

EXPERIMENTAL STUDY

Volume regulation of muscle cells in the carp *Cyprinus carpio* in response to hypernatremia

Martemyanov VI¹, Poddubnaya NY²

Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Yaroslavl oblast, Russia.
martem@ibiw.yaroslavl.ru

ABSTRACT

BACKGROUND: Hypernatremia supports the movement of water from the intracellular to the intercellular space. This shift leads to cells shrinkage and disruption of intracellular processes, representing risk factors of morbidity and mortality in clinical circumstances. On the other hand, hypernatremia triggers protective mechanisms that counteract damage of cells.

OBJECTIVES: To determine in experiments *in vivo* the ranges of sodium content regulation in the blood plasma of carp characterizing the norm and hypernatremia. To identify the patterns volume regulation of skeletal muscles cells in response to hypernatremia.

METHODS: Carps were acclimating for 3 weeks to a different salinity in the range of 0–12 g/L. In the plasma and muscle tissue the concentration of sodium, potassium, calcium and magnesium was determined by the method of flame spectrophotometry. Water content in the muscles has been additionally determined.

RESULTS: Carps acclimated in the salinity range of 0–6 g/L, maintained the concentration of sodium in blood plasma within of the range of 129–135 mmol/L (normonatremia). In the salinity zone of 6–12 g/L concentration of sodium in the blood plasma of fish has increased to 207 mmol/L (hypernatremia). Hypernatremia was causing the increase of the sodium, potassium, calcium and magnesium content in carp muscles and the drop of water level.

CONCLUSIONS: Muscle tissue of carp adapts to hypernatremia by means of increasing inorganic ions by 70.8 % and organic osmolytes by 29.2 % (Fig. 2, Ref. 51). Text in PDF www.elis.sk.

KEY WORDS: blood plasma, muscle tissue, hypernatremia, volume regulation of muscle cells.

Introduction

Normally, the level of sodium in human blood plasma is maintained in the narrow range of concentrations (1, 2). Higher concentrations of sodium in the blood plasma reveal elderly people and children with symptoms of diarrhea (3–5). Hypernatremia is often revealed by the patients of intensive therapy departments creating the risk for mortality (1, 6–8). Hypernatremia leads to an increase of the internal environment osmolality (9) causing a drop in osmotic pressure between the extracellular and intracellular body fluid. Cells of various organs and tissues of the body must have adaptive mechanisms to this situation. Since taking of human tissue samples is prohibitive, adapting mechanisms of the body cells to hypernatremia are studied on animals, usually *in vitro*.

Adaptation mechanisms of the erythrocytes of carp *Cyprinus carpio* and the river flounder *Platichthys flesus* to hypernatremia

in experiments *in vitro* and *in vivo* differ significantly (10). This shows that for adequate understanding of the processes that occur when different types of body cells are adapting to the increase of sodium concentration in the extracellular fluid, data obtained *in vivo* is required. It is especially important to take this into account in clinical examinations and developing of treatment methods from hypernatremia consequences for humans.

It is determined that in freshwater hydrobionts in the zone of critical salinity (10–15), and in euryhaline species during transition from freshwater to seawater (16–20) the level of sodium and chloride ions in the blood plasma increases. This situation is an ideal model for studying adaptation mechanisms of various organs and tissues of organisms to increasing sodium concentration of extracellular fluid *in vivo*.

Work objective – to determine in experiments *in vivo* the ranges of sodium regulation in the blood plasma of carp which characterize the norm (normonatremia) and hypernatremia. To identify the patterns volume regulation of skeletal muscles cells in response to hypernatremia.

Materials and methods

The experiments were performed with carps of the age 1+ in winter period at water temperature of 13–14 °C. The groups of six specimens were placed in 300-L aquaria filled with artemesian water (contents of sodium, potassium, calcium, and magne-

¹Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Yaroslavl oblast, Russia, and ²Cherepovets State University, Cherepovets, Vologda oblast, Russia

Address for correspondence: V.I. Martemyanov, Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, 152742 Borok, Yaroslavl oblast, Russia,

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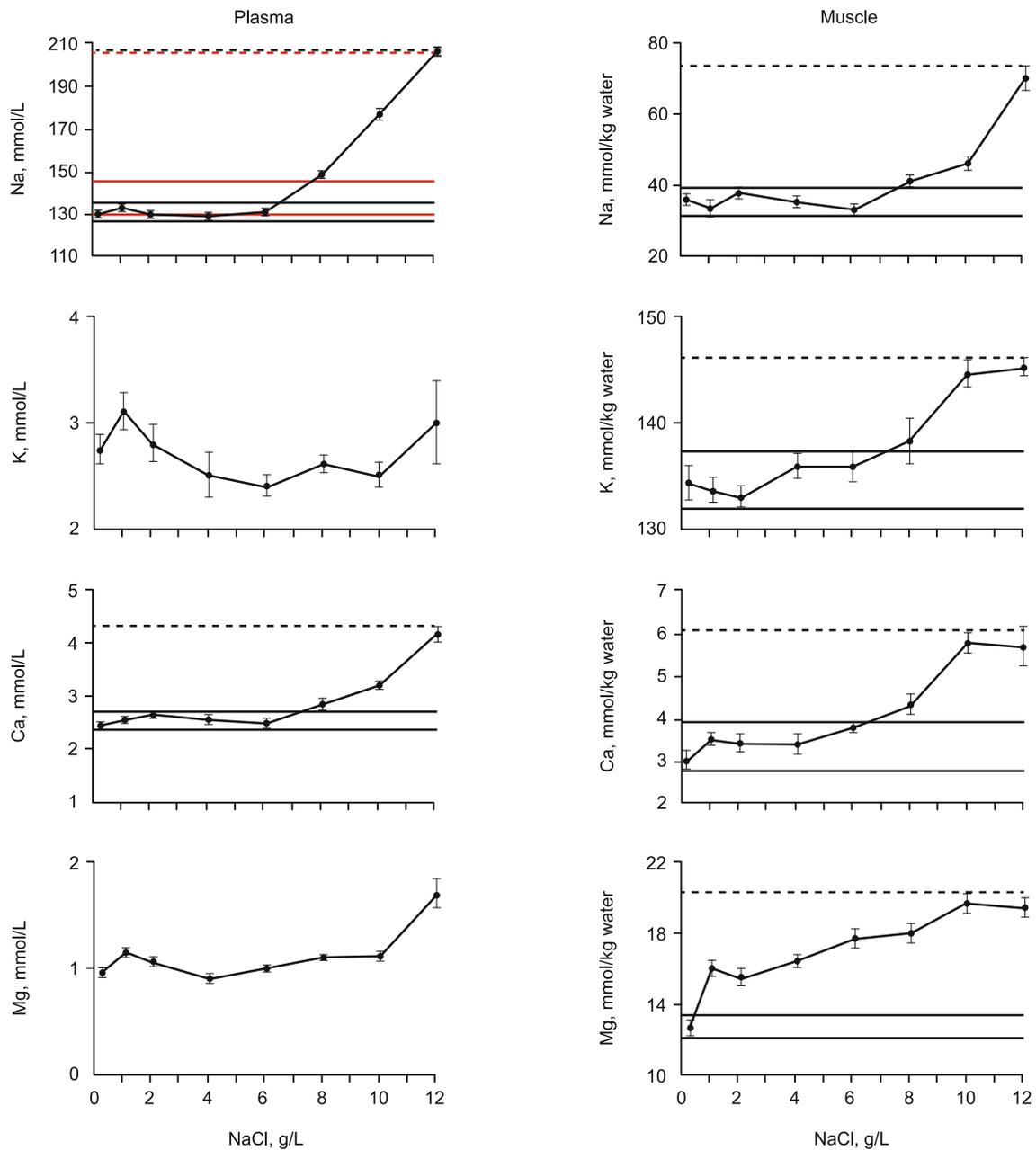


Fig. 1. Regulatory ranges of sodium, potassium, calcium, magnesium in the blood plasma and carp muscle tissue are at norm (borders are marked with solid black lines) and at hypernatremia (the upper limits are indicated by dashed lines of black color). Red solid lines show the boundaries of normonatremia, and the dashed line shows the border limit of hypernatremia in the human blood plasma.

sium were 0.46, 0.04, 2.00, and 0.48 mM/L, respectively). In one aquarium, the fish specimens were kept in artesian water; in the other seven NaCl of 0.5 g/L was added daily. When NaCl concentrations reached 1, 2, 4, 6, 8, 10, and 12 g/L respectively, salting was stopped and the fish specimens were maintained in the given constant conditions for not less than 3 weeks. Following this acclimation period, the fish were quickly caught and the blood was sampled from the caudal artery using a Pasteur pipette. Muscle tissue was sampled in the zone of the 2nd–4th ribs. The subse-

quent procedures of blood and muscle sampling were performed as described previously (21).

Concentration of sodium and potassium in the samples was measured using propane by a Flapho-4 spectrophotometer; concentration of calcium and magnesium was measured in an air-acetylene flame in absorption regime by an AAS-1 atomic absorption photometer (CarlZeiss, Iena, Germany). Ion concentration in the plasma is defined in mmol/L, in the muscle – in mmol/kg of water. Water content in the muscle is defined in percentage.

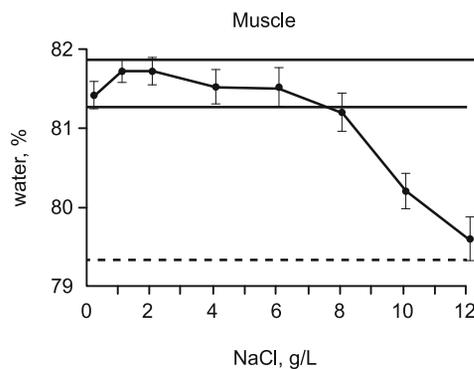


Fig. 2. The range of the water level regulation in carp muscles at norm (the borders are separated by solid lines) and at hypnatremia (the border is marked with a dashed line).

Average results and their standard deviations have been presented. Accuracy of the differences was evaluated with the help of Student coefficient with confidence probability of $p \leq 0.05$. Relationship between the environment salinity and the studied parameters have been evaluated based on the Spearman rank correlation coefficient (r_s); for sampling less than 5 – based on the accuracy approximation coefficient (R^2).

Results

Carp acclimated in the salinity range of 0–6 g/L maintained the concentration of sodium in the blood plasma within the range of 129–135 mmol/L (normonatremia), and in the muscle tissue 32–39 mmol/kg water (Fig. 1, the range boundaries are highlighted by solid black lines). In the salinity zone of 6–12 g/L, sodium concentration in blood plasma of fish has increased exponentially ($R^2 = 0.996$) to 207 mmol/L, and in the muscle tissue to 73 mmol/kg water (the upper boundaries of the ranges are indicated by dashed lines).

Concentration of potassium in the blood plasma of carps did not depend on the environmental salinity ($r_s = -0.065$). Carps acclimated in the salinity range of 0–6 g/L maintained the potassium concentration in the range of 132–137 mmol/kg water (Fig. 1, the range boundaries are highlighted by solid lines) in the muscle tissue, and in the salinity zone of 6–10 g/L increased to 146 mmol/kg of water. In the salinity zone of 10–12 g/L, the level of potassium in the muscle of carps did not change.

In carps acclimated in the range of 0–6 g/L, the calcium content in the blood plasma did not depend on the salinity of the medium, being maintained in the range of 2.4–2.7 mmol/L (the boundaries are marked by solid lines), and in the salinity zone of 6–12 g/L has significantly increased ($R^2 = 0.961$) to 4.3 mmol/L (marked with a dashed line). Carps acclimated in the salinity range of 0–6 g/L, maintained calcium concentration in the muscle tissue in the range of 2.8–3.9 mmol/kg water (Fig. 1, solid lines), and in the salinity zone of 6–10 g/L increased to 6.1 mmol/kg water (indicated by a dashed line). In the salinity zone of 10–12 g/L, the level of calcium in carp muscle did not change.

Carp acclimated in the range of 0–10 g/L maintained magnesium concentration in the blood plasma within 0.9–1.1 mmol/L, and in the critical salinity of 12 g/L sharply increased to 1.8 mmol/L. Carps acclimated in fresh water regulated concentration of magnesium in the muscles in the range of 12.1–13.3 mmol/kg water (Fig. 1, marked with solid lines), and in the salinity zone 1–10 g/L increased to 20.2 mmol/kg water (marked with a dashed line). In the salinity zone of 10–12 g/L the level of magnesium in the muscle of carps did not change.

In the range of 0–6 g/L the water content in carp muscle did not depend on the salinity of the environment, maintaining within 81.2–81.9 %, and in the salinity zone 6–12 g/L significantly decreased to 79.3 % (Fig. 2).

Discussion

Concentration of sodium in the blood plasma of carp is maintained normally within a certain narrow range (normonatremia) (Fig. 1, the boundaries are separated by solid lines of black color), and when hypnatremia rises to the maximum limit value (indicated by a dashed line of black color).

According to different authors, in humans the value of the lower limit of normonatremia rather differs, reported values are 130 mmol/L (22, 23), 134 mmol/L (24), 135 mmol/L (1, 2). The threshold value dividing the upper limit of normonatremia and the lower limit of hypnatremia is 145 mmol/L (1, 2). Summarizing the available results, it should be acknowledged that concentration of sodium in the blood plasma of healthy people (range of normonatremia) is commonly maintained in the range of 130–145 mmol/L (Fig. 1, the boundaries are marked with red solid lines). In the state of hypnatremia, concentration of sodium in human blood plasma can be increased up to 206 mmol/L (25, Fig. 1, red dashed line). The comparison shows that the range of sodium content in the internal environment, compatible with the vital activity of cells in various organs and tissues of carp and human, is similar. It can be expected that the adaptation mechanisms of various types of body cells to an increase of sodium concentration in the internal environment of carp and humans will act in a similar way.

The osmotic concentration of blood plasma and muscle tissue of the body are in equilibrium (21). In the state of hypnatremia, the content of ions in the blood plasma of carp increased: sodium by 72 mmol/L, and calcium by 1.6 mmol/L, in total amounting to 73.6 mmol/L. In muscles, the sodium concentration increased by 34 mmol/kg water, potassium by 9 mmol/kg water, calcium by 2.2 mmol/kg water, magnesium by 6.9 mmol/kg water, in total amounting to 52.1 mmol/kg water. Difference of the total ion concentration between the plasma and muscle tissue is 21.5 mmol. This deficiency of ions in carp muscle tissue was compensated by accumulation of organic osmolytes. Maintenance of the structure and function of macromolecules, including proteins, compatible with vital activity of the cell can be achieved only in narrow ranges of intracellular concentration of inorganic ions (26). Intracellular accumulation of electrolytes is limited because high ion concentrations interfere with structure and function of macromolecules, including proteins (27, 28).

Reaching maximum value of potassium, calcium, magnesium in carp muscles (Fig. 1, dashed lines, specific values are presented in the results) was observed at salinity of 10 g/L. Carps acclimated in the salinity zone of 10–12 g/L did not increase the level of these ions in muscles, thus preventing their damaging effects on intracellular processes.

In addition to the ionic mechanisms, volume regulation cells specifically accumulate organic osmolytes to increase intracellular osmolality (26–30). Organic osmolytes need to be non-perturbing solutes that do not interfere with protein function even when occurring at high intracellular concentrations (28, 29, 31, 32).

Increase of glycine, histidine and alanine concentration in the muscle of carp has been observed in the zone of critical salinity (33). Compensation of intracellular osmotic concentration in muscles of toads due to organic substances in state of increase salinity has comprised from 53 % of *Bufo viridis* and 57 % of *Bufo boreas* (34). Adaptation of *Tilapia mossambica* to hyperosmotic conditions was accompanied by the cleavage of proteins and accumulation of amino acids in muscles (35). When euryhaline teleosts go from the fresh water to the sea water, there is an 82 % increase in the amino-acid content of the thick-lipped mullet *Crenimugil labrosus* muscle and a 66 % increase in that of the flounder *Paralichthys lethostigma* muscle (36). In the experiments *in vivo* (37) and *in vitro* (20) cell volume regulation in heart ventricles of the euryhaline flounder *Platichthys flesus* is carried out under hyperosmotic conditions by means of increased intracellular concentration of potassium and taurine.

Thus, in the zone of critical salinity increase of osmotic concentration of the freshwater hydrobionts internal environment has been observed, mainly due to the increase of sodium and chloride ions. Carp muscle tissue adapts to the increase of extracellular fluid osmotic concentration due to accumulation of inorganic ions (mainly sodium, potassium and magnesium) and organic osmolytes. Despite these adaptations, recovery of initial volume of muscle cells has not been achieved. This is demonstrated by decrease of the water level in carp muscles acclimated in the critical salinity zone (Fig. 2). Decreased intracellular fluid volumes of the carps in the critical salinity zone were determined using carboxyl-14C-inulin (12).

When cultivating freshwater ciliate *Tetrahymena pyriformis* in solutions with sodium content of 36.5 and 223 mmol/L, average cell volume has decreased from 16–18.3 μm^3 to $9.5 \pm 0.47 \mu\text{m}^3$. At the same time, water level in the cells has decreased from 80.7 % to 71.2 % (38). In the zone of critical salinity of 12–14 g/L fingerling of grass carp *Ctenopharyngodon idella* has decreased the total body weight by 8.5–11.3 %; water content in the muscle tissue has decreased from 80% in fresh water to 74.4 % at 14 g/L (39). Within the tolerant salinity range, the total body water content of carp and tubenose goby is supported at stable level, demonstrating standard physiological functioning of the osmotic regulation. Hypohydremia is observed when the organism is exposed to water of critical salinity, indicating problems with osmotic regulation (14). Decrease of water level in the muscles of two toad species acclimated to hyperosmotic salinity has been observed (34). Hypernatremic dehydration was observed in infants (40) and elderly people (41).

At transition of different species of euryhaline fish from freshwater to the marine environment increase of extracellular fluid osmolality occurs due to increase of sodium and chloride ions (16–20). During transfer of killifish *Fundulus heteroclitus* from freshwater to seawater the plasma osmolality increased, muscle cell shrinkage occurred. During prolonged acclimation cell water content returns to a higher value (17). Water content of the muscles did not change at adaptation of *Tilapia mossambica* to hyperosmotic conditions (18). Constancy of the water content in the muscles reflects ability of the cells to regulate volume. In the experiments *in vivo* (37) and *in vitro* (20) cell volume regulation in heart ventricles of the euryhaline flounder *Platichthys flesus* is carried out under hyperosmotic conditions. The water content of the heart ventricle cells remained constant.

Thus, the muscle tissue of freshwater organisms adapts to hypernatremia due to accumulation of inorganic ions (mainly sodium, potassium and magnesium) and organic osmolytes. Despite these adaptations, the restoration of the original volume of muscle cells, definitive normonatremia, is not achieved. Hypernatremic dehydration in humans indicates a pattern of regulation of cell volume similar to that of freshwater organisms. In euryhaline species in the transition from fresh water to sea water, the water content in the muscles does not change, indicating the restoration of the volume of muscle cells to the original level.

Mechanisms involved in volume regulation of cell in response to hyperosmotic solutions have been studied in *in vitro* experiments. It was shown that hyperosmotic solutions cause volume reduction of different cell types in the initial period due to the exit of water by osmotic gradient (20, 42–48). Movement of water causes activation of several ion transporters in cell membranes. Activation of the $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter leads to the intracellular transfer of sodium chloride and potassium chloride. Activation of Na^+/H^+ exchange and $\text{Cl}^-/\text{HCO}_3^-$ exchange leads also to sodium chloride entry into the cells. Sodium ion entering the cells is extruded through $\text{Na}^+/\text{K}^+ - \text{ATPase}$ in exchange for potassium (27, 29, 49–51). The overall balance of these processes is an increase of cell potassium and chloride compensating for the increase of extracellular osmolality.

In experiments *in vivo* the carp muscle tissue has adapted to hypernatremia by accumulation of not only potassium, but also sodium and magnesium (Fig. 1). Muscular cells of two species of toads (34) and of crucian carp (21) adapted to hypernatremia by means of accumulation of sodium and potassium ions.

The results show that the pattern of the cell volume regulation in response to hypernatremia in experiments *in vitro* and *in vivo* is different. This is important to take into account in clinical studies. Changes in cellular inorganic ion levels can alter resting membrane potential, the rates of enzymatically catalyzed reactions and membrane solute transport that is coupled to ion gradients. Different patterns of cell volume regulation, realized *in vitro* and *in vivo*, will affect the intracellular processes in different ways. Consequently, the cell volume regulation pattern realized *in vivo* will be adequate for the organism. Since the pattern of the muscle tissue cell volume regulation, realized *in vivo* in response to hypernatremia, functions in different vertebrate species (carp, crucian

carp, two species of toads) in a similar way, a similar reaction of humans should be expected.

Conclusion

The method of acclimating freshwater hydrobionts to the salinity of environment within total interval compatible with the vital activity of organisms of different species allows to determine the ranges of normonatremia and hypernatremia regulation. To identify patterns and limits of changes in various inorganic ions and organic osmolytes involved in volume regulation of various types of body cells in response to hypernatremia. To assess the effect of inorganic ions on intracellular processes. The muscle tissue of carp adapts to hypernatremia by means of accumulation of inorganic ions (mainly sodium, potassium and magnesium) and organic osmolytes. Despite these adaptations, restoration of the original volume of muscle cells, characteristic for normonatremia, is not achieved. Hypernatremic dehydration is observed in the human body and freshwater fish. The range of sodium content in the internal environment, compatible with the vital activity of the cells of various organs and tissues of the body of carp and human, is similar. It can be expected that the mechanisms of muscle cells adaptation to an increase in the sodium concentration in the internal environment of a human body will be carried out in the same way as in the body of carp.

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