or 100% - (C) in dechlorinated and aerated water for 20 days. After a period of acclimation the fish were divided in three experimental groups each consisting of five fish placed into separate aquarium: (1) control fish (C); (2) fish exposed during 1 hour and 15 minutes of progressive decrease of O_2 to 4.2 mg/L water or 56±1% of dissolved oxygen (H1) in comparison to the controls; (3) fish exposed during 2 hours of progressive decrease of O_2 to 1.95 mg/L water or 26±1% of dissolved oxygen (H2) in comparison to the controls. Two aquariums (H1 and H2) were covered in order to reduce oxygen concentration. Fish

remained under these conditions until the end of treatment when they were weighed and sacrificed one by one every five minutes each group at the time.

The concentration of oxygen in the water was determined by using HI 9143-Microprocessor auto cal dissolved oxygen meter (Hanna instruments).

The fish were sacrificed by a sharp blow on the head, always between 8 and 10 a.m. Fresh blood was immediately collected into heparinized test tubes. The number of red blood cells (RBCs) was determinate in whole blood by using Neubauer-chamber for counting and expressed in the number of RBC x 10^{12}/L of blood. The haemoglobin (Hb) concentration in erythrocytes was estimated by the cyanmethaemoglobin method [33] and expressed in mmol/L. The haematocrit (Htc) value was obtained by the standard microhaematocrite method using microhaematocrit tubes, and expressed in L/L. The concentration of glucose was measured by Hultman [34] colorimetric method, and expressed in mmol/L. The lipid peroxides (LP) concentration was determined in the whole blood according to Ohkawa et al. [35] and expressed in nmol/mL blood.

For the determination of antioxidant defense enzyme activities the liver tissue was dissected and placed in ice-cold 155 mmol NaCl and washed with the same solution. The liver tissue was then minced and homogenized in 10 volumes of 25 mmol surcose containing 10 mmol Tris-HCl, pH 7.5 at 1500 rpm using a Thomas Sci. Co glass homogenizer (Teflon pestle), 8-10 up-and-down strokes. Homogenates were then centrifuged at 4^{o}C at 100 000 x g for 90 min. Total SOD activity was assayed in the supernatant by the epinephrine method [36]. For the determination of CAT activity the assay was performed as described by Beutler [37]. The activity of GSH-Px was evaluated by following the oxidation of NADPH at 340nm with t-butylhydroperoxide [38]. GST activity toward 1-chloro-2,4-dinitrobenzene as substrate was determined according to Habig [39]. The GR activity was determined as described by Glatzle et al. [40]. All examined antioxidant defense enzyme activities were expressed per g of wet mass (U/g w.m.).

All obtained data were statistically analyzed and expressed as mean ± SE. Statistical differences between all experimental and control groups were estimated by Student's paired *t*-test [41]. The value of $p<0.05$ was taken as the least degree of significance.

RESULTS

In Table 1 the number of RBCs, Hb concentration, Htc value concentrations of glucose and LP are presented. Acute hypoxia causes a significant increase of the number of RBCs ($p<0.01$), Hb concentration ($p<0.02$) and Htc value ($p<0.02$) in H1 group of carps. A progressive reduction of oxygen concentration (H1) significantly decreases the glucose concentration ($p<0.01$) in the blood of fish. The concentration of LP was significantly increased ($p<0.005$) in the blood of H1 experimental group of carps.

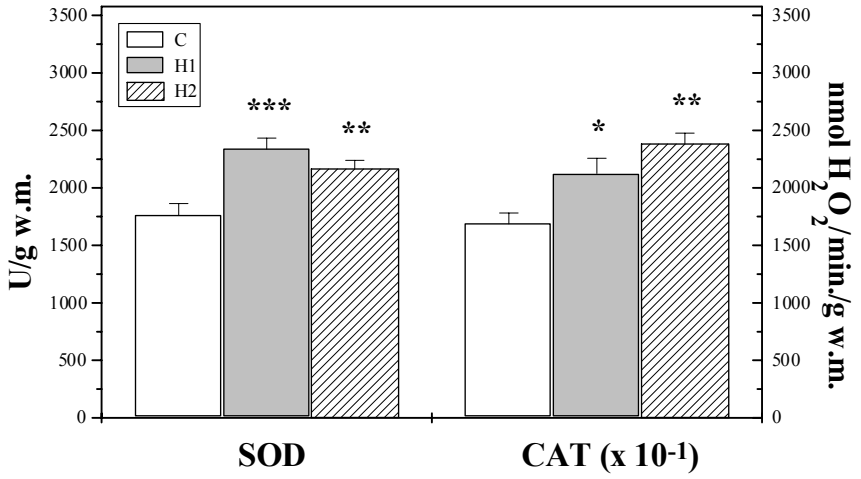
Table 1. Some blood parameters of control carps (C), carps exposed of progressive decrease of O_2 to 56±1% during 1 hour and 15 minutes (H1) and carps exposed of progressive decrease of O_2 to 26±1% during 2 hours (H2). The results were compared in respect to the control animals.

BLOOD	C	H1	H2
RBC (10^{12}/L blood)	1.87 ± 0.02	2.91 ± 0.16***	1.89 ± 0.04
Hb (mmol/L blood)	5.59 ± 0.24	6.73 ± 0.15**	5.94 ± 0.60
Htc (L/L blood)	0.34 ± 0.01	0.40 ± 0.01**	0.35 ± 0.01
Glucose (μmol/mL blood)	6.81 ± 0.40	3.71 ± 0.06***	6.75 ± 0.29
LP (nmol/mL blood)	0.80 ± 0.14	1.85 ± 0.05****	1.02 ± 0.19

Means ± SE from 5 animals in each group.
Significantly different from controls (C): **p< 0.02; ***p<0.01; ****p<0.005

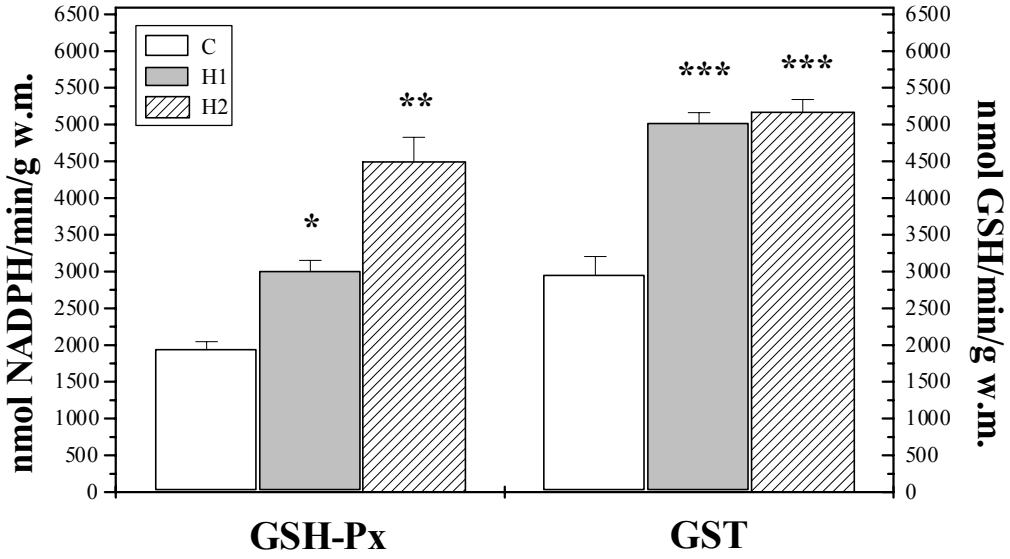
The activity of SOD, presented in Figure 1, was significantly increased in both H1 and H2 experimental groups of fish (p<0.01 and p<0.02, respectively) in respect to the controls. These hypoxia episodes increase the CAT activity (p<0.05. p<0.02). GSH-Px activity was significantly increased (Figure 2) in the liver of fish exposed to acute hypoxia (H1 and H2) in respect to the control carps (p<0.05 and p<0.02). The activity of GST (Figure 2) was significantly increased in H1 and H2 groups of fish (p<0.01, p<0.01, respectively) as well. GR activity was significantly increased only in H1 experimental group of fish (p<0.01) when compared to the controls (Figure 3).

Figure 1. The activities of superoxide dismutase (SOD, U/g w.m.) and catalase (CAT, μmol H_2O_2/min/g w.m.) in the liver of control carps (C), carps exposed of progressive decrease of O_2 to 56±1% during 1 hour and 15 minutes (H1) and carps exposed of progressive decrease of O_2 to 26±1% during 2 hours (H2).



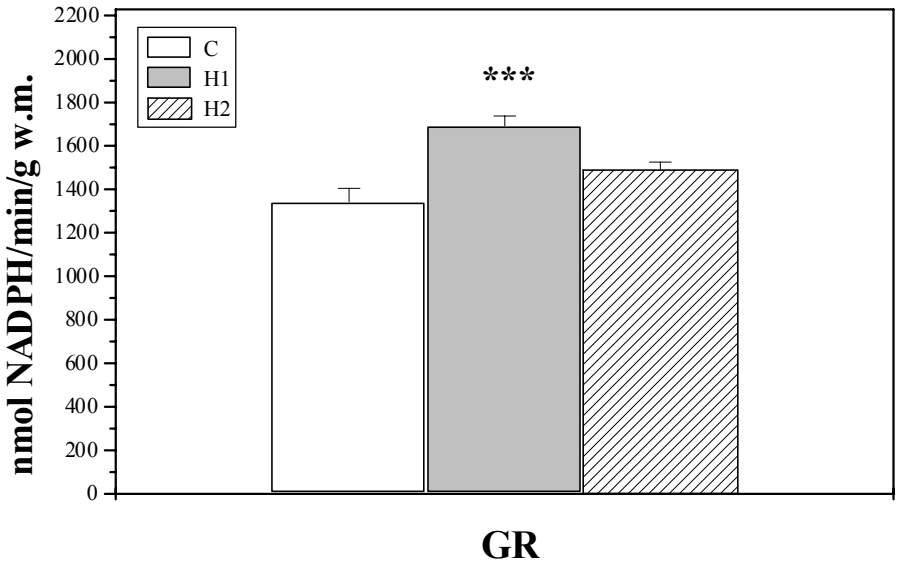
Means ± SE from 5 animals in each group.
The results were compared in respect to the control animals.
Significantly different from (C): *p<0.05; **p <0.02; ***p<0.01.

Figure 2. Glutathione peroxidase (GSH-Px, nmol NADPH/min/g w.m.) and glutathione-S-transferase (GST, nmol GSH/min/g w.m.) activities in the liver of control carps (C), carps exposed of progressive decrease of O_2 to 56±1% during 1 hour and 15 minutes (H1) and carps exposed of progressive decrease of O_2 to 26±1% during 2 hours (H2).



Means ± SE from 5 animals in each group.
The results were compared in respect to the control animals.
Significantly different from (C) : *p<0.05; **p<0.02; ***p

Figure 3. Glutathione reductase activity (GR, nmol NADPH/min/g w.m.) in the liver of control carps (C), carps exposed of progressive decrease of O_2 to 56±1% during 1 hour and 15 minutes (H1) and carps exposed of progressive decrease of O_2 to 26±1% during 2 hours (H2).



Means ± SE from 5 animals in each group.
The results were compared in respect to the control animals.
Significantly different from (C) : ***p<0.01.

DISCUSSION

It is known that reduction of water oxygen concentration alters haematological [12, 21], biochemical and physiological parameters [42, 43, 44, 45, 46]. In our experimental conditions limitations of amount of dissolved oxygen causes an increase in RBCs number, haemoglobin concentration (Hb) and haematocrit value (Htc) in the blood of fish (H1 and H2) (Table 1). Increased RBCs count in the circulation is primary haematological response to hypoxia, which is documented in the goldfish [11, 47]. According to Houston and Murad [47] erythrocyte division in response to hypoxia offers only a limited advantage in increasing the blood O_2-carrying capacity to sinthesize haemoglobin. Speckner et al. [48] suggest that mature carp erythrocytes retain this ability under reduced oxygen concentrations. Claireaux et al. [49] showed that a greater amount of available haemoglobin improving blood oxygen and appear to be a fast adaptive response to acute hypoxia. Haemoglobin oxygen binding affinity and increased ability of the blood to transport available oxygen are produced under the same mechanism of erythrocyte alkalization [50]. Similar observations showed by Van Raaij et al. [20] indicate that deep hypoxia has the quantitative importance of the adrenergic response of carp erythrocytes. The metabolic response to hypoxia may vary, depending on the physiological state of the animal, level of activity and temperature [51, 52]. Results of our study show significantly lower glucose concentration in H1 group under progressively reduced oxygen concentration, indicating the stimulation of anaerobic glycolysis (Table 1). These data are similar to previous investigations on fish obtained by other authors [53, 44] and also in amphibians [54, 55]. Our results show a significantly increased lipid peroxidation in the blood of carps exposed to the first degree of acute hypoxia. The acute hypoxia induces cell damage in the liver and gills of teleosts [26, 56] or amphibians [57]. Other environmental stress factors [32] can also produce intensive peroxidation of lipids of freshwater teleosts.

The activities of SOD, CAT, GSH-Px and GST were significantly increased in the liver of both H1 and H2 carp groups. This may be a consequence of the activatory effects of reduced oxygen concentration on the activities of antioxidative defense system enzymes (Figure 1,2). Similar results were observed in other teleosts [26, 4], which showed that partial reduction of molecular oxygen produces the superoxide anion radical and hydroxyl radical in all tissues of fish and in some mammalian tissues [58, 59, 60] as well. An increased activity of examined enzymes in the liver of hypoxic carps (Figure 1) and mammalian species [61] is natural physiological response to survive an oxidative insult. The activity of CAT was also increased (Figure 1) in the liver of hypoxic carps as well as in the liver of other fish species such as goldfish (*Carassius auratus*) [24, 61, 62]. These results are in conflict with those reported by Wilhem et al. [21], showing the elimination of generated H_2O_2 by simple diffusion. However, some caution has to be taken in the interpretation of Wilhelm's results since different culture conditions and different species were used. Increased activity of GSH-Px (Figure 2) under hypoxic conditions, which is observed also by other authors [24] in some tissues of

freshwater teleosts, indicates a protective role of this enzyme in the liver of carps. Barth et al. [63] showed that increased activity of GSH-Px may be a consequence of intensive lipid peroxidation.

According to our data (Figure 2) the activity of GST was significantly increased in the liver of carps exposed to acute hypoxia and may be of importance to minimize cell damage during reintroduction of oxygen [64]. Similar results were observed in tissues of other teleosts [27,28]. Gadagbui and James [65] showed a marked affinity of isolated GST from channel catfish whole intestine.

GR have a very important role in the process of translation of oxidized glutathione (GSSG) to his reduced form (GSH) [66]. Our results and data of Gallager and Digiulio [27] showed increased GR activity as a response to oxidative stress under hypoxic conditions, which follow an increased GSH-Px activity [67] as well.

CONCLUSIONS

Acute hypoxia causes significant changes in some blood parameters which results in increased RBCs count, Htc value and Hb concentration. Decreased glucose concentration demonstrates a certain degree of anaerobic metabolism. Increased LP can be taken as positive indicator of oxidative stress under hypoxic conditions.

Reduction of oxygen concentration in water induces an oxidative stress indicating a higher activity of antioxidant defense enzymes such as SOD, CAT, GSH-Px, GST and GR in the liver of carp (*Cyprinus carpio* L.).

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