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Species-specific sensitivity to selenium-induced impairment of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*)

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ABSTRACT

Species differences in physiological and biochemical attributes exist even among closely related species and may underlie species-specific sensitivity to toxicants. Rainbow trout (RT) are more sensitive than brook trout (BT) to the teratogenic effects of selenium (Se), but it is not known whether all tissues exhibit this pattern of vulnerability. In this study, primary cultures of RT and BT adrenocortical cells were exposed to selenite (Na_2SO_3) and selenomethionine (Se-Met) to compare cell viability and ACTH-stimulated cortisol secretion in the two fish species. Cortisol, the primary stress hormone in fish, facilitates maintenance of homeostasis when fish are exposed to stressors, including toxicants. Cell viability was not affected by Se, but selenite impaired cortisol secretion, while Se-Met did not (RT and BT EC_{50}>2000 mg/L). RT cells were more sensitive (EC_{50} = 8.7 mg/L) to selenite than BT cells (EC_{50} = 90.4 mg/L). To identify the targets where Se disrupts cortisol synthesis, selenite-impaired RT and BT cells were stimulated with ACTH, dbcAMP, OH-cholesterol, and pregnenolone. Selenite acted at different steps in the cortisol biosynthesis pathway in RT and BT cells, confirming a species-specific toxicity mechanism. To test the hypothesis that oxidative stress mediates Se-induced toxicity, selenite-impaired RT cells were exposed to NAC, BSO and antioxidants (DETCA, ATA, Vit A, and Vit E). Inhibition of SOD by DETCA enhanced selenite-induced cortisol impairment, indicating that oxidative stress plays a role in Se toxicity; however, modifying GSH content of the cells did not have an effect. The results of this study, with two closely related salmonids, provided additional evidence for species-specific differences in sensitivity to Se which should be considered when setting thresholds and water quality guidelines.

Introduction

Fundamental differences in biochemical and physiological attributes exist, even among closely related species. Rainbow trout (*Oncorhynkiss mykiss*), a species highly sensitive to environmental stressors and toxicants, digest macronutrients more efficiently than Atlantic salmon (*Salmo salar*) (Krogdahl et al., 2004), have higher hepatic glutathione (GSH) reserves than brook trout (*Salvelinus fontinalis)* (Miller et al., 2009), and have lower phase I biotransformation V_{max} for sulfoxidation than channel catfish (*Ictalurus punctatus*) or tilapia (*Oreochromis* sp.) (Gonzalez et al., 2009). Such differences in the basic biology of organisms may mediate species-specific sensitivity to toxicants. For example, the greater sensitivity to waterborne cadmium or copper of rainbow trout compared to yellow perch (*Perca flavescens*) can be explained by differences in the affinity and function of gill binding sites and transport fluxes of the toxicants and of ions such as Ca^{2+} and Na^{+} (Taylor et al., 2003; Niyogi and Wood, 2004). Species-specific sensitivity to toxicants in fish have been also documented for pesticides (VanDolah et al., 1997; Quinn et al., 2010), pharmaceuticals (Gonzalez et al., 2009), and selenium (Holm et al., 2005).

Selenium (Se), an essential constituent of glutathione peroxidase, deiodinase and thioredoxin reductase, can be toxic at levels slightly above homeostatic requirement (Janz et al., 2010). It occurs at varying levels in the bedrock, with highest concentrations measured in marine shales (Haygarth, 1994). Anthropogenic activities such as coal mining and agriculture enhance weathering of seleniferous rock, increasing Se levels in the aquatic environment (Hamilton, 2004). Selenium bioaccumulates in the liver and gonads of fish, and uptake occurs primarily through diet, not the water column (Stewart et al., 2010). Selenite is the most acutely toxic form of Se to fish, followed by selenate and organic selenomethionine (Se-Met). Selenium is teratogenic in fish (Coyle et al., 1993; Hamilton et al., 2005; Rigby et al., 2010) and species-specific sensitivities to Se have been documented. Rainbow trout have higher larval deformity rates than brook trout or cutthroat trout (*Oncorhynchus clarki)* when exposed to elevated Se in

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the environment (Holm et al., 2005). It is not known whether processes other than larval development exhibit species-specific sensitivities to Se, and what cellular characteristics and mechanisms underlie these differences.

During the metabolism of both selenite and Se-Met, reactive oxygen species (ROS), leading to increased oxidative damage, are produced (Palace et al., 2004; Misra and Niyogi, 2009); however, the metabolic pathways for selenite and Se-Met differ. Selenite is reduced by GSH to hydrogen selenide, which may then react with oxygen to produce ROS (Seko et al., 1989). This mechanism of oxidative stress only requires the presence of GSH and oxygen. In contrast, the production of ROS by Se-Met is more complex. Selenomethionine must be first metabolized to methylselenol by methioninase, then methylselenol reacts with GSH to produce the ROS (Palace et al., 2004). Thus GSH, usually acting as an antioxidant protecting cells from damage (Kelly et al., 1998), plays a role in ROS production by selenite and Se-Met. Se-induced oxidative damage in human hepatoma cells was observed in both cells depleted of GSH and those with artificially elevated GSH levels (Shen et al., 2000), suggesting a dual role for GSH in Se toxicity. Although Palace et al. (2004) provided evidence that GSH augments Se toxicity in fish embryos, the universality of oxidative stress and GSH in Se toxicity has not been demonstrated thus far.

The teleost adrenocortical cell model has been used to assess adrenal toxicity of several organic and inorganic toxicants (Hontela and Vijayan, 2009), but the adrenotoxicity of Se, either as selenite or Se-Met, has not been investigated. The adrenocortical cells are located in the head kidney, the adrenal tissue of teleost fish (Hontela, 2005), a system which has been well characterized *in vitro* (Lacroix and Hontela, 2001; Aluru et al., 2005; Fuzzen et al., 2010). When a fish perceives a stress, adrenocorticotropic releasing hormone (ACTH) binds a membrane receptor in the steroidogenic adrenocortical cell and activates the cAMP-protein kinase A signaling pathway to stimulate, via the StAR protein, the uptake of cholesterol by the mitochondria (Fig. 1). Cholesterol is transformed to pregnenolone, and then a series of cytochrome P450 enzymes in the endoplasmic reticulum and the mitochondria transform pregnenolone to cortisol (Hontela, 2005).

The steroidogenic pathway leading to cortisol can be manipulated with steroid precursors and signaling molecules to determine the specific step(s) disrupted by a toxicant (Bisson and Hontela, 2002; Hontela and Vijayan, 2009). Such mechanism-based data might be extrapolated to other steroidogenic cholesterol-dependent pathways, including synthesis of testosterone and estrogens. It may also be used to compare species-specific sensitivities to toxicants and investigate the mechanisms of toxicity (Lacroix and Hontela, 2004). The present study tested the hypotheses that Se is adrenotoxic, and that adrenocortical cells of rainbow trout (RT) are more sensitive to Se than the adrenocortical cells of brook trout (BT), as has been proposed for the teratogenic effects of Se. The specific objectives were to (1) determine, *in vitro*, the effect of sodium selenite and Se-Met on cortisol secretion by adrenocortical cells in RT and BT, (2) identify the step(s) disrupted by Se in the steroidogenic pathway leading to cortisol secretion, and (3) investigate the role of oxidative stress in Se toxicity.

Materials and methods

Chemicals. Porcine adrenocorticotropin (ACTH I-39), collagenase/dispase, DNAase, minimal essential medium (MEM), bovine serum albumin (BSA), sodium bicarbonate ($NaHCO_3$), L-buthionine-[*S*,*R*]-sulfoximine (BSO), N-acetyl-L-cysteine (NAC), sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), dextrose, sodium selenite (Na_2SeO_3), selenomethionine (Se-Met), potassium phosphate (KH_2PO_4), ethanol, ethylenediaminetetraacetic acid (EDTA), metaphosphoric acid, 2,6-di-*tert*-butyl-4-methylphenol (BHT), 3-amino-1,2,4-triazole (ATA), sodium diethyldithiocarbamate trihydrate (DETCA), α-tocopherol, trypan blue, retinol, pregnenolone, N^6,2′-o-dibutyryladenosine 3′,5′-cyclic monophosphate (dbcAMP), and OH-cholesterol were purchased from Sigma-Aldrich (Oakville, Ontario). Hepes was purchased from Fisher Scientific (Ottawa, Ontario) and 3-aminobenzoic acid ethyl ester (MS-222) was purchased from MP Biomedicals (Solon, Ohio).

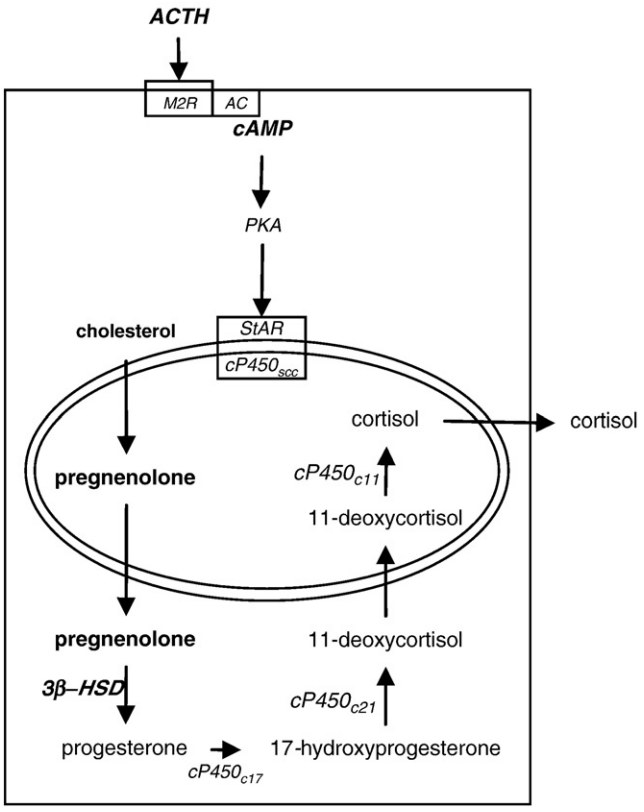

Fig. 1. Cortisol biosynthesis pathway in an adrenocortical steroidogenic cell of the teleost fish. ACTH = adrenocorticotropic hormone, M2R = melanocortin 2 receptor, AC = adenylyl cyclase, cAMP = cyclic adenosine monophosphate, PKA = protein kinase A, StAR = steroidogenic acute regulatory protein, $cP450_{scc}$ = cytochrome P450 side chain cleavage, 3β-HSD = 3β-hydroxysteroid-Δ5-steroid dehydrogenase, $cP450_{c17}$ = cytochrome P450 17α-hydroxylase-17,20 lyase, $cP450_{c21}$ = cytochrome P450 21-hydroxylase, and $cP450_{c11}$ = cytochrome P450 11β-hydroxylase. Compounds in bold were used in the pathway experiment.
Adapted from Hontela (2005) and Hontela and Vijayan, (2009).

Fish. Animal use protocols were approved by the University of Lethbridge Animal Care Committee in accordance with national guidelines. Juvenile rainbow trout, *O. mykiss*, (109.9 ± 4.9 g) and juvenile brook trout, *S. fontinalis*, (54.8 ± 2.2 g) were obtained from the Allison Creek Brood Trout Station (Blairmore, Alberta). Fish were kept in a 1000 L tank (semi-static system, 25% daily water renewal, 7 mg/L oxygen, 161 mg/L $CaCO_3$) at 14 °C for the duration of experiment. Fish were fed extruded floating steelhead food pellets (Nelson's Silver Cup Fish Feed, Allison Creek Brood Trout Station, Blairmore, Alberta) between 0900 and 1000 hours *ad libitum*. Fish were allowed a minimum of two weeks to acclimate to laboratory conditions before experiments began.

Cell culture. Fish were sacrificed with 1 g/L MS-222, bled from the caudal vasculature, and perfused through the heart with 0.7% NaCl. The head kidney was removed and a rough homogenate made (pieces ~1 mm^3). The tissue was then digested with collagenase/dispase (2 mg/mL) and DNAase (1.2 mg/mL) in MEM (pH = 7.4, supplemented with 5 g/L BSA and 2.2 g/L $NaHCO_3$) for 1 h at 23 °C. The cell suspension was filtered with Nitex monofilament cloth (30 μm) and the cell concentration adjusted to 75×10^6 cells/mL.