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Methylmercury-induced changes in gene transcription associated with neuroendocrine disruption in largemouth bass (*Micropterus salmoides*)

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ABSTRACT

Methyl-mercury (MeHg) is a potent neuroendocrine disruptor that impairs reproductive processes in fish. The objectives of this study were to (1) characterize transcriptomic changes induced by MeHg exposure in the female largemouth bass (LMB) hypothalamus under controlled laboratory conditions, (2) investigate the health and reproductive impacts of MeHg exposure on male and female largemouth bass (LMB) in the natural environment, and (3) identify MeHg-associated gene expression patterns in whole brain of female LMB from MeHg-contaminated habitats. The laboratory experiment was a single injection of 2.5 μg MeHg/g body weight for 96 h exposure. The field survey compared river systems in Florida, USA with comparably lower concentrations of MeHg (Wekiva, Santa Fe, and St. Johns Rivers) in fish and one river system with LMB that contained elevated concentrations of MeHg (St. Marys River). Microarray analysis was used to quantify transcriptomic responses to MeHg exposure. Although fish at the high-MeHg site did not show overt health or reproductive impairment, there were MeHg-responsive genes and pathways identified in the laboratory study that were also altered in fish from the high-MeHg site relative to fish at the low-MeHg sites. Gene network analysis suggested that MeHg regulated the

Abbreviations: 11-KT, 11-ketotestosterone; ABCB7, ATP-binding cassette sub-family B (MDR/TAP), member 7; AMACR, alpha-methylacyl-CoA racemase; AR, androgen receptor; BEST, Biomonitoring of Environmental Status and Trends; BRD4, bromodomain containing 4; CASP3, caspase 3, apoptosis-related cysteine peptidase; CAT, catalase; CCND2, cyclin D2; CD9, CD9 molecule; CDC73, cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (*S. cerevisiae*); CGA, glycoprotein hormones, alpha polypeptide; CIDEA, cell death-inducing DFFA-like effector a; CLDN4, claudin 4; CNS, central nervous system; CP, ceruloplasmin (ferroxidase); CPE, carboxypeptidase E; CREB1, cAMP responsive element binding protein 1; CTSD, cathepsin D; CYP19A1, cytochrome P450, family 19, subfamily A, polypeptide 1; CYP24A1, cytochrome P450, family 24, subfamily A, polypeptide 1; CYP26A1, cytochrome P450, family 26, subfamily A, polypeptide 1; E2, 17β-estradiol; ESR1, estrogen receptor 1; ESR2, estrogen receptor 2 (ER beta); EROD, ethoxyresorufin-O-deethylase; F2, coagulation factor II (thrombin); FDR, false discovery rate; FGA, fibrinogen alpha chain; FOS, FBJ murine osteosarcoma viral oncogene homolog; FSHB, follicle stimulating hormone, beta polypeptide; FST, follistatin; GAD1, glutamate decarboxylase 1 (brain, 67 kDa); GAD2, glutamate decarboxylase 2 (pancreatic islets and brain, 65 kDa); GH1, growth hormone 1; GNAQ, guanine nucleotide binding protein (G protein), q polypeptide; GNAS, GNAS complex locus; GnRH, gonadotropin-releasing hormone; GNRHR, gonadotropin-releasing hormone receptor; GSEA, gene set enrichment analysis; HSPA8, heat shock 70 kDa protein 8; IGF1, insulin-like growth factor 1 (somatomedin C); KDR, kinase insert domain receptor (a type III receptor tyrosine kinase); LDLR, low density lipoprotein receptor; LH, luteinizing hormone; LHB, luteinizing hormone beta polypeptide; LMB, largemouth bass; LTF, lactotransferrin; LYZ, lysozyme; MBP, myelin basic protein; MeHg, methylmercury; MYF5, myogenic factor 5; MYOD1, myogenic differentiation 1; NAWQA, National Water-Quality Assessment Program; NFKBIA, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NPY, neuropeptide Y; NR1H3, nuclear receptor subfamily, 1 group H, member 3; NRIP1, nuclear receptor interacting protein 1; PARP1, poly (ADP-ribose) polymerase 1; PCK2, phosphoenolpyruvate carboxykinase 2 (mitochondrial); PECR, peroxisomal *trans*-2-enoyl-CoA reductase; PENK, proenkephalin; PITX1, paired-like homeodomain 1; PITX2, paired-like homeodomain 2; PLG, plasminogen; POMC, proopiomelanocortin; PPARG, peroxisome proliferator-activated receptor gamma; PRDX4, peroxiredoxin 4; PRL, prolactin; PROP1, PROP paired-like homeobox 1; PTPN6, protein tyrosine phosphatase, non-receptor type 6; PTTG1, pituitary tumor-transforming 1; RASAL1, RAS protein activator like 1 (GAP1 like); SCG2, secretogranin II; SDHC, succinate dehydrogenase complex, subunit C, integral membrane protein, 15 kDa; SERPINC1, serpin peptidase inhibitor, clade C (antithrombin), member 1; SGCE, sarcoglycan, epsilon; SNEA, sub network enrichment analysis; SRD5A1, steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1); SSTR2, somatostatin receptor 2; STAR, steroidogenic acute regulatory protein; STC1, stanniocalcin 1; T, testosterone; TAGLN, transgelin; TBP, TATA box binding protein; TCF21, transcription factor 21; TGFB1, transforming growth factor, beta 1; TH, tyrosine hydroxylase; THRA, thyroid hormone receptor, alpha (erythroblastic leukemia viral (v-erb-a) oncogene homolog, avian); TPT1, tumor protein, translationally-controlled 1; TSPO, translocator protein (18 kDa); VEGFA, vascular endothelial growth factor A; Vtg, vitellogenin.

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expression targets of neuropeptide receptor and steroid signaling, as well as structural components of the cell. Disease-associated gene networks related to MeHg exposure, based upon expression data, included cerebellum ataxia, movement disorders, and hypercalcemia. Gene responses in the CNS are consistent with the documented neurotoxicological and neuroendocrine disrupting effects of MeHg in vertebrates.
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1. Introduction

Mercury is a toxicant of global concern because it is transported through the atmosphere. Atmospheric mercury originates from both natural sources and anthropogenic sources, such as coal-burning power plants. Aquatic systems become contaminated with inorganic mercury from atmospheric deposition, as well as from point sources such as industrial activities and mining. Bacteria in aquatic sediments convert inorganic mercury to methyl-mercury (MeHg), which readily bioaccumulates through aquatic food webs. Thus, fish, wildlife, and humans are at risk of exposure to MeHg. Developmental exposure to MeHg is well known to have neurotoxic effects in mammals (Clarkson and Magos, 2006). More recently, the neuroendocrine-disrupting effects of MeHg exposure have been highlighted in aquatic vertebrates (Crump and Trudeau, 2009; Waye and Trudeau, 2011).

Exposure of fish to environmentally-relevant concentrations of MeHg can cause negative effects on reproductive performance. Tilapia (*Oreochromis niloticus*) exposed to MeHg chloride exhibited reduced steroid hormone concentrations and abnormal gonad development in females (Arnold, 2000). In another study, plasma concentrations of the steroid hormones 17β-estradiol (E2) and 11-ketotestosterone (11-KT) were suppressed in female and male fathead minnows (*Pimephales promelas*), respectively, following a low dietary exposure to MeHg (Drevnick and Sandheinrich, 2003). The same study found retarded gonad development in females and reduced spawning success. Thus, there appears to be clear evidence that mercury, at environmentally-relevant concentrations (<1 μg/g in diet), can cause altered reproductive performance in teleost fishes. However, it is unclear if the inhibition of reproduction is primarily due to direct effects on neuroendocrine function in the CNS or due to effects on peripheral reproductive tissues.

The mechanisms of MeHg toxicity include oxidative stress, binding of methylmercury to sulfhydryl groups of proteins, and the alteration of intracellular calcium concentrations (Ceccatelli et al., 2010). Thus, MeHg has multiple molecular targets, including those that are involved in the neuroendocrine system (Crump and Trudeau, 2009; Tan et al., 2009). To improve understanding of the molecular signaling cascades affected by MeHg, transcriptomics studies have been conducted in both mammals (Hwang et al., 2011; Padhi et al., 2008) and fish (Liu et al., 2013; Richter et al., 2011). These studies have demonstrated that genes related to nerve cell differentiation, myelination, iron ion homeostasis, glutathione transferase activity, regulation of muscle contraction, protein folding, cell redox homeostasis, and steroid biosynthetic process are susceptible to alteration in response to MeHg exposure. Although there is a growing body of literature that characterizes the molecular signaling cascades in the central nervous system (CNS) and peripheral tissues of vertebrates that are responsive to MeHg, additional comparative studies are warranted due to the significant human health risks associated with MeHg neurotoxicity and neuroendocrine disruption.

The objectives of this study were to identify gene networks in the CNS of female largemouth bass (*Micropterus salmoides*, LMB) that were responsive to MeHg treatments, assess the general health and reproductive endocrine status of LMB in environments with MeHg contamination, and to ascertain whether any changes in the expression of genes or gene networks that were responsive to MeHg exposure in a controlled laboratory study were also affected in the brain of female LMB collected from high-MeHg sites in the natural environment. Sites chosen for this research were located on the St. Marys, Santa Fe, Wekiva, and St. Johns River systems of central and northern Florida, representing a range of mercury contamination.

2. Materials and methods

2.1. Laboratory exposures and field collections

Female LMB were injected with either a control injection of 20 mM Na_2CO_3 (pH 6.98) or an injection of MeHg at 2.5 μg/g body weight in 20 mM Na_2CO_3 (pH 6.98) as previously described (Richter et al., 2011). Fish were maintained at CERC in flowing well water, by treatment. Water quality was monitored throughout the holding period. After 96 h, the fish were anesthetized with ethyl 3-aminobenzoate methanesulfonate (MS-222, Sigma–Aldrich) and the hypothalamus was dissected and flash frozen with liquid nitrogen. All animal use was reviewed and approved by the Columbia Environmental Research Center Institutional Animal Care and Use Committee.

We conducted fish health assessments at three study locations in Florida in conjunction with the USGS National Water-Quality Assessment (NAWQA) Program. The three sites were the Wekiva, Santa Fe, and St. Marys Rivers in Florida, USA. Site characteristics of the Santa Fe and St. Marys River sites have been previously described (Chasar et al., 2008, 2009). Additional LMB whole brain samples for the microarray study (details below) were collected from the St. Johns River near Welaka, FL. The St. Johns River is relatively free of Hg contamination. Fish sampling and site details for LMB in this river system can be found in Martyniuk et al. (2013). All field research was conducted in accordance with the procedures described by the American Society of Ichthyologist and Herpetologists (ASIH), American Fisheries Society (AFS), and American Institute of Fishery Research Biologists (AIFRB).

2.2. Field health assessments and tissue collection

Fish examination and tissue collection procedures in the field followed the methods of Schmitt et al. (1999). Weights (±1 g) and lengths (±1 mm) of all fish were recorded. External lesions and a necropsy-based health assessment of LMB were performed according to the protocols set forth in the USGS Biomonitoring of Environmental Status and Trends (BEST) Program (Schmitt and Dethloff, 2000). Visible lesions and gross pathological anomalies (fin erosions, skin ulcerations, eye disorders, visible tumors, and skeletal deformities) were scored based upon their occurrence and prevalence.

Following field examination, tissue, blood, and plasma samples were collected for analyses of total mercury and biomarkers that included ethoxyresorufin-O-deethylase (EROD) activity, vitellogenin (Vtg), and sex steroids E2, 11-KT and testosterone (T). Blood samples were obtained via caudal venipuncture using a heparinized

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