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Regulation of iodothyronine deiodinases and sodium iodide symporter mRNA expression by perchlorate in larvae and adult Chinese rare minnow (*Gobiocypris rarus*)

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ABSTRACT

Perchlorate is a widespread contaminant in the aquatic environment. In the present work, the expressions of deiodinase enzymes (*d1*, *d2*, and *d3*) and sodium iodide symporter (*nis*) genes were determined after larval and adult rare minnow (*Gobiocypris rarus*) exposed to 5 and 50 μg/L perchlorate for 21 days. The results showed that deflation of swim bladder development was observed in larvae at 50 μg/L perchlorate treatment. An up-regulation of the *d2* and *nis* mRNA levels were observed in the larve and in brain of adults. Meanwhile the expressions of *d3* mRNA levels were significantly down-regulated in the liver. These results indicate the changes in *d2*, *nis*, and *d3* mRNA expression brings about increased outer-ring deiodination, idodine uptake, and a further decrease of inner-ring deiodination, respectively reflecting auto-regulation of hypothalamic-pituitary-thyroid (HPT) axis in adult after perchlorate exposure. The larval fish development could be affected by perchlorate at environmentally relevant concentrations.

1. Introduction

As previously reported, many endocrine disrupting chemicals (EDCs) have been released into the aquatic environment (Petrovic et al., 2004; Gao et al., 2008a,b). In recent years, there has been an increasing concern on the perchlorate contamination due to its stability and nonreactivity in water (Charnley, 2008; Park et al., 2006). In several western states of America, concentrations of perchlorate ranged from 8 μg/L to 3.7 g/L in ground and surface waters (Urbansky, 1998). Maximum contaminant level of perchlorate for drinking water is set 6 μg/L by the California Environmental Protection Agency (California EPA, 2004). In China, measurable concentrations of perchlorate in tap water, groundwater, surface water, and bottled water were in the range of 0.037–54.4 μg/L, and mean concentrations were about 2 μg/L (Liu and Mou, 2003; Shi et al., 2007; Wu et al., 2010). In addition, tissue concentrations of perchlorate are greater than that in the water in a few fish species exposed to perchlorate (Theodorakis et al., 2006).

The thyroid hormones (THs), triiodothyronine (T_3) and thyroxine (T_4) have a wide range of biological effects in physiological processes of fish (Crane et al., 2004; Porazzi et al., 2009). Perchlorate is known to disrupt thyroid function via competitive inhibiting thyroidal iodide uptake (McLanahan et al., 2009), and to effect on the development of the central nervous system in mammalian (Gilbert and Sui, 2008). Previous studies have been found that perchlorate can impair THs production and increase follicular epithelial cell height, hyperplasia, and hypertrophy in fish (Bradford et al., 2005; Mukhi et al., 2005). The sodium iodide symporter (NIS) is an integral plasma membrane glycoprotein of thyroid hormone synthesis (Dohan et al., 2003; Schroder-van der Elst et al., 2004). Iodothyronine deiodinases play a crucial role in the mechanism of thyroid hormone action. Three deiodinases have been found in fish: type 1 (D1), type 2 (D2), and type 3 (D3), which control the conversion of T_4 to the more active T_3 or to the inactive metabolites rT_3 and T_2 (Orozco and Valverde, 2005). With the advances in molecular approaches, the application of toxicogenomics could be a powerful tool for evaluating the effects and discovering molecular mechanisms underlying toxic response (Lema et al., 2008; Wintz et al., 2006). The deiodinase enzymes mRNA levels in fish are sensitive molecular biomarkers to thyroid disrupting chemicals (Li et al., 2009b; Picard-Aitken et al., 2007). However, effects of exposure to the environmental pollutant perchlorate on the expression of iodothyronine deiodinases and sodium iodide symporter genes in fish have not been throughout investigated and discussed.

Our present study aimed to investigate the effects of environmentally relevant concentrations of perchlorate on plasma THs levels and the expression of TH-related genes (*d1*, *d2*, *d3*, and *nis*)

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of larvae and adult rare minnow, and to discuss the possible molecular mechanisms underlying toxic response.

2. Materials and methods

2.1. Chemicals

Magnesium perchlorate was purchased from Sigma (Chemical Co., USA). Stock solutions of magnesium perchlorate were prepared in distilled water. Working solutions were freshly diluted by the stock solutions with dechlorinated tap water. To obtain the final concentration for exposure, the appropriate amount of the stock solutions were added to the aquarium water via polytetrafluorethylene (PFTE) and isoversinic tubes (Abimed, Langenfeld, Germany).

2.2. Culture conditions and experimental design

The brood stock of rare minnow (*Gobiocypris rarus*) was raised in flow-through system with dechlorinated tap water (pH 7.2–7.6; hardness 44.0–61.0 mg $CaCO_3$/L) at a constant temperature (25 ± 1 °C) with a photoperiod of 16:8 h (light:dark) and has been used for testing chemicals in our laboratory for more than seven years (Zha et al., 2007). Adult fish were fed a commercial pellet food (Trea, Germany) (0.1% body mass per day), while larvae and fry were provided newly hatched brine shrimp (*Artemia*) nauplii two times daily. Waste and residue were removed daily while the test equipment and chambers were cleaned once a week.

2.2.1. Experiment 1

Newly hatched larvae ($n = 90$) (about 72 h post fertilization) were the offspring from the same pair of brood stock and were randomly divided into three groups. Larvae were exposed to perchlorate (control, 5, and 50 μg/L; nominal concentrations) for 21 days. Samples of larval fish ($n = 5$) were taken weekly to observe for morphological effects and then stored at −80 °C until RNA extractions. After 21 days exposure, all remaining larvae were euthanized, and measured for the body length and body weight.

2.2.2. Experiment 2

Healthy adult rare minnows ($n = 90$), four months old and the offspring from the same pair of brood stock, were randomly divided into three groups. Fish were exposed under flow-through conditions to various experimental groups: control, 5, and 50 μg/L perchlorate treatments. Samples of liver and brain were taken weekly ($n = 5$), and all remaining fish were euthanized after 21 days exposure. The blood was collected from each fish in a heparinized microcapillary tube and centrifuged immediately at 8000*g*, 4 °C for 10 min. The plasma was kept frozen at −80 °C until use. Gonad and liver of each fish were removed and weighed after the blood was collected. Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as following: GSI = gonad weights (g)/body weights (g) × 100; HSI = liver weights (g)/body weights (g) × 100. Further, the liver and brain were flash-frozen in liquid nitrogen and stored at −80 °C until analysis.

2.3. Plasma THs measurement

Plasma T_4 and T_3 were measured using commercial radioimmunoassay (RIA) following the method described previously (Li et al., 2009b). Briefly, a sample volume of 50 μL was used for T_4 and T_3 measurement. The RIAs for T_4 and T_3 were validated for use with rare minnow samples by demonstrating parallelism between a series of diluted and spiked plasma samples in relation to the standard curve. The inter-assay coefficients of variation for both T_4 and T_3 were less than 5% and intra-assay coefficients of variation less than 10%. Cross reactivity between T_4 and T_3 antibodies was less than 0.5%. Assay sensitivities were 3 and 0.25 ng/mL for T_4 and T_3, respectively.

2.4. Real-time PCR

RNA-isolation, reverse-transcription, and real-time PCR were performed following methods described previously (Li et al., 2009a). The forward and reverse primers for *d3* (GenBank accession No. is GU290040) were as follows: 5′-CCAAATGCGCCGTCTTGAAG-3′; 5′-TGTGCGTGTCCGACTCCAAC-3′. All the samples were analyzed in triplicate and the mean value of these triplicate measurements were used for calculations of mRNA expressions. Results were analyzed according to delta-delta Ct method. The *d1*, *d2*, *d3*, and *nis* mRNA expression was normalized for *β-actin* mRNA expression. Gene expression data are presented as fold change relative to control at the same sampling time.

2.5. Statistics

All statistical analyses were performed with the SPSS (version 13.0; USA). All quantitative data are expressed as the mean ± SE of the mean (SEM). Statistical analysis of the data was performed using analysis of variance, followed by Bonferroni's Multiple Comparision Test. A probability of $p \leqslant 0.05$ was considered statistically significant.

3. Results and discussions

3.1. The exposure experiment in larvae

3.1.1. Mortality, growth, and morphology

No mortality was observed during the exposure period. There were no significant differences in body weight and body length between treatments and control (Table 1). No effects on the body pigmentation were observed at the end of exposure (21d), whereas the deflation of swim bladder development was observed at 50 μg/L perchlorate treatment (Fig. 1). Previous studies have shown that the treated with 59 μg/L perchlorate inhibited development and metamorphosis in *Xenopus laevis* (Goleman et al., 2002). In zerbafish, body length of larvae were significantly decreased at 100 and 250 mg/L perchlorate treatments (Mukhi et al., 2007). These results indicate that perchlorate may affect the development of aquatic organisms at early life stage at environmentally relevant concentrations.

3.1.2. Quantitation of d1, d2, d3, and nis mRNA by real-time PCR

The expressions of *β-actin*, *d2*, and *nis* were initially observed at 7 days post-hatching (DPH) and *d1* at 21 DPH, whereas the *d3* mRNA was not detected at high levels in larvae at 21 DPH (data not shown). The expressions of the *d2* and *nis* in whole larvae were shown in Fig. 2. The mRNA expressions of *d2* and *nis* were significantly down-regulated in 50 μg/L perchlorate treatment after 7-day exposure. The *d2* and *nis* mRNA levels were significantly up-regulated in 50 μg/L perchlorate treatment after 14-day exposure. At the end of 21-day exposure, the mRNA expression of the *d2* was significantly up-regulated, whereas there were no significant variations in *nis* mRNA levels. Similar results were also obtained in *X. laevis* tadpoles, which the expression of *d2* and *nis* mRNA levels were up-regulated about six and sixty folds, respectively, in thyroid tissue after 12-day treatment with 20 mg/L perchlorate (Opitz et al., 2009). Present data suggested that the up-regulation of *d2* and *nis* mRNA levels reflect the auto-regulation of hypothalamic-pituitary-thyroid (HPT) axis after perchlorate exposure.