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Exposure to environmental levels of waterborne cadmium impacts corticosteroidogenic and metabolic capacities, and compromises secondary stressor performance in rainbow trout

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ABSTRACT

The physiological responses to waterborne cadmium exposure have been well documented; however, few studies have examined animal performances at low exposure concentrations of this metal. We tested the hypothesis that longer-term exposure to low levels of cadmium will compromise the steroidogenic and metabolic capacities, and reduce the cortisol response to a secondary stressor in fish. To test this, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to 0 (control), 0.75 or 2.0 μg/L waterborne cadmium in a flow-through system and were sampled at 1, 7 and 28 d of exposure. There were only very slight disturbances in basal plasma cortisol, lactate or glucose levels in response to cadmium exposure over the 28 d period. Chronic cadmium exposure significantly affected key genes involved in corticosteroidogenesis, including melanocortin 2 receptor, steroidogenic acute regulatory protein and cytochrome P450 side chain cleavage enzyme. At 28 d, the high cadmium exposure group showed a significant drop in the glucocorticoid receptor and mineralocorticoid receptor protein expressions in the liver and brain, respectively. There were also perturbations in the metabolic capacities in the liver and gill of cadmium-exposed trout. Subjecting these fish to a secondary handling disturbance led to a significant attenuation of the stressor-induced plasma cortisol, glucose and lactate levels in the cadmium groups. Collectively, although trout appears to adjust to subchronic exposure to low levels of cadmium, it may be at the cost of impaired interrenal steroidogenic and tissue-specific metabolic capacities, leading to a compromised secondary stress performance in rainbow trout.

1. Introduction

Fish encounter elevated cadmium (Cd) concentrations in their aquatic environment due to both natural and anthropogenic sources, which usually results in elevated Cd bioaccumulation in tissues (McGeer et al., 2011). In vertebrates, Cd has no known physiological function, but can exert toxicity at sublethal concentrations (McGeer et al., 2011). The primary route of entry of Cd in fish is through their gills and/or gastrointestinal tract and accumulates predominately in the kidney, gills and liver, and to a lesser extent in the brain (Kamunde, 2009; McGeer et al., 2011). The effects of chronic exposure to waterborne Cd at sublethal concentrations include disturbances in whole-body or plasma ion homeostasis, modifications in tissue-specific enzyme activities and metabolic capacity, as well as endocrine disruption (McGeer et al., 2011).

A well-known endocrine disrupting effect of sublethal Cd exposure involves the impairment of cortisol stress axis functioning in fish (Brodeur et al., 1997; Ricard et al., 1998; Lacroix and Hontela, 2004; Hontela and Vijayan, 2008). The corticosteroid stress response in teleosts involves the activation of the hypothalamus-pituitary-interrenal (HPI) axis culminating in the release of cortisol into circulation (Aluru and Vijayan, 2009). The primary hormonal step in HPI activation involves the secretion of corticotropin-releasing factor (CRF) that stimulates the pituitary to produce adrenocorticotrophic hormone (ACTH), a proopiomelanocortin-derived hormone (Aluru and Vijayan, 2009). ACTH activates melanocortin 2 receptor (MC2R), a G-protein coupled receptor leading to corticosteroid biosynthesis (Metz et al., 2006; Aluru and Vijayan, 2008). The key steps in cortisol biosynthesis is thought to include the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR), as well as the conversion of cholesterol to pregnenolone catalyzed by the cytochrome P450 side chain cleavage enzyme (P450scc) (Payne and Hales, 2004; Aluru and Vijayan, 2006). The terminal step in

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corticosteroid biosynthesis involves the conversion of deoxycortisol to cortisol by 11-β hydroxylase (Payne and Hales, 2004; Aluru and Vijayan, 2006).

The target tissue cortisol action is mediated by activation of either the glucocorticoid receptor (GR) and/or the mineralocorticoid receptor (MR) (Prunet et al., 2006; Takahashi and Sakamoto, 2013). In teleosts, unlike mammals, there are multiple copies of GRs but a functional role for these isoforms has not been established (Prunet et al., 2006; Aluru and Vijayan, 2009). The cortisol response during stress allows for physiological adjustments that are essential to restore homeostasis (Mommsen et al., 1999; Aluru and Vijayan, 2009). A primary role for stressor-induced cortisol elevation is to allow for metabolic adjustments, including stimulation of gluconeogenesis in the liver to restore homeostasis (Mommsen et al., 1999). While Cd has been shown to target the HPI axis and disrupt cortisol production (Hontela and Vijayan, 2008), the effect of subchronic exposure to environmentally relevant levels of Cd on target tissues involved and the mechanisms of action are far from clear.

We tested the hypothesis that subchronic exposure to Cd disrupts the metabolic capacity and compromises the cortisol stress performance to a secondary stressor in juvenile rainbow trout (*Oncorhynchus mykiss*). This was tested using a water-borne exposure in a flow-through system as described in Milne (2010). Trout were exposed to either 0 (control), 0.75 (low exposure) or 2.0 μg/L (high exposure) Cd for 28 d and then subjected to a 5 min handling disturbance as described before (Ings et al., 2011). We examined plasma cortisol, glucose and lactate levels as markers of stress response, while transcript abundances of MC2R, StAR and P450scc in the head kidney tissue were used as markers of steroidogenic capacity. Liver and gill metabolic capacities were determined by measuring the activities of glycolytic (hexokinase, glucokinase, pyruvate kinase and lactate dehydrogenase), gluconeogenic (phosphoenolpyruvate carboxykinase) and oxidative (citrate synthase) enzymes, while GR and MR protein expressions in the liver and brain were used to assess target tissue cortisol responsiveness.

2. Materials and method

2.1. Chemicals

Tricaine methanesulfonate (MS-222) and sodium bicarbonate were purchased from Syndel Laboratories Ltd., (Vancouver, BC, CAN). Borosilicate and scintillation tubes, monobasic and dibasic sodium phosphate, potassium bicarbonate, perchloric acid, potassium chloride and sodium bicarbonate were purchased from Fisher Scientific (Fairlawn, NJ, USA). Scintillation cocktail and cortisol antibody were purchased from MP biomedicals (Solon, OH, USA). [1,2,6,7-3H]-cortisol tracer and ECl plus Western Detection System were purchased from GE healthcare (Upsala, Sweden). D-glucose and Tween 20 were purchased from Bioshop (Burlington, ON, CAN). Cadmium chloride and 96-well plates were purchased from VWR (Mississauga, ON, CAN). Thimerasol, activated charcoal, dextran (from *Leuconostoc mesenteroides*), amyloglucosidase, imidazole, beta-nicotinamide adenine dinucleotide disodium salt (β-NADH), phosphenolpyruvate (PEP), lactate dehydrogenase (LDH), pyruvate kinase, adenosine triphosphate (ATP), adenosine diphosphate (ADP), deoxyguanosinediphosphate (2-DGDP), imidazole, magnesium chloride, and sodium bicarbonate were purchased from Sigma (St. Louis, MO, USA). Manganese chloride was purchased from J.T. Baker Chemidal CO (Phillipsburg, NJ, USA). Protease inhibitor tablets were purchased from Roche (Mannheim, Germany). Protein ladder and Sybr green for quantitative real-time PCR (qPCR) were purchased from Bio-Rad (Hercules, CA, USA).

2.2. Experimental animals and Cd exposure

Juvenile rainbow trout (27 ± 8 g) were obtained from Rainbow Springs Hatchery (Thamesford, ON, CAN) and held at Wilfrid Laurier University under protocols approved and verified by the Laurier Animal Care Committee, which adheres to and is certified by the Canadian Council on Animal Care as described in detail in Milne (2010). Initially, 420 fish were randomly divided among two 220 L polyethylene tanks (RTS Plastics, New Hamburg, ON, CAN) and acclimated for two weeks to flowing water (140 mg/L $CaCO_3$ with Ca, Mg and Na concentrations of 868 ± 28, 480 ± 16 and 338 ± 16 uM (mean ± SEM), pH 7.2 and 11 °C) that was a mixture of local well water and reverse osmosis product water. Fish were fed 2% body weight daily as a single meal (Bio Oregon Protein Inc., Warrenton, OR) and a photoperiod (16 light:8 dark) was maintained throughout the experiment.

Following the acclimation period, trout were non-selectively distributed to six 220 L polyethylene tanks (70 per tank) supplied with flowing (700 mL/min) water. Continuously flowing well and reverse osmosis water to achieve the desired chemistry (see previous paragraph) were mixed in a 60 L polyethylene head tank and then delivered to three smaller 10 L polypropylene exposure head tanks before distribution to fish tanks. Concentrated stock solutions of Cd (as $CdCl_2$) were metered (QG6 pump, Fluid Metering Inc, New York) into two of the exposure head tanks to achieve the desired waterborne Cd concentration (either 0.75 or 2.0 μg Cd/L). Test solution (or unmodified (control) water) delivery from the exposure head tanks was split to replicate fish tanks ($n = 2$ tanks of 70 fish each for control, 0.75 or 2 μg Cd/L). Measured total Cd concentrations were determined using graphite furnace atomic absorption spectrometry (see Milne, 2010) and were 0.03 ± 0.0002 μg Cd/L, 0.71 ± 0.101 μg Cd/L and 1.85 ± 0.119 μg Cd/L for control, low and high exposures, respectively. Exposures were initiated by spiking the tanks with appropriate volumes of concentrated stock solution to achieve the target exposure concentrations and throughout the exposure all header and fish tanks were aerated. The concentrated Cd stock solutions were renewed weekly and water pH, conductivity and temperature were also measured daily using a pH meter (Seven Go, Mettler Toledo, Fisher Scientific, Mississauga, ON, CAN) and a conductivity meter (YSI 30, Yellow Springs Instruments, Yellow Springs, OH, USA). Feeding was stopped 24 h prior to sampling.

2.3. Sampling

The Cd exposure study was carried out for 28 d and fish were sampled at 1, 7 and 28 d post-exposure. At each sampling time 8 fish (4 fish per replicate exposure tank) were sampled, except on day 28 for the 2.0 μg Cd/L group, where only 6 fish (3 from each tank) were sampled. Sampling consisted of euthanizing the fish quickly with an overdose of MS-222 (1 g per 10 L) buffered with sodium bicarbonate. Fish were bled by severing the caudal peduncle and blood was collected into heparinized tubes. Blood-containing tubes were centrifuged at 10,000 × *g* for 2 min to separate the plasma and stored at −70 °C until cortisol, glucose and lactate analysis. Tissues (brain, liver and head kidney) were sampled on 1, 7 and 28 d post-Cd exposure and stored frozen at −70 °C to assess enzyme activities, metabolite level, protein expression and mRNA abundances later.

2.4. Acute stressor exposure

After 28 d exposure, the remaining fishes in the tanks for each group were subjected to a handling disturbance for 5 min as described previously (Ings et al., 2011). Fish were sampled either prior to (28 d = 0 h) or at 1 and 24 h post-handling stressor.