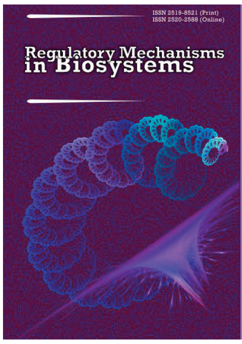

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Change in magnesium concentration in erythrocytes in fish under stress

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At present, the role of erythrocyte magnesium in the respiratory processes of fish (and other animals) under conditions of stress load is not known. This article presents the results of research on change in the concentration of magnesium in erythrocytes under the action of stressors of different quality and quantity for bream (*Abramis brama* L.), silver crucian carp (*Carassius auratus gabelio* Bloch) and tench, (*Tinca tinca* L.) in Rybinsk Reservoir. The concentration of magnesium ions was analyzed on an atomic absorption spectrometer AAS-1 manufactured by Carl Zeiss (Germany) in the absorption regime in an air-acetylene flame. For the first time, the dependence of the change in magnesium concentration in erythrocytes on the intensity of the stress load of different nature on the example of fishes was revealed. Weak and moderate strength effects (low doses of epinephrine, norepinephrine, small changes in water temperature, saline injection, prick into the abdominal cavity, short-term removal of fish from the water, short-term keeping of fish in a limited volume of water) increased the concentration of magnesium in erythrocytes up to 2.5 times. At the same time, an increase in the affinity of hemoglobin for oxygen and a decrease in oxygen consumption were observed. However, strong stressors such as catching, transporting fish to the laboratory (representing complex effects of hypoxia, limited water volume, mechanical effects, etc.), as well as a sharp and large change in water temperature, high doses of adrenaline reduced the concentration of magnesium in erythrocytes 3 times. At the same time, the hemoglobin affinity for oxygen decreased and oxygen absorption increased. However, before the death of fish (from exhaustion), with a low level of magnesium in erythrocytes the affinity of hemoglobin for oxygen increased and the intensity of gas exchange decreased. The research conducted allow us to consider the change in the concentration of magnesium ions in erythrocytes – which are positive modulators of the affinity of hemoglobin for oxygen – in fish exposed to stress as one of the mechanisms for reducing the gas exchange intensity for weak and medium short-term stress effects and increasing it for strong short-term ones. Especially important is the role of erythrocyte magnesium as a molecular mechanism for reducing oxygen uptake and, consequently, increasing anabolism and, thus, increasing the growth and development of animals under the action of mild, short-term stressors, i.e. with eustress. In addition, the concentration of magnesium in erythrocytes can serve as an indicator of the state of fish. A high level of this cation in erythrocytes (1.5–2.0 times higher and more than normal) is characteristic for strong, healthy animals in a state of eustress or physiological stress, and extremely low values of this indicator (1.5–2.0 and more times below the norm) are an indicator of reversible or permanent ill-being (distress or pathological stress). Weakened, exhausted animals are not capable of a response to eustress or physiological stress. The possible causes of low magnesium concentrations in human erythrocytes are discussed.

Keywords: bream; tench; crucian carp; affinity of hemoglobin to oxygen; gas exchange intensity; eustress; distress.

Introduction

An important property of erythrocytic magnesium is a positive modulating effect on affinity of hemoglobin for oxygen (Flatman, 2003; Wells, 2009), which manifests particularly in interspecies differences in concentrations of magnesium in erythrocytes of fish, which differ by intensity of gas exchange and affinity of hemoglobin for oxygen, and also differences in seasonal dynamic of concentrations of magnesium in red blood cells and affinity of hemoglobin for oxygen. Active swimmers which consume lots of oxygen have a lower level of erythrocytic magnesium and lower affinity of hemoglobin for oxygen compared to fish which are more resistant to oxygen deficiency (Soldatov, 1997; Zaprudnova & Kamshilov, 2008; Vornanenet al., 2009; Wells, 2009). During spawning (i.e. during stress), fish were recorded as having the highest level of magnesium in erythrocytes during the year and the highest affinity of hemoglobin for oxygen. Minimum content of magnesium in red blood cells and minimum affinity of hemoglobin for oxygen was recorded in summer, i.e. during maximum values of moving activity of fish and water temperature (Zaprudnova et al., 2016). Intensity of gas exchange and affinity of hemoglobin for oxygen changes also during stress (Wendelaar Bonga, 1997; Nikinmaa, 2003; Zaprudnova & Kamshilov 2010). Despite active study of physical-biochemical processes of fish during stress (Faught et al., 2016; Rodnick, & Planas, 2016; Takei & Hwang, 2016), at present there are no data on participation of erythrocytic magnesium related to breathing, in stress reaction of fish. There is no information on this issue for other vertebrates, including mammalia, despite intense study of the effect of magnesium on the organism of humans (see the special journal Magnesium Research published since 1988 and the international conferences described in it, and also numerous publications in other journals of physiological-biochemical and medical orientation). At the same time, most studies have concentrated on the positive effect of magnesium on the nervous and cardio-vascular systems of humans (Nechifor, 2008; Rosanoff & Wolf, 2016; Boyle et al., 2017). Often, the level of erythrocytic magnesium is used for determining deficiency of this ion in the organism, but the reason for change in concentration of magnesium in red cells is found usually to be related to some disease (Cox, et al., 1991; Widmer et al., 1995; Widmer et al., 1998; Kopitsyna et al., 2015).

The problem of stress is significantly related to such fundamental capacities of living organisms as ability to change the response reaction in relation to the extent (force and duration) of effect and initial condition of the organism. This dependency has been reflected in the theories of favourable stress (eustress) and damage stress (distress) (Dhabhar, 2008; Schreck, 2010; Schreck & Tort, 2016) or of physiological and pathological stress (Arshavskii, 1982). Pathological stress (or distress) occurs in response to activity of strong and/or long stressors and is followed by increase in catabolic processes, leading to decrease in resistance of an organism. Pathological stress is the stress as it is most commonly understood. Physiological stress occurs in response to short term stressors of low or average strength. During physiological stress, the intensity of anabolic processes and non-specific resistance of the organism increase. Earlier (Zaprudnova, 2017), it was demonstrated that under the effect of not strong (weak and medium strength) short term stressors (physiological stress), fish were observed to have changes in concentration of cations of sodium, calium, calcium and magnesium in the internal environment towards increased ion concentration gradients on membranes of the cells and tissues, in particular hypernatremia in the environment on average equaled 10%. At effect of strong and/or long term stressors (pathological stress), changes in concentration of cations in the internal environment were orientated towards decrease of ion concentration gradients on membrane of the cells and tissues. During acute reversible and acute lethal stress, hyponatremia equaled up to 30% and 50% respectively, and during subacute and chronic lethal stresses – up to 20% and 10% respectively. The objective of this study was the dynamic of the concentration of magnesium in erythrocytes among some freshwater fish suffering stress of different intensity.

Materials and methods

We studied mainly mature or nearly mature individuals of bream (*Abramis brama* L.) in the Rybinsk Reservoir. Some of the tests were replicated on mature and nearly mature individuals of tench (*Tinca tinca* L.) and silver crucian carp (*Carassius auratus gabelio* Bloch). The body length of the studied bream, tench and silver crucian carp ranged from 350–386, 334–362 and 155–280 mm respectively. After caudectomy, blood was put in test tubes moistened with heparin and centrifuged at 1800 g 30 min. All plasma with the upper layer of leukocytes and erythrocytes was removed. Erythrocytes were dissolved in 500 times distilled water and held in refrigerator at 4 ºC no less than 2 days until full hemolysis. Concentration of magnesium ions was analyzed in absorptional regime on an atomic-absorptive spectrometer AAS-1 manufactured by Carl Zeiss (Jena, Germany) in air-acetylene flame.

The norm was considered to be the condition of fish adapted for no less than two weeks to the laboratory conditions in deep shaded basins (proportion of body weight and water was 1 : 200 and more). The control condition of fish was the level of natremia (Zaprudnova, 2017). As insignificant short term stressors (1–7), we used: intraperitoneal injection of 0.3–1.5 mg/kg of adrenalin (stressor 1), injection of normal saline (2), introduction of 0.2–1.3 mg/kg of noradrenalin (3), a prick with a needle in the abdominal cavity (4), extraction of fish from water for 1–2 min (5), gradual change in water temperature by 4–5 °C (6), putting fish for 20 min into a limited volume of water: the proportion of body weight and water equaled 1 : 15 (stressor 7, Table 1). As strong short term (2–20 min) stressors (8–11), we used a sharp increase in water temperature by 17–23 °C (stressor 8), introduction of high doses of adrenalin: 5–6 mg/kg (9), as short term strong complex stressor, we also used 15–20 min removal of fish from a natural water body in summer at the temperature of 18–23 °C (stressor 10a) and at water temperature of 2–9 °C in autumn-winter (10b). The work also analyzes literature data (Martemyanov, 1999) on roach (*Rutilus rutilus* L.), obtained in winter. The longer term strong complex stressors were removal of fish from their natural environment followed by transportation of fish to the laboratory during 1.0–1.5 h in a limited volume of water in summer (water temperature equaled 19–24 °C) in conditions of autogenic hypoxia (decrease in the content of oxygen in water to 3 mg/l) and increase in water temperature by 5–7 °C, when there was observed practically full exhaustion of hormones in chromaffin tissue (stressor 11a) and in autumn at lower temperatures (4–9–16 °C) − stressor 11b. Individuals which died in the conditions of stressor 11b were selected into a separate group 11c. Stressor 12 was 1.5 h imitation of transportation in summer of fish which were adapted to laboratory conditions (Table 2). Stressor 13 – subacute stress: 9–15 days retention in laboratory conditions exposed to increased light and noise disturbance factors, with high placement density of (1 : 30), with periodic mechanical disturbance and elements of autogenic hypoxia. Stressor 14 – chronic stress: 1.0–1.5 months of retention in laboratory conditions with increased light and noise disturbance, with possible periodic low hypoxia. Also, fish in conditions of chronic stress were sometimes affected by low short term stressors: 1 (adrenalin in low doses), 2 (normal saline), 4 (prick), 7 (limited volume of water, Table 3). During the tests, fish were not fed.

The results of studying the concentration of magnesium in erythrocytes of fish were compared with the intensity of gas exchange and the value of affinity of hemoglobin for oxygen, obtained earlier (Zaprudnova & Kamshilov, 2010) for the same species of fish and the same or similar stress conditions.

The work presents the average values of concentrations of manganese ions and mean error. The check of the norm of distribution in the selections was made using the Shapiro-Wilk criterion. The reliability of the differences were assessed using Student's criterion.

Results

Under the impact of insignificant short term stressors (in the research presented – stressors 1–7), i.e. under physiological stress, concentration of sodium in blood plasma increased by 9.2%, recovered after 1–2 days. The level of magnesium in erythrocytes in the abovementioned conditions increased, reaching maximum values at the water temperature of 17–18 °C after 2 h after impact (Table 1). Subsequently, concentration of magnesium in erythrocytes gradually reduced, returning to pre-stress values after 1–2 days. The amount and duration of deviation of erythrocytic cation increased from low to average stressors. Injection of hormones, introducing normal saline, injection and prick (stressors 1–4) should be probably classified as average stressors: recovery of the level of erythrocytic manganese occurred only after 2 days, and concentration of magnesium in red blood cells increased by 2.33–2.56 times. Under the impact of the stressors 5–7 which were, obviously, weaker than the first four, concentration of magnesium in erythrocytes increased by 1.83–2.20 times, and return to the normal values was observed after 24 hours. In summer, fish of the Rybinsk Reservoir, while rising from the bed to the surface heated by the sun, every day have the same load as at the impact of stressor 6. As the water temperature decreased, the reaction to the stressor slowed: in winter, at the temperature of acclimation of 0.2 °C, increase in the concentration of manganese of red blood cells by 1.82–2.11 times was observed a day after injection of adrenalin (stressor 1) and prick to the body (stressor 3). And, on the contrary, in summer, at the water temperature of 24 °C, maximum deviation of the level of magnesium in erythrocytes occurred already an hour after injecting adrenalin (stressor 1) and normal saline (stressor 2): by 2.35 times on average.

Affinity of hemoglobin for oxygen of bream 1.5–2.0 h after the effect of short term not strong (weak and medium strength) stressors (low doses of adrenalin, saline injections, pricking, insignificant increase in water temperature, short term retention in small volume of water) increased by 1.34–1.71 times. In such conditionsб consumption of oxygen by bream, tench and and silver crucian carp decreased by 1.27–1.60 times (Zaprudnova & Kamshilov, 2010).

At acute reversible stress (stressors 8–12), i.e. at pathological stress, the concentration of sodium in blood plasma decreased by 28.3%. At the same time, the level of magnesium in erythrocytes decreased by 1.55–2.98 times. (Table 2). After 3–5 days (not studied before), we recorded normal level of this cation in erythrocytes: 9.6–11.1 mmol/l and recovery to pre-stress concentrations of sodium in blood plasma. During acute lethal stress (stressor 11c), the level of magnesium in erythrocytes was practically no different from that of fish which survived similar conditions.

Catching fish in the autumn-winter period caused a lower stress effect (stressor 10b) than in summer at higher temperatures (stressor 10a), and, therefore caused a smaller decrease in the level of magnesium in erythrocytes. Transportation of the fish was performed at a rather high temperature in autumn, therefore there are practically no differences between transportation of fish in summer. In the fish which adapted to the laboratory conditions, and therefore were less sensitive to stressors, during the effect of stressors of an equal strength (stressor 12), deviation of concentration of magnesium in red blood cells manifested less clearly, though the differences in relation to the norm were similarly significantly high (P < 0.001).

Table 1
Concentration of magnesium in erythrocytes (mmol/l) of bream after the impact of insignificant short term stressors (total number of the studied fish – 115)

Stressors^{x}	Time since end of exposure to stressors, hours					
	0.5	2.0	3.5	5.0	24	48
1 (adrenalin, 0.3–1.5 mg/kg)	14.5 ± 0.19***	26.1 ± 0.17***	22.3 ± 0.31***	18.1 ± 0.12***	16.1 ± 0.04***	10.1 ± 0.26***
2 (normal saline)	15.6 ± 0.20***	24.3 ± 0.15***	20.4 ± 0.19***	15.6 ± 0.26***	15.4 ± 0.08***	10.2 ± 0.15***
3 (noradrenalin, 0.2–1.3 mg/kg)	16.4 ± 0.18***	25.4 ± 0.22***	n.a.	14.7 ± 0.21***	n.a.	n.a.
4 (prick)	14.2 ± 0.15***	23.8 ± 0.30***	n.a.	16.2 ± 0.12***	n.a.	n.a.
5 (extraction from water for 1–2 min)	n.a^{xx}.	22.5 ± 0.21***	n.a.	n.a.	9.9 ± 0.14***	n.a.
6 (gradual increase in temperature of water by 4–5 °C)	n.a.	18.7 ± 0.29***	n.a.	n.a.	10.3 ± 0.16***	n.a.
7(limited volume of water)	13.9 ± 0.16***	n.a.	n.a.	n.a.	n.a.	n.a.

Note: ^{x} – norm see in Table 2, *** – P < 0.001, compared to the norm; n.a. – not analyzed.

Table 2
Concentration of magnesium in erythrocytes (mmol/l) of some freshwater fish after exposure to significant stressors (the number of the studied bream – 54, tench – 32, crucian carp – 20)

Stressors	Bream	Tench	Crucian carp	Roach (Martemyanov, 1999)
Norm	10.2 ± 0.21	11.0 ± 0.30	12.4 ± 0.28	9.8–10.7
8 (sharp change in water temperature by17–23 °C)	4.1 ± 0.37*** 3.7 ± 0.51***	3.6 ± 0.42***	5.5 ± 0.40***	n.a.
9 (adrenalin 5–6 mg/kg)	3.8 ± 0.26***	3.9 ± 0.29***	n.a.	n.a.
10a (removal from the natural environment in summer)	4.4 ± 0.35*** 3.6 ± 0.40***	4.8 ± 0.41***	n.a.	n.a.
10b (removal from the natural environment in autumn and winter)	6.3 ± 0.38*** 4.9 ± 0.36***	6.2 ± 0.37***	6.3 ± 0.42***	6.6–7.0***
11a (catching and transportation to the laboratory in summer)	3.2 ± 0.45***	n.a.^{x}	n.a.	n.a.
11b (catching and transportation to the laboratory in autumn)	4.1 ± 0.44***	4.3 ± 0.33***	4.6 ± 0.28***	n.a.
11c (lethal stress)	3.5 ± 0.46***	n.a.	n.a.	n.a.
12 (imitation of transportation in summer)	6.9 ± 0.39***	7.1 ± 0.36***	7.5 ± 0.41***	n.a.

Note: *** – P < 0.001 compared to the norm; ^{x} – n.a., not analyzed.

Table 3
Concentration of magnesium in erythrocytes (mmol/l) of some fish exposed to subsevere and chronic stress and after impact of additional insignificant stressors (the number of the studied bream – 29, tench – 14, crucian carp – 20)

Stressors^{x}	Bream	Tench	Crucian carp
13 (subsevere stress)	5.3 ± 0.40***	5.0 ± 0.38***	n.a.
14 (chronic stress)	7.2 ± 0.19***	7.4 ± 0.23***	7.6 ± 0.30***
14 (chronic stress) + 1 (adrenalin 0.3−1.5 mg/kg)	5.6 ± 0.32$^{\underline{***}\ **}$	n.a.^{xx}	n.a.
14 (chronic stress) + 2 (normal saline)	4.8 ± 0.37$^{\underline{***}\ ***}$	n.a.	n.a.
14 (chronic stress) + 4 (prick)	6.9 ± 0.35$^{\underline{***}}$	n.a.	5.6 ± 0.38$^{\underline{***}\ **}$
14 (chronic stress) + 7 (limited volume of water)	7.0 ± 0.36$^{\underline{***}}$	7.1 ± 0.33$^{\underline{***}}$	6.1 ± 0.43$^{\underline{***}\ *}$ 6.9 ± 0.39$^{\underline{***}}$

Note: ^{x} – norm see in Table 2; *** – P < 0.001, ** – P < 0.01, * – P < 0.05 under line – compared to the initial level at chronic stress, rest – compared to the norm, ^{xx} – n.a., not analyzed.

Affinity of hemoglobin for oxygen after exposure to strong short term stressors (catching, transporting, steep and high increases in water temperature) decreased by 1.23–1.35 times, and the consumption of oxygen increased on average by 1.50 times. However, the fish in a dying condition affinity of hemoglobin for oxygen increased compared the fish which were just caught: for example, bream – up to two times. Therefore, in these conditions, consumption of oxygen decreased: by 1.28–1.51 times (Zaprudnova & Kamshilov, 2010).

Subsevere and chronic stresses are also classified as pathological stress. Fish experiencing subsevere stress live 5 to 20 days, under chronic stress – 1–3 months. Having subsevere stress (stressor 13), hyponatremia equaled 19.5%, and concentration of magnesium in erythrocytes decreased by 2.06 times (Table 3). In fish with chronic stress (stressor 14), hyponatremia equaled 9.4%, and concentration of magnesium in erythrocytes was 1.51 times lower than the norm. However, affinity of hemoglobin for oxygen was close to the normal values: lower by 1.12–1.15 times. Also, reduced consumption of oxygen was recorded (Zaprudnova & Kamshilov, 2010).

In the conditions of chronic stress, 1.0–1.5 h after additional insignificant stress, the fish (when significant deviations in the concentration of erythrocytic magnesium should be observed) were in the half of the cases recorded as having a reliable decrease in the studied parameter, and in the rest – differences were not reliable for the initial level of manganese in red blood cells at chronic stress (Table 3). After additional insignificant stress, the level of natremia of fish decreased by 4.3–11.1%.

Discussion

The present research revealed the dependency of magnesium concentration in red blood cells of fish on intensity of stressor loads of different types: level of erythrocytic magnesium increased under the effect of insignificant and average stressors (at physiological stress) and decreased – under effect of significant loads (at pathological stress). The obtained data indicated also a broader range of fluctuations in magnesium concentrations in erythrocytes of stressed fish: a five-seven times change in the level of this cation. At the same time, similarly in both groups of significant and insignificant stressors, deviations became clearer after increasing load in duration and amount of magnesium concentration. Perhaps, there should be some load of intermediary value between average and significant stressors which does not cause deviations of magnesium concentrations in erythrocytes (condition of areactivity). It is possible that this phenomenon is related to absence of reaction of erythrocytic magnesium of people to intravenous injection of adrenalin (Ryzen et al., 1990). However, absence of reaction to the stressor in the abovementioned work could be also related to a particular latency in change of magnesium level in red blood cells during influence of an insignificant load. Practically instantly, during stress, change occurred only in concentration of erythrocytic sodium, along with the level of catecholamines which regulate it (Zaprudnova & Kamshilov, 2008).

The results of our work concur well with numerous studies by Konstantinov (1993) and his students on many species of fish, and also on amphibians in 1980–2000. The authors demonstrated that during insignificant fluctuations of practically all familiar abiotic factors (temperature, illuminance, oxygen, salinity, pH), i.e. at eustress or physiological stress, increase in growth and development of animals occurs, which is followed by decrease in consumption of oxygen and food. The present work states that at eustress or physiological stress, increase in concentration of magnesium of erythrocytes as a positive modulator occurs, contributing to increase in affinity of hemoglobin for oxygen and, therefore, reduction in gas exchange and, in consequence, increase in anabolism, growth and development of fish. And, by contrast, decrease in concentration of magnesium in erythrocytes during short term significant stress effect (alarm reaction of anxiety of general adaptation syndrome; distress or pathological stress) contributes to decrease in affinity of hemoglobin for oxygen and therefore increase in gas exchange, and, as a result, increase in catabolism. Under more prolonged effect of stressors (stage of exhaustion of general adaptation syndrome, subacute and chronic stress), one should take into account the effect on the affinity of hemoglobin for oxygen and, therefore, intensity of gas exchange, decrease in the level of ATP in erythrocytes (negative modulator of affinity of hemoglobin for oxygen) due to general exhaustion of the organism. Perhaps, this particular factor is related to relatively high affinity of hemoglobin for oxygen and decreased intensity of gas exchange, which were recorded before death among fish (at the end of pathological stress) with low concentration of magnesium in erythrocytes. It could be assumed that because of relatively high affinity of hemoglobin for oxygen and decrease in oxygen consumption during chronic stress, a condition of metabolic depression is maintained.

With deterioration of the condition (exhaustion, weakening, illness), and also against the background of the existing load, the fishes did not react to insignificant short stressors in relation to type of physiological stress, i.e. there was observed no increase in magnesium concentration in erythrocytes and no changes in the level of cations in the internal body environment towards increase in ion concentration gradients on the membrane of cells and tissues (condition of areactivity). Moreover, in these conditions, changes by type of pathological stress can occur, i.e. decrease in concentration of magnesium in erythrocytes and change in the level of cations in the internal environment towards decrease in ion concentration gradients on the membrane of cells and tissues. Earlier (Zaprudnova, 2017), at effect of insignificant doses of noradrenaline and pricking, the fish suffering ichthyophthiriasis had hyponatremia. In other words, the pattern and extent of reaction to a stressor depended also on initial functional condition of the organism.

On the basis of the conducted studies, magnesium ions in erythrocytes as positive modulators of affinity of hemoglobin for oxygen are recommended to be considered as one of the molecular mechanisms which regulate processes of gas exchange, anabolism and catabolism of fish under stress. An especially significant role of erythrocytic magnesium is played by increase in anabolism and, therefore, growth and development of animals under effect of insignificant, short term stressors, i.e. under eustress or physiological stress.

Also, a significant role in mechanisms of excessive anabolism and increase in resistivity of an organism to the impact of unfavourable factors during eustress (or physiological stress) is played by energy of increased ion concentration gradients on the membrane of cells and tissues (Zaprudnova, 2017). Ion concentration gradients on the cellular membrane belong to the parameters of energetic condition of the organism. In particular, sodium potential belongs to the main "energetic currency" on the external membrane of animals` cells (Skulachev et al., 2010). Finally, ion concentration gradients can be considered an expression and mechanism of maintaining the stable metabolism of the organism. In natural conditions, insignificant short term stressors create the required condition of physiological stressors which cause a stimulating effect on all vital processes. Strong anabolic processes of fish during spawning also occur due to using energy of increased ion concentration gradients on the membrane of cells and tissues, and also minimization of energy discharge due to decrease in oxygen consumption. The latter is possible due to the high affinity of hemoglobin for oxygen because of increase of magnesium (the positive modulator) in erythrocytes (Zaprudnova & Kamshilov, 2016).

Energy released as result of decrease in ion concentration gradients at the effect of significant and/or long term stressors is used for maintaining life in extreme conditions.

Concentration of magnesium in erythrocytes of freshwater and marine fish is similar. For example, the fluctuation range of this parameter in marine fish equaled 9.8–12.8 mmol/l (Soldatov, 1997). However, it is interesting that the values of magnesium in erythrocytes of humans are lower compared to fish: 1.4–2.3 mmol/l in the norm. This parameter is extremely low for many other mammals, for example, cattle < 1 mmol/l (Cox et al., 1991; Hinds et al., 1994; Flatman, 2003; Kopitsyna et al., 2015). It could be presumed that for humans, this reflects the condition of stress in regular life due to high psychological-emotional loads, intoxications, etc, which are typical for most of the population (Segerstrom & Miller, 2004). Moreover, people with psychologically borderline disorders have an erythrocytic magnesium level < 0.3 mmol/l (Kopitsyna et al., 2015). The conditions of laboratory animals before the study in vivaria are usually insufficiently favourable. Also, one should take into account the effect of stress procedures which are implemented prior to drawing blood from people and other mammals, which are more sensitive to stress than fish. With psychologically ill people, these procedures can have an especially frightening effect. It could be presumed that significantly different levels of erythrocytic magnesium (and even their oppositely orientated changes) among people with identical psychological illnesses mentioned in the works of a number of authors (Cox et al., 1991; Hinds et al., 1994; Widmer et al., 1995; Widmer et al., 1998; Kopitsyna et al., 2015) to a larger extent can be a manifestation of non-specific change of this cation in different stress conditions of patients rather than the specific effect of disease. From this perspective, six-fold differences (Flatman, 2003) in the concentrations of magnesium in red blood cells of swine and cattle could be explained. However, there is obviously a specific relationship between magnesium in red blood cells and some diseases, for example, sickle cell anemia (Flatman, 2003).

It should be recognized that until recently, the level of erythrocytic magnesium as a diagnostic parameter of animals (and humans) has remained underestimated. However, this question should be analyzed in more detail. In particular, the high variability of concentrations of erythrocytic magnesium of fish under stress (increases of up to 2.5 times and decreases of up to 3 times), to some extent limits the opportunity of its use as an accurate indicator of deficiency of this cation in the organism. An important recommendation for obtaining more reliable information on the condition of an organism is regular analysis of the level of magnesium in red blood cells of animals (and humans) in different functional conditions. The results of our studies on fish demonstrated that high levels of this cation in erythrocytes (1.5–2.0 times higher and more than the norm) is distinctive for strong, healthy animals in a condition of eustress or physiological stress, and extremely low values of this parameter (1.5–2.0 times and more lower than the norm) are an indicator of both reversible and constant ill-being (distress or pathological stress). Absence of reaction of concentration of erythrocytic magnesium to insignificant short term stressors towards increase, and more important – change towards decrease in the level of this cation also indicates ill-being. Diagnosing the condition of animals in relation to the magnesium concentration in the internal environment of an organism is still complicated. First of all, this is related to the fact that during physiological stress, short term change in magnesium concentration in blood plasma occurs towards increase in concentration gradients on the membrane of cells, i.e. hypomagnesemia, and during pathological stress, by contrast, more or less prolonged hypermagnesemia (Zaprudnova, 2017). Therefore, the observed hypomagnesemia of humans after intravenous injection of adrenalin is mistakenly determined by some authors (Ryzen et al., 1990) as a negative effect. A low level of erythrocytic magnesium is a more reliable characteristic of ill-being: temporarily or constant.

Conclusion

For the first time, the relationship of change in the concentration of magnesium in erythrocytes to the intensity of stress loads of different type was determined on the example of three species of fish: bream, tench, silver crucian carp. Insignificant and average loads (eustress or physiological stress) increased the concentration of magnesium in erythrocytes, while the same factors but in higher doses, and also complex effects of strong stressors (distress or pathological stress) decreased the content of magnesium in erythrocytes. During eustress, we observed increase in affinity of hemoglobin for oxygen and decrease of consumption of oxygen, and during distress – shift of dissociation curve to the right and intensification of gas exchange. On the basis of the conducted study, ions of magnesium in erythrocytes (as positive modulators of affinity of hemoglobin for oxygen) are recommended to be considered as one of the molecular mechanisms regulating the processes of gas exchange and metabolism of fish under stress. An especially significant role of these ions is intensifying anabolism and, therefore, growth and development of animals exposed to the influence of not strong (weak and medium strength), short term stressors,i.e. to eustress or physiological stress. However, before death, the fish (at the end of pathological stress) at low concentration of magnesium in erythrocytes had increased affinity of hemoglobin for oxygen, and reduced intensity of gas exchange. Perhaps, in this situation, the essential role in the shift of dissociation curve to the left is played by decrease in the level of ATP – negative modulator of affinity of hemoglobin for oxygen – due to the general exhaustion of organism. Concentration of magnesium in erythrocytes can be an indicator of the condition of fish, in particular, an extremely low level of magnesium in red blood cells always indicates temporary or constant ill-being. Weakened, exhausted, diseased animals are not able to react in response to type of eustress or physiological stress.

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