Level of natremia as an index of the condition of the organism of animals under stress

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In the diagnosis of stressful conditions in humans and other animals, ionic indicators remain practically unused. In this work, we studied the changes in the concentrations of sodium ions in the blood plasma of freshwater fish under stress caused by stressors of different quality and quantity. Most of the experiments were carried out on adult bream (Abramis brama L) from the Rybinsk Reservoir. Separate experiments were duplicated on adult individuals of roach (Rutilus rutilus L.), pike (Esox lucius L.), and blue bream (Abramis ballerus L.).

The concentration of cations in the blood plasma was determined using a Flapho-4 flame photometer. Under the action of mild and short-term stressors of different qualities, the sodium concentration in the internal environment altered toward an increase in concentration gradients on the cell membrane (eustress or physiological stress). Hypernatremia was approximately 10%. Under the action of strong and/or prolonged stressors of different strength, the sodium concentration in the internal environment changed toward a decrease in concentration gradients on the cell membrane (distress or pathological stress). Hyponatremia was 50% in the conditions of acute lethal stress, 20% in subacute lethal stress, 10% or more in chronic lethal stress. During strong acute reversible stress, hyponatremia could reach 30%. Analysis of the material on mammals allowed us to conclude that the adaptation mechanisms in fish and higher vertebrates are similar. In this work, for the first time, the state of the system of electrolyte balance of animals under stress was analyzed from the standpoint of the leading role of ionic concentration gradients on the cell membrane (mainly sodium) in the energetics (level of disequilibrium) of the organism. We propose a concept that in normal and extreme conditions fish use two different defense reactions (or adaptation strategies): active and passive, consisting, respectively, in increasing or decreasing the level of disequilibrium (energy) in the organism. The hyponatremia recorded by numerous authors, which accompanies diseases in humans, is evidently a nonspecific reaction of the organism and serves as an indicator of reduced energy of the organism. It is suggested that the sodium level in the internal environment of the organism be used for diagnosing the stress state of animals.

Keywords: fishes; mammalians; energy of the organism; ion concentration gradients; active and passive defense reaction; eustress; distress.

Materials and methods

The main object of the studies was mature and close-to-maturity bream (Abramis brama L.) of the Rybinsk Reservoir. Separate experiments were performed on adult individuals of pike (Esox lucius L.), roach (Rutilus rutilus L.) and zope (Abramis ballerus L.) from the same water body.
Experiments were performed in summer, when water temperature equaled 17–20 °C in the conditions of acute, subacute and chronic stress with lethal outcome, and also acute reversible stress of different degree of strength and duration. Low stress (Fig. 1) was caused by putting acclimatized fish into limited water volume (ratio of body weight to water was 1:20) with continuous aeration. Strong reversible acute stress was caused by short-term capture of fish in the natural environment (no longer than 15 min) and their transportation to the laboratory for 1.5–2.0 hours in a limited volume of water (Fig. 2a, 3a) and rapid change in the temperature by 18 °C (Fig. 3a). Acute lethal stress lasting 5 to 12 h was caused by complex action of several strong factors: capture, transportation in a limited volume of water with decreasing oxygen content (to 2.5 mg/L) and increasing temperature (to 25 °C, Fig. 2b, 3b) and irritation of all these factors on acclimated fish (Fig. 3b). Subacute stress with lethal outcome lasting 5–18 days was caused by quite strong stressors, which fish could not adapt to: post-traumatic effects of long trawling and hard transportation conditions (Fig. 2c, 3c) and retention of fish in containers (Fig. 3c). Chronic lethal stress lasting 1–3 months was caused by retention of fish in the laboratory conditions against the background of increased light and noise irritators (Fig. 2d, 3d) and in non-favourable conditions of ponds of experimental base (Fig. 3d). Each experimental point for bream was represented by 11–24 fish, and by 5–9 individuals of other species.

Concentration of cations in blood plasma was analyzed using Flapho-4 flame photometer manufactured by Carl Zeiss (Jena, Germany). The study presents the mean values of concentrations of ions and standard error (x ± SE). The normal distribution for the selections was tested using the Shapiro-Wilk criterion. We determined that all the selections had normal distribution. Significance of the differences was assessed using the t-test.

Results

In the conditions of influence of weak and short-term stressors (Fig. 1), the content of blood sodium of fresh-water fish may increase 10% on average. Approximately to the same extent, the sodium concentration may decrease during chronic stress with lethal outcome (Fig. 2d, 3d). During subacute stress with lethal outcome, caused by powerful stressors, to which fish was not capable to adapt, hyponatremia reached 20% (Fig. 2c, 3c). During severe acute stress, loss of sodium from the internal environment reached nearly 50%, thus resulting in the quick death of the animals (Fig. 2b, 3b). Maximal possible hyponatremia, in which fish survive, observed during acute reversible stress, may equal 30% (Fig. 2a, 3a). There is a direct dependence between the level of hyponatremia and the strength of stressors.

Fig. 1. Dynamics of the sodium concentration in blood plasma of some fish during eustress (physiological stress, mmol/L): *** – P < 0.001 compared with the norm

Outside of the short-term weak actions, there were seen changes toward increase in concentration gradients on the cell membranes (hypernatremia, eu-stress), while in the conditions of strong or prolonged actions – toward decrease (hyponatremia, distress). At the same time, hyponatremia was seen in all unfavourable conditions.

As demonstrated earlier (Zaprudnova, 2012, 2017), changes the concentrations of other main cations in the internal environment (potassium, calcium, magnesium) during acute stress reaction to short-term weak stressors are oriented toward increase in concentration gradients on the cell membranes (hypokalemia, hypercalcaemia, hypomagnesemia), during strong or long-term actions – toward decrease (hyperkalemia, hypocalcaemia, hypermagnesemia). Under subacute and chronic stress, before fish died, the concentrations of these ions in blood plasma (serum) were close to the norm. Thus, hyponatremia may be considered the most expressed and reliable ion indicator of malfunctioning of the organism. Many other biochemical parameters also changed noticeably following acute stress loads, while weakly reacting or not responding to subacute and chronic stress at all (for example, glucose, catecholamine s, etc.).

Discussion

General mechanisms of adaptation were found in the system of electrolyte balance in fresh-water fish, related to the amount (strength and duration) of the stressor. The stressors were classified according to quality. However, the pattern of their action, as demonstrated in this and the earlier articles (Zaprudnova, 2012, 2017), depended on the intensity of the action:

Fig. 2. Dynamics of sodium concentration in blood plasma of bream in distress (pathological stress, mmol/L): a – acute reversible stress (duration of 1.5–2.0 h); b, c, d – respectively acute, subacute, chronic lethal stress up to the point of death of fish (dark circles indicate the condition before death); **P < 0.01, *** – P < 0.001 compared with the norm

Fig. 3. Concentration of sodium in blood plasma of some fish in distress (pathological stress, % of the norm, horizontal line): a – acute reversible stress lasting 1.5–2.0 h; b, c, d – the data seen before the fish died, respectively in the conditions of acute, subacute, chronic lethal stress; 1, 2, 3, 4 – bream, roach, pike and blue bream respectively
Changes in the concentrations of ions in blood plasma occurred under short-term weak stressors during spawning, and under long-lasting stressors after spawning (Zaprudnova & Martemyanov, 1988).

In medicine, the important (leading) role of sodium concentration in blood serum (plasma) is first of all emphasized for the support of osmolality of the internal environment, single-cell environment, and ultimately, volume-regulation of each cell. This study has for the first time analyzed the conditions of the system of electrolyte balance of animals under stress from the perspective of the leading role of ion-concentrating gradients in the energetics of the organism. It would be more correct to talk about electrochemical gradient on the cellular membrane, but we only have concentration of sodium in the internal environment. Sodium ions take part in energization of the external membrane of animal cells: work of the sodium pump results in the functioning of most of the transport functions of cells (from epithelial to cartilage and brain cells), which are implemented not due to the consumption of energy of ATP particularly, but rather the energy of ion gradients or membrane potential (Ugolev, 1985; Natochin, 2002; Skulachev et al., 2010). Therefore, we may assume that support of constant ion composition of the internal environment of the organism (mainly sodium) is, to a larger degree, the support of the energetic support of the organism, i.e. one of the most important, and perhaps priority functions of the organism – activates in extreme conditions, when the evolutionally important role belongs to erythrocytic manganese during influence of short-term weak stressors as one of the molecular mechanisms of reduction of oxygen uptake, and therefore, strengthening of anaerobism, and thus increase in growth and development of animals (Zaprudnova, 2019).

The presented provisions on the two strategies of adaptation in fish in normal and extreme conditions correlate with the provisions of Selye’s theory of eustress and distress, or Arshavsky’s theory of physiological and pathological stress (Dhabhar, 2008; Schreck, 2010; Schreck & Tort, 2016).

Based on the proposed concept of two protective reactions in normal and extreme conditions or adaptation strategies – active and passive, comprising respectively increase or decrease in the level of imbalance (in energetics) of the organism, one may formulate the main principles of diagnostics of fish in natural and artificial environments according to the condition of electrolyte balance. The deviations toward decrease in ion concentration gradients across the membrane of cells and the tissues may be considered as indicators of one or the other extreme conditions (pathology), whereas changes in opposite direction: toward increase in concentration gradients as a sign of pathology that may develop in the conditions of extreme stress. The first sign of illness is malfunctioning of functioning system in the studied parameters (the finest level of biological organization). Transition through the phase of hypercompensation (stimulation) to the phase of reduction (depression) is a general pattern in response reaction of biological systems of different organization level to increasing stimuli or its incessant action at the same intensity. The condition of reactivity occupies an intermediate position between the phases of hypercompensation and reduction. At the same time, as mentioned above, the level of natremia is the most reliable indicator of the condition of the organism outside the 4 major cations. The most characteristic signs of active protection reaction (physiological stress, or eustress) include hypernatremia, and passive (pathological stress or distress) includes hyponatremia. Assessment of the level of natremia should take into account not only amplitude, but also time factor as well. It is important to determine the extent of stability (in time) of hyponatremia in the diagnostics of pathology itself (actual) in order to exclude short-term (reverse) hyponatremia during acute stress. The advantage of this parameter is low variability and some latency (retardation of changes) in the organism.

Analysis of information in the literature about the condition of the system of electrolyte balance in mammals (chiefly humans) in stress conditions and diseases, and also the extent of the pressure an experimental object of research experiences, suggests a conclusion about the similarity of mechanisms of adaptation to stress in fish and higher vertebrates: during short-term weak loading, deviations occurring in the system of electrolyte balance are oriented toward increase in ion concentration gradients across the membrane, and toward decrease during strong or long-term loading. The most revealing work concerning this aspect was performed by Indian scientists on rats in the conditions of high hypoxia. The authors determined that raising the animals to 2–5 km above sea level led to hypernatremia, then at the height of 7.5 km, absence of reaction to the impact was observed, and further elevation to the height of 10.5 km led to hyponatremia and death of the animals (Purshottam & Ghosh, 1971).

As the main cause of changes in the content of ions in the tissues, the authors mention acute hypoxia. In this and other similar earlier studies, no attempts were made to determine general mechanisms of adaptation. The authors of the mentioned study (Purshottam & Ghosh, 1971) also presented literature information about the results of studies of influence of high altitude hypoxia on content of sodium and potassium ions in the tissues of rats, dogs and human volunteers, conducted by Soviet and foreign researchers in the 1930–1960s. The analysis of these literature data allows us to conclude that they are coherent with our concept of two strategies of adaptation of animals in normal and extreme conditions: i.e. de-
pending on strength of stressors (amount of hypoxia or height above sea level, and also time of exposure), the pattern of response reaction can take its course according to the type of hypercompensation or reduction, and also no changes may occur at all (condition of inertivity), as a result of restoration of the functions during multi-hour observations among other reasons.

The most numerous studies of ion-regulation performed on people in extreme situations were carried out by domestic and American authors on cosmonauts (and astronauts) during flights. At the same time, the researchers considered weightlessness as the main (disturbing) factor and drew no conclusions on general mechanisms of adaptation (Gazenko et al., 1980; Grigorev et al., 1996; Natochin, 2000). In short, the patterns of changes in the human system of electrolyte balance during space flights, determined by the authors, are coherent with the dependence of the pattern and extent of response reaction in fish on the amount (strength) of stressor and initial functioning condition of the organism, which we determined. Most often, after long flights, the cosmonauts were observed to have hypokalemia and hypercalcemia. In a number of cases, hypernatremia and increase in osmolality of blood were also observed. That means, all of those are “symptoms” of physiological stress. Hypernatremia and hypercalcemia were usually observed only during the very first days after return to Earth. However, hypocalcaemia increased a week later, reaching 50%. For us it is obvious that in the conditions of hyperpolarized inhibition, there occurred powerful restorative (anabolic) processes in cosmonauts who had lost weight during the flights (particularly, decrease in the volume of thighs was recorded). However, right after the take off (during transition to weightlessness), hyponatremia was observed (i.e. shifts according to the type of pathological stress). Low hypernatremia (1.5%) was seen after landing after short flights. We may assume that decrease in concentration of sodium in blood plasma would have been more notable if before landing the cosmonauts had not received food rich in sodium chloride. Moreover, during flights and as a result of the flights, the individual variability of the surveyed parameters increased. For example, after landing, the coefficient of sodium variability in blood plasma doubled. Individual differences in reactions to extreme conditions of flights were so high that in a number of cases, members of one crew were observed to have changes not only in the amount, but also in the orientation of response reaction.

That means that in some individuals, the shifts in the system of electrolyte regime of balance occurred as a type of physiological stress, while in others, a clear tendency was seen in the change in a number of parameters toward pathologization. Mass media reports of approximately twenty years ago inform us that one of the cosmonauts suffered myocardial infarction during the flight. Selye classified it to idiothetic or pluocratic diseases, i.e. caused by non-specific (stress) irritants. To those categories, he also identified cancer, arthritis, osteochondrosis, stroke and other. In other words such diseases are called adaptation diseases. Under very strong loads (space flights), it was possible to classify people based on the magnitude and orientation of ion changes according to the extent of stressors. On fish, it was shown that in the initial period of the disease, the changes in concentration of ions in the tissues were directed (acceleration) and during the late stages of the disease – decrease (reduction). This is relevant for both contagious (Larnarski, 1984; Zapadnouva, 2013) and non-contagious diseases (Natochin et al., 1995).

Changes in the concentration of ions in patients (the organs) suffering adaptation diseases also occur according to the type of reduction, i.e. toward decrease in concentration gradients across the membrane of cells and tissues. Selye observed decrease in concentrations of potassium and increase in sodium in the cardiac muscle in patients suffering myocardial infarction. Further, such a dynamic in ion content of the heart muscle, as well as changes according to the type of reduction in the internal environment, were seen by many researchers. The authors determined direct dependence of the degree of damage to the heart muscle on the decrease in the concentration gradients of sodium and potassium across the membranes of cells. With improvement of the clinical condition of patients, ion composition in the tissues normalized. As known, during oncogenesis, ion composition of diseased cells changes according to the reduction type (Malenkov, 1976).

However, during strokes (brain ischemia or brain hemorrhage), also related to adaptation diseases, both hypernatremia and hypernatremia were recorded in earlier studies (Vilenski et al., 1998; Carcel et al., 2016; Murphy, 2016). Perhaps, in this case, we need to consider specific changes in the system of electrolyte exchange. We may assume that during ischemia or brain bleed, the nervous structures related with hormone regulation of electrolyte exchange (hipothalamus, pituitary gland) become damaged, for example, vasopressin is being generated insufficiently (antidiuretic hormone) thus weakening the action of the hormone towards the corresponding cell receptors. Hypernatremia may also occur during depression (Ozderni, 2013). During this disease, the deficit of the influence of antidiuretic hormone on renal structures is likely related to excessive influence of it on the corresponding brain structures.

Therefore, a question arises of why dependence of the pattern of response reaction of the electrolyte balance system on the amount (strength and duration) of stressor has not been seen in higher vertebrates? There may be two main reasons for this. First of all, in medicine and other spheres of biology, on higher vertebrates, i.e. directly or indirectly related to medicine, the priority is still the study of specific component of response reaction (i.e. quality to the disadvantage of quantity) and significantly less attention is paid to the study of general mechanisms of adaptation. First of all, there are specific reasons of the disease, associated for example with excess or deficiency of the action of hormones that regulate electrolyte balance of the organism, and also with disorders in consumption of water and salt, both arbitrary and not. The second reason is the high level of homeostasis in mammals, which manifests both in narrower range of
deviations of ion content from the normal values and in the rarely applied

nism prefers to maintain sodium homeostasis (absorbing or containing

low water temperatures (Zaprudnova, 2005). In mammals, even during

duction type in fish are more common and occur more often than in hig-

brates, dependence of the changes in the system of electrolyte balance on

the system of electrolyte balance. In other words, in lower aquatic verte-

considered as one of most the important signs of progressing evolution of

in acute lethal cases is lower than in fish (Purshottom & Ghosh, 1971).

That being said, the broad range of fluctuations of normal values of

sodium concentrations in blood plasma in humans (130–150 mmol/L),

reported in many books over several decades, is surprising. Possibly, the

currently proposed normal range of natremia in humans is also quite wide:

135–145 mmol/L. This is even stranger because we observed fish that

belong to lower vertebrates to have a narrow range of the normal values of

natremia: in bream, for example, it equaled 130–133 mmol/L in the nor-

mal laboratory conditions and ecologically clean districts of the Rybinsk

Reservoir (Zaprudnova, 2018). Even slight deterioration in the mainte-

nance conditions of fish in the laboratory caused low (2–4%) hyponatrem-

ia (Zaprudnova, 1999), and bream in ecologically unfavourable districts
could be observed to have low hypo- and hypernatremia, and normona-
tremia (condition of areactivity) depending on time and intensity of the

action of the stressor (Zaprudnova, 2018). After significant exacerbation

of circulatory conditions as a result of large industrial accidents, sodium

concentration in the blood plasma of bream could drop down to

80 mmol/L (Martenyanyov, 2014). However, we should recognize that in

the studies on humans, there already are emerging researches reporting a

narrower range of normal values of natremia. Therefore, sodium concen-

tration in blood plasma equaling 136 and 138 mmol/L was considered a

marker of mortality and risk of cardio-vascular diseases in elderly men

(Wannamethee et al., 2016).

In the times of Selye, the role of retention of sodium and water in the

organism during stress, evoked by elevation of aldosterone in blood and

accompanied by strengthening of inflammatory processes in the tissues

and rise in blood pressure, has been unclear. Later, aldosterone was found to

be associated with an increased risk of death from cardio-vascular dise-

ases (Tomashchitz et al., 2010). The adaptive significance of this hormone

may be explained from the perspective of energetic role of sodium poten-
tial. Among hormones in fish, a function similar to aldosterone, aimed at

preservation of sodium in the organism under stress, was performed by

prolactin and cortisol influencing the gill epithelium.

The results of our own researches on fish and analysis of the literature

material on the condition of the system of electrolyte balance in mammals

during stress and diseases allows us to conclude that maintenance of con-

stancy of ion content in the internal environment (mainly sodium), is, to a

significant degree, the support of energetic of the organism, i.e. one of the

most important and maybe prior mechanisms of survival under pressure

(stress). While not denying the existence of specific reasons for hyponat-

tremia, we consider it in most cases as a system reaction, or it indicates
decrease in the energetics of the organism. This is especially evident du-

ing adaptation diseases. The level of sodium in plasma (serum) of blood

may be a reliable indicator of the organism’s condition.

Conclusions

During the influence of strong or long-term stressors of various quali-
yty, sodium concentration in the internal environment of fish changed
toward decrease in concentration gradients across the cellular membrane
(hyponatremia, distress or pathological stress), during the influence of

weak and short-term – toward increase in ion concentration gradients
(hyponatremia, eustress or physiological stress). Judging from the litera-
ture data, changes in ion composition of the internal environment of higher
vertebrates during stress are similar to those of fish. In the present research,
for the first time the analysis of the condition of the system of electrolyte
balance of animals during stress was made from the perspective of the
leading role in the organism’s energetics of ion concentration gradient
across the membrane of cells. The study suggests that there are two diffe-
rent strategies of adaptation depending on intensity of the action of stres-
or: active and passive. Hyponatremia that accompanies many diseases in
people (mainly, diseases of adaptation) is supposedly a non-specific re-
ation of the organism and an indicator of decreased energetics of the orga-
nism. Sodium level in the internal environment of the organism is pro-
posed for diagnostics of stress condition of animals.

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