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SHORT COMMUNICATIONS

Effect of Magnetic Storm on the Sensitivity of Juvenile Roach Intestinal Glycosidase to Heavy Metals (Cu, Zn) and the Herbicide Roundup

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Abstract—The effect of a magnetic storm (frequency range of 0–5 Hz) on the activity and sensitivity of underyearling intestinal glycosidase (maltase and amylolytic activity) to heavy metal ions (Cu^{2+} and Zn^{2+}, concentrations of 0.1– 25 mg/L) and the herbicide Roundup (0.1– 50 μg/L) have been studied in vitro during early the embryogenesis of roach *Rutilus rutilus* (L.). The impact of the magnetic storm on roach embryos 48–72 h post fertilization increases the sensitivity of digestive glycosidases (except for maltase in the presence of Cu) to Cu, Zn, and Roundup.

Keywords: roach, digestive glycosidases, magnetic storm, heavy metals, Cu, Zn, Roundup herbicide

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INTRODUCTION

Aquatic organisms are subject to the environmental impact of a range of natural and anthropogenic factors. The geomagnetic field is one of the most important ecological factors that has served as a background for the evolution of living beings. Changes in the geomagnetic field related to the effect of the disturbed Sun wind currents on the magnetosphere of the Earth are called "magnetic storms" (MSs). Usually the intensity of Mss does not exceed 1% compared to the intensity of the geomagnetic field. However, even such weak impacts may cause serious biological effects [7]. The exposure of common *Cyprinus carpio* L. and crucian *Carassius auratus* (L.) carps to MSs changed the activity of intracellular Ca^{2+}-dependent cysteine proteinases and other digestive intestinal hydrolases [7]. At that, an MS weakly affects the activity of proteinases but rather strongly decreases the activity of glycosidases, especially in hungry fish [4]. Salts of heavy metals and organic xenobiotics are among the anthropogenic factors heavily loading natural ecosystems. Copper and zinc belong to the group of priority pollutants of ambient waters, with concentrations reaching several milligrams per liter in the areas subject to technogenic pollution. These chemical elements are essential micronutrients participating in many biochemical reactions, but at high concentrations they become toxic to aquatic biota. Entering the organism with water and food, Cu and Zn affect fish digestive enzymes, both directly and indirectly. Roundup is the most well-known herbicide based on the isopropyl amine salt of glyphosate [N-(phosphonomethyl) glycine]. This pesticide is used for the extermination of weeds on crops, in collector–drainage canals, irrigation systems, and ponds. In vitro and in vivo experiments revealed that exposure to 0.1–50 μg/L Roundup changes the activities of digestive hydrolases in several freshwater bony fishes [1, 2].

Some factors may modify the sensitivity of a living being to the effects of other factors. For instance, a decrease in the activities of digestive hydrolases in roach underyearlings was a remote consequence of exposure during early embryogenesis to Cu^{2+} ions (0.001 mg/and 0.01 mg/L). Exposure to a low-frequency magnetic field (72.5 Hz, 150 μT) during the same period levels the inhibiting effect of Cu^{2+} and leads to the adaptive increase in the enzyme-substrate affinity at combined effect of both factors [3]. In most fish species all stages of embryonic development occur in the environment and various factors affect fish by the earliest stages of ontogenesis. This necessitates the assessment of remote consequences of the effects of various factors during the embryonic period. There are no data on the remote effects of exposure to MS during early ontogenesis on the activity of fish digestive hydrolases and their sensitivity to heavy metals and organic pollutants.

The goal of the present paper is to study the effect of typical MSs during early embryogenesis on the activity and sensitivity of glycosidases in roach under-

Table 1. Amylolytic activity (μmol/g min) in the roach *Rutilus rutilus* intestine of the control and MS-exposed fish upon the in vitro effects of Cu^{2+} and Zn^{2+} ions

Metal	Concentrations of ions, mg/L					
	0	0.1	1	5	10	25
Cu	$\frac{24.1 \pm 1.7^a}{27.3 \pm 1.2^a}$	$\frac{25.0 \pm 0.8^b}{23.4 \pm 1.0^b}$	$\frac{26.6 \pm 0.6^a}{21.9 \pm 0.6^b}$	$\frac{19.0 \pm 0.8^c}{8.87 \pm 0.9^c}$	$\frac{13.4 \pm 0.6^d}{2.04 \pm 0.2^d}$	$\frac{2.35 \pm 0.1^e}{1.62 \pm 0.4^d}$
Zn	$\frac{24.1 \pm 1.7^a}{27.3 \pm 1.2^a}$	$\frac{22.7 \pm 0.8^{a,b}}{21.6 \pm 1.0^b}$	$\frac{20.3 \pm 0.6^{b,c}}{15.4 \pm 1.0^d}$	$\frac{20.7 \pm 0.8^{b,c}}{18.1 \pm 0.4^c}$	$\frac{20.2 \pm 1.0^c}{16.3 \pm 0.8^{c,d}}$	$\frac{19.4 \pm 0.8^c}{14.9 \pm 0.5^d}$
Cu + Zn	$\frac{24.1 \pm 1.7^a}{27.3 \pm 1.2^a}$	$\frac{20.9 \pm 0.6^b}{17.1 \pm 1.0^b}$	$\frac{19.1 \pm 1.0^b}{16.8 \pm 0.9^b}$	$\frac{10.7 \pm 0.8^c}{8.03 \pm 0.6^c}$	$\frac{7.49 \pm 0.7^d}{0.78 \pm 0.6^d}$	$\frac{1.34 \pm 0.1^e}{0.12 \pm 0.1^e}$

Here and in Tables 2 and 3, the means and errors of means are given; on the top is the control; on the bottom is treatment; different letters in the superscript indicate the statistical significance of differences between the parameters in each row.

yearlings to the effects of Cu^{2+} and Zn^{2+} ions and Roundup herbicide.

MATERIALS AND METHODS

Fertilized roach eggs were exposed to MSs. The gametes were obtained from eight females and six males caught by a seine net on the spawning ground in the Rybinsk Reservoir in May 2012. The eggs were fertilized using the dry method, after which the eggs were transferred into two crystallizers (about 3000 eggs in each) filled with riverine water (temperature of 16–18°C); the water in crystallizers was replaced twice daily. One crystallizer with developing roach embryos was kept for the whole span of the experiment in the condition of a natural geomagnetic field and served as control. The second crystallizer, 48 h post fertilization, was placed into the experimental device [7] in which MS (0–5 Hz) was simulated for 24 h. The selected time period of roach embryogenesis (48–72 h post fertilization) covered various stages of organogenesis. The experiment was carried out during the period of quiet range geomagnetic status. After yolk sack resorption, 500 larvae from treatment and control were transferred into ponds with natural food resources. Four months later, 20 specimens of underyearlings were caught in each pond, their body length and weight were measured, and these fish were subject to biochemical analyses. To determine the activity of glycosidases, medial sections of intestine dissected from 20 specimens were homogenized and analyzed as described in more detail in [7]. To study the toxicant effects in vitro, the homogenates were initially incubated with sulfates of copper ($CuSO_4 \cdot 5H_2O$) and zinc ($ZnSO_4 \cdot 7H_2O$) or their 1 : 1 mixture, as well as with Roundup for 1 h. The concentrations of Cu^{2+} and Zn^{2+} ions as calculated by total content of metal in the salt were 0.1, 1.0, 5.0, 10.0, and 25.0 mg/L. The concentrations of Roundup as per glyphosate were 0.1, 1.0, 10.0, 25.0, and 50.0 μg/L. Amylolytic activity reflecting sum activities of the starch-hydrolyzing enzymes (α-amylase EC 3.2.1.1, glucoamylase EC 3.2.1.3 and maltase EC 3.2.1.20) was assessed by gain of hexoses using modified Nelson's technique [5]. To determine maltase activity by the glucooxidase technique, a Fotoglucosa clinical biochemistry kit (OOO Impact, Russia) was used. The activities of enzymes were expressed as micromoles of reaction products produced for 1 min of incubation per 1 g wet tissue weight (μmol/g min). The results are given as means and errors of means ($M \pm m$). The normality of distribution of analyzed parameters was examined using Kolmogorov–Smirnov test. The statistical significance of differences was assessed using unifactor analysis (ANOVA followed by LSD-test) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Exposure to a MS sans Cu^{2+} and Zn^{2+} ions and Roundup did not change the activity of glycosidases ($p > 0.05$), but in most cases their sensitivity to the effects of tested toxicants increases (Tables 1–3). Upon exposure to MSs, amylolytic activity in the presence of Cu^{2+} ions was 14–94%; of Zn^{2+} ions, 21–46% lower than in control (0 mg per 1 L) over the whole concentration range (Table 1). In the control, a decrease in amylolytic activity was recorded in the narrower range of metal concentrations: 21–90% at incubation to Cu^{2+} ions and 14–19% to Zn^{2+} ions. Incubation with mixtures of Cu^{2+} and Zn^{2+} ions decreases the level of amylolytic activity by 37–100% of control, following exposure to MS and 13–94% without such exposure. In most cases the extent of retardation of amylolytic activity directly depended on the metal concentration. The directions of changes in the activity of maltase in the presence Cu^{2+} and Zn^{2+} ions differed (Table 2). In the control, maltase activity decreased following incubation with Cu^{2+} (10 mg/L

Table 2. Activity of maltase (μmol/g min) in the roach *Rutilus rutilus* intestine of the control and MS-exposed fish at the in vitro effects of Cu^{2+} and Zn^{2+} ions

Metal	Concentrations of ions, mg/L					
	0	0.1	1	5	10	25
Cu	18.9 ± 0.5[a] / 16.7 ± 0.9[a]	24.0 ± 0.3[c] / 16.1 ± 1.1[a]	18.1 ± 0.2[a, b] / 16.9 ± 0.3[a]	18.5 ± 0.7[a] / 16.7 ± 0.1[a]	17.1 ± 0.3[b] / 18.4 ± 0.4[b]	12.1 ± 0.7[c] / 16.6 ± 0.2[a]
Zn	18.9 ± 0.5[a] / 16.7 ± 0.9[a]	15.6 ± 0.3[c] / 16.7 ± 0.4[a]	16.7 ± 0.5[c] / 20.0 ± 0.4[c]	21.7 ± 0.7[b] / 20.5 ± 0.7[c]	18.9 ± 0.6[a] / 18.3 ± 0.4[b]	19.6 ± 0.5[a, b] / 19.6 ± 0.2[c]
Cu + Zn	18.9 ± 0.5[a] / 16.7 ± 0.9[a]	19.1 ± 0.8[a] / 16.4 ± 0.2[a]	20.6 ± 0.2[b] / 18.0 ± 0.5[a, b]	22.3 ± 0.5[c] / 19.4 ± 1.1[b]	23.7 ± 0.5[c] / 21.4 ± 0.5[c]	25.5 ± 0.2[d] / 26.7 ± 0.4[d]

Table 3. Activity of glycosidases (μmol/g min) in the roach *Rutilus rutilus* intestine of the control and MS-exposed fish upon the in vitro effect of Roundup

Activities of glycosidases	Concentration of Roundup, mg/L					
	0	0.1	1	10	25	50
Amylolytic	24.1 ± 1.7[a] / 27.3 ± 1.2[a]	21.5 ± 0.5[b] / 17.4 ± 0.6[c]	21.2 ± 0.6[b] / 18.0 ± 1.0[b, c]	21.9 ± 0.3[b] / 20.0 ± 0.8[b]	24.1 ± 0.7[a] / 20.0 ± 0.7[b]	21.3 ± 0.6[b] / 19.9 ± 0.9[b]
Maltase	18.9 ± 0.5[a] / 16.7 ± 0.9[a]	25.9 ± 0.8[b] / 26.9 ± 0.7[b]	25.3 ± 0.6[b] / 27.0 ± 1.0[b]	28.2 ± 0.6[c] / 27.7 ± 0.4[b]	28.4 ± 0.4[c] / 27.4 ± 0.2[b]	32.0 ± 0.6[d] / 28.3 ± 0.7[b]

and 25 mg/L) and Zn^{2+} (0.1 mg/L and 1 mg/L) ions. In the experiment the activity increased 10% and 10–23% following incubation with Cu^{2+} (10 mg/L) and Zn^{2+} ions (1–25 mg/L). No metal concentration/effect dependence was revealed. At a combined effect of Cu^{2+} and Zn^{2+} ions, the activity of maltase increased by 9–35% in the control and 8–60% in the experiment.

Exposure to MS during embryogenesis increases the sensitivity of glycosidases to the effect of Roundup in underyearlings. This effect was more pronounced at low (0.1–1.0 μg/L) herbicide concentrations (Table 3). In the control, amylolytic activity decreased by 10–21%, following incubation with herbicide; in the experiment, it decreased by 27–36% compared to the control. The activity of maltase in the control underyearlings was increasing sequentially by 34–69% along with an increase in Roundup concentration, while in the experimental fish it was increasing by 61–69%. An earlier study on the effects of organic compounds (chlorophos and nitrozoguanidine) on the fish embryos also revealed changes in the sensitivity of glycosidases in juvenile roach to the in vitro effects of Cu^{2+} and Zn^{2+} ions [8]. Changes in the sensitivity of glycosidases may be determined by the effects of natural and anthropogenic factors on the synthesis of pancreatic (α-amylase) and specifically intestinal enzymes. Several isoforms of α-amylase, glucoamylase, and maltase exist. This is why it possible that the glycosidases functioning in the intestine of roach underyearlings of control and experimental groups are molecularly diverse.

CONCLUSIONS

Exposure of roach eggs to an MS for 48–72 h post fertilization did not result in remote changes in enzymatic activity, but the sensitivity of glycosidases to the effects of heavy metals (Cu and Zn) and Roundup in roach underyearlings increased. The sensitivities of the starch-hydrolyzing enzymes increase to a higher extent when compared to the specifically membrane enzyme, maltase.

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