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Nickel induces hyperglycemia and glycogenolysis and affects the antioxidant system in liver and white muscle of goldfish *Carassius auratus* L.

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ABSTRACT

The toxicity of nickel to mammals is well studied, whereas information on nickel effects on fish is scant. Goldfish exposure to 10–50 mg L^{-1} of waterborne Ni^{2+} for 96 h showed reduced glycogen levels by 27–33% and 37–40% in liver and white muscle, respectively, accompanied by substantial increases in blood glucose levels (by 15–99%). However, indices of oxidative damage to proteins (carbonyl proteins) and lipids (lipid peroxides) were largely unaffected by nickel exposure. In liver, the activities of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx), were not affected by Ni^{2+} treatment, while catalase activity was elevated by 26%. In white muscle, however, substantial increases in SOD (by 38–147%) and GPx (by 2.5–5.5-fold) activities appeared to compensate for decreased catalase activity (by 59–69%) in order to resist Ni-induced oxidative perturbations. Both hepatic and muscular glutathione reductase activities were suppressed by 10–30% and 12–21%, respectively, after goldfish exposure to all Ni^{2+} concentrations used. However, the activity of glucose-6-phosphate dehydrogenase was remarkably enhanced (by 1.6–5.4-fold) in white muscle of Ni-exposed fish, indicating a strong potential increase in NADPH production under Ni exposure. Thus, the exposure of goldfish to 10–50 mg L^{-1} of Ni^{2+} for 96 h induces glycogenolysis and hyperglycemia, showing some similarities with a hypoxia response, and leads to a substantial activation of defense systems against reactive oxygen species in liver and white muscle in tissue-specific and concentration-dependent manner.

1. Introduction

Nickel is the 22nd most abundant element on earth and is ubiquitous in marine and freshwater ecosystems. Nickel concentrations increase in aquatic systems receiving inputs from urban and industrial effluents (Pyle and Couture, 2012). Human activities, including mining, smelting, refining, alloy processing, scrap metal reprocessing, fossil fuel combustion, and waste incineration, contribute acutely to nickel contamination of aquatic and terrestrial ecosystems (Eisler, 1998). Anthropogenic Ni can enter aquatic systems via fallout from airborne particulate matter, surface runoff near industrial and urban areas, industrial effluents released directly into aquatic systems, or wastewater treatment facilities (Schaumloffel, 2005). As a result of anthropogenic activities, Ni concentrations in freshwater environments contaminated with Ni reach up to 2500 μg L^{-1} whereas in unpolluted freshwater they usually range from 0.1 to 10 μg L^{-1} (reviewed in Eisler, 1998).

Nickel is considered to be potentially hazardous to living organisms, because of its genotoxicity, immunotoxicity, mutagenicity and carcinogenicity (reviewed in Denkhaus and Salnikow, 2002; Eisler, 1998; Kasprzak et al., 2003). The toxicity of nickel compounds has been well established for mammalian model organisms (Athar et al., 1987; Cartañá et al., 1992; Misra et al., 1990; Rodriguez et al., 1990, 1991) or cell cultures (Chen et al., 2003; Kowara et al., 2005; Salnikow et al., 1994), whereas fish have received only minor attention (De Luca et al., 2007; Ptashynski et al., 2002). However, the evidence of extensive freshwater pollution with Ni suggests that investigations of Ni effects on fish are probably essential.

Injuries to cellular metabolism may result from the cumulative effects of Ni-induced oxidative damage. Nickel has been hypothesized to mediate oxidative damage to DNA, lipids and proteins either directly or via inhibition of antioxidant defenses (reviewed in Kasprzak, 1991, 1995; Klein et al., 1991). Oxidative stress, caused by Ni, is either a consequence of reactive oxygen species (ROS) formation via Fenton/Haber–Weiss reactions involving the Ni^{2+}/Ni^{3+} redox couple (Klein et al., 1991; Torreilles and Guerin, 1990), or results from depletion of intracellular free radical scavengers such as GSH (Salnikow et al., 1994), due to the formation of Ni-GSH

Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase; GPx, glutathione peroxidase; GST, glutathione-S-transferase; GR, glutathione reductase; G6PDH, glucose-6-phosphate dehydrogenase; CP, carbonyl proteins; LOOH, lipid peroxides; L-SH, low molecular mass thiols; H-SH, high molecular mass thiols

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complexes (Krężel et al., 2003). In mammals, the induction of ROS is one of the most important mechanisms of Ni toxicity (Inoue and Kawanishi, 1989; Klein et al., 1991; Torreilles and Guerin, 1990). ROS readily interact with DNA, proteins, and other important biomolecules, causing cellular and molecular damage and physiological dysfunction. Several antioxidant antioxidant enzymes, including superoxide dismutase (SOD, EC 1.15.1.1), catalase (EC 1.11.1.6), and glutathione peroxidase (GPx, EC 1.11.1.9), and associated enzymes (glutathione-S-transferase GST, EC 2.5.1.18), glutathione reductase (GR, EC 1.6.4.2) and glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) are available to mitigate this damage, and induction of these enzymes is often an indicator of oxidative stress (Lushchak, 2011). However, nickel can inhibit responses of antioxidant systems by suppressing the activities of antioxidant enzymes such as catalase (Misra et al., 1990; Rodriguez et al., 1990), glutathione peroxidase (Athar et al., 1987; Misra et al., 1990), glutathione reductase (Cartañá et al., 1992; Misra et al., 1990), and glucose-6-phosphate dehydrogenase (Cartañá et al., 1992). Nickel-induced oxidative stress has received little study in fish, in contrast to mammals where links between Ni exposure, oxidative stress and DNA damage have been reported (reviewed in Pyle and Couture, 2012).

In fish, waterborne nickel can be taken up across the gills (Tjälve et al., 1988), transported in the blood and accumulated in different organs (Tjälve et al., 1988), leading to deleterious effects. Furthermore, fish chronically exposed to waterborne Ni showed alterations in carbohydrate metabolism. Increasing waterborne Ni concentrations resulted in marked decreases in liver and muscle glycogen stores with concomitant increases in plasma glucose and lactate concentrations (Canli, 1996; Chaudhry, 1984; Chaudhry and Nath, 1985; Ghazaly, 1992; Jha and Jha, 1995). Although the fluctuations in carbohydrate metabolism may reflect a general stress response to Ni exposure, potentially mediated through a Ni-induced stimulation of glucocorticoids, the hyperlactacidemia may be a further reflection of a Ni-induced reaction, similar to a hypoxia response (Pyle and Couture, 2012). Similar to the actions of Co, Ni can stabilize the alpha subunit of the hypoxia-inducible transcription factor (HIF-1α) and trigger the expression of HIF-1 mediated genes (Andrew et al., 2001). Thus, fish suffering from Ni-induced metabolic perturbations might have to switch to anaerobic ATP production, which is less efficient and consumes more glucose than aerobic ATP production. Thus, Ni might induce glucocorticoid release to mobilize liver and muscle glycogen reserves, causing plasma hyperglycemia, as a means of supporting anaerobic metabolism in response to Ni (Pyle and Couture, 2012).

Given the increased global demand for Ni and corresponding potential for increased anthropogenic inputs into the environment, it is important to develop a deeper understanding of the basic toxicity of Ni to fish (Pyle and Couture, 2012). Freshwater fish, occupying upper levels of trophic chains, can accumulate non-degradable pollutants, like metals, in different tissues, including liver and white muscle (Adeyeye et al., 1996). The Ni-induced metabolic perturbations in these tissues can serve as indicators of freshwater ecosystem contamination and constitute health hazard of fish consumers. Taking into account everything mentioned above, the present study aimed to investigate the effects of waterborne Ni^{2+} on free-radical processes and glycogen/glucose levels in the two main glycogen-storing tissues, liver and white muscle, of a stress-resistant freshwater fish species, the goldfish *Carassius auratus*.

2. Material and methods

2.1. Reagents

Phenylmethylsulfonyl fluoride (PMSF), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), oxidized glutathione (GSSG), glucose-6-phosphate (G6P), ethylenediamine-tetraacetic acid (EDTA), xylenol orange, cumene hydroperoxide, ferrous sulphate, 2,4-dinitrophenylhydrazine (DNPH), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), KH_2PO_4, Tris(hydroxymethylaminomethane) and N,N,N′,N′-tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich Corporation (St. Louis, USA). $NADP^+$ and NADPH were obtained from Reanal (Budapest, Hungary). All other reagents were from local suppliers in Ukraine and Russia and they were of analytical grade.

2.2. Animals and experimental conditions

Goldfish (*C. auratus*), weighing 40–65 g, were obtained from local suppliers twice, in June and November 2010. In both cases, fish were acclimated to laboratory conditions for four weeks in a 1000 L tank under natural photoperiod in aerated dechlorinated tap water. Water parameters were 23–24 °C and 7.0–7.6 mg L^{-1} O_2 during summer and 19–20 °C and 9.0–9.5 mg L^{-1} O_2 during the autumn. The pH and hardness (determined as Ca^{2+} concentration) of the water was pH 7.6–7.8 and 39–40 mg L^{-1}, respectively, and did not differ between the experimental periods. Fish were fed commercial cyprinid pellets (Koi Grower, The Netherlands) containing 44% protein, 11% fat and 150 mg kg^{-1} vitamin C to apparent satiation. Fish were fed during the acclimation period, but were fasted for four days prior to and during experimentation to prevent extensive excretion. All experiments were conducted in accordance with national and institutional guidelines for the protection of animal welfare.

Experiments were carried out in 120 L glass aquaria (containing 100 L of water) in a static mode under the same conditions following standard acute toxicity test procedure. Groups of 5–6 fish were placed in aquaria with different nominal concentrations of Ni^{2+} ions: 10, 25 and 50 mg L^{-1} (added as $NiCl_2 \cdot 6H_2O$) and exposed to these conditions for 96 h (no mortality occurred from these exposures). Concentrations were chosen, taking into consideration a LD_{50} for nickel of 98 mg L^{-1} reported for *C. auratus* after 48 h exposure (Eisler, 1998). The experiments were carried out in three independent runs (two in summer and one in winter), but the total number of fish for each parameter investigated was 5–6. Fish in the control group were maintained in the same manner, but without the addition of nickel chloride to the water. Aquarium water was not changed over the 96 h course in order to avoid stressing the animals. Levels of dissolved oxygen, temperature and pH were monitored every 24 h. After fish exposure, blood was quickly taken from caudal vessels using 50 mM EDTA as an anticoagulant. Subsequently, fish were sacrificed, liver and white muscle were quickly dissected out, rinsed in ice-cold 0.9% NaCl, frozen and stored in liquid nitrogen until use.

2.3. Nickel concentration in aquarium water

The concentration of nickel in aquaria during fish exposure was measured spectrophotometrically using the reaction of Ni(II) with dimethylglyoxyme (2,3-butanedione dioxime) (Mitchell and Mellon, 1945) with some modifications. The test samples contained 0.02 ml of 1% dimethylglyoxyme and a volume of aquarium water calculated to contain approximately 20 μg of nickel; this was adjusted to a final volume of 2.0 ml with distilled water. Samples were incubated for 15 min at room temperature and then the absorbance of the bright red Ni(II)dimethylglyoxymate complex that was formed was measured at 540 mn with continuous mixing of samples because of the low solubility of the complex. The concentration of nickel in test samples was determined using a linear regression of data from a standard curve with different concentrations (3–25 μg mL^{-1}) of Ni^{2+}.

2.4. Determination of glucose concentration in Blood and level of glycogen in liver and white muscle

Blood was separated into plasma and erythrocytes by centrifugation (1500 g, 15 min, 4 °C) and plasma was collected. The aliquots of plasma (0.1 ml) were mixed with 30% trichloroacetic acid (TCA) (final TCA concentration 10%) and centrifuged (3000 g, 7.5 min, 21 °C) to pellet proteins. The supernatants were removed and used for determination of glucose concentration.

Glycogen from liver and white muscle was extracted following the modified procedure of Roe et al. (1961). Samples of frozen liver and white muscle were homogenized in 10% TCA (1:10, w/v) and centrifuged (3000 g, 7.5 min, 21 °C) to pellet proteins. The resulting supernatants were mixed with ethanol (1:3, v/v) and precipitated glycogen was separated by centrifugation (4500 g, 12 min, 21 °C). Glycogen pellets were re-suspended in water (0.5:1, v/v water/homogenate) and then 30% KOH was added (1:3, v/v resuspended glycogen/KOH). The mixtures were held in a boiling water bath for 20 min with 30 s mixing every 5 min to allow complete glycogen digestion.

Glycogen concentrations in tissues and glucose concentrations in plasma were determined by the anthrone method (Seifter et al., 1950) with minor modifications. Aliquots of plasma (50 μl) and tissue glycogen hydrolysates (40–50 μl) supplemented with water to volumes of 0.5 ml and were mixed with 1 ml of freshly prepared 0.2% anthrone (dissolved in 94% sulphuric acid) with subsequent incubation in tightly covered test-tubes in boiling water for 10 min. After cooling, the absorbance of samples was determined at 620 nm. Glucose concentration in samples