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Sexually dimorphic transcriptomic responses in the teleostean hypothalamus: A case study with the organochlorine pesticide dieldrin

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ABSTRACT

Organochlorine pesticides (OCPs) such as dieldrin are a persistent class of aquatic pollutants that cause adverse neurological and reproductive effects in vertebrates. In this study, female and male largemouth bass (*Micropterus salmoides*) (LMB) were exposed to 3 mg dieldrin/kg feed in a 2 month feeding exposure (August–October) to (1) determine if the hypothalamic transcript responses to dieldrin were conserved between the sexes; (2) characterize cell signaling cascades underlying dieldrin neurotoxicity; and (3) determine whether or not co-feeding with 17β-estradiol (E_2), a hormone with neuroprotective roles, mitigates responses in males to dieldrin. Despite also being a weak estrogen, dieldrin treatments did not elicit changes in reproductive endpoints (e.g. gonadosomatic index, vitellogenin, or plasma E_2). Sub-network (SNEA) and gene set enrichment analysis (GSEA) revealed that neuro-hormone networks, neurotransmitter and nuclear receptor signaling, and the activin signaling network were altered by dieldrin exposure. Most striking was that the majority of cell pathways identified by the gene set enrichment were significantly increased in females while the majority of cell pathways were significantly decreased in males fed dieldrin. These data suggest that (1) there are sexually dimorphic responses in the teleost hypothalamus; (2) neurotransmitter systems are a target of dieldrin at the transcriptomics level; and (3) males co-fed dieldrin and E_2 had the fewest numbers of genes and cell pathways altered in the hypothalamus, suggesting that E_2 may mitigate the effects of dieldrin in the central nervous system.

1. Introduction

Dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,67,8,8a-octahydro-1,4,5,8-dimethanonaphthalene) is a neuroactive organochlorine pesticide (OCP) that is a persistent organic pollutant found primarily in sediment in aquatic habitats. Despite restricted use in recent years, dieldrin remains a concern for aquatic organisms such as teleost fishes because there is a high capacity for dieldrin to bioaccumulate in tissues. This has been demonstrated for fish exposed in the laboratory (Lamai et al., 1999;

Abbreviations: A2M, alpha-2-macroglobulin; AMACR, alpha-methylacyl-CoA racemase; APOA1, apolipoprotein A-I; AR, androgen receptor; BCL2L1, BCL2-like 1; CAT, catalase; CCNA2, cyclin A2; CCND2, cyclin D2; CCNE1, cyclin E1; CEL, carboxyl ester lipase (bile salt-stimulated lipase); CGA, glycoprotein hormones, alpha polypeptide; CTNNB1, catenin (cadherin-associated protein), beta 1, 88 kDa; CYB5A, cytochrome b5 type A (microsomal); CYP19A1, cytochrome P450, family 19, subfamily A, polypeptide 1; CYP26A1, cytochrome P450, family 26, subfamily A, polypeptide 1; DBI, diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein); DRD1, dopamine receptor D1; DRD2, dopamine receptor D2; EP300, E1A binding protein p300; ESR2, estrogen receptor 2 (ER beta); F9, coagulation factor IX; FGB, fibrinogen beta chain; FN1, fibronectin 1; FOS, v-fos FBJ murine osteosarcoma viral oncogene homolog; FOXC1, forkhead box C1; FSHB, follicle stimulating hormone, beta polypeptide; FST, follistatin; GH1, growth hormone 1; GNRHR, gonadotropin-releasing hormone receptor; GSTA2, glutathione S-transferase alpha 2; HMGCR, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase; HOMER1, homer homolog 1 (Drosophila); HSD3B2, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2; IGF1, insulin-like growth factor 1 (somatomedin C); KLKB1, kallikrein B, plasma (Fletcher factor) 1; KRAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; LHB, luteinizing hormone beta polypeptide; MET, met proto-oncogene (hepatocyte growth factor receptor); MYOD1, myogenic differentiation 1; NFKBIA, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NPY, neuropeptide Y; NR5A1, nuclear receptor subfamily 5, group A, member 1; NR5A2, nuclear receptor subfamily 5, group A, member 2; NR0B1, nuclear receptor subfamily 0, group B, member 1; P2RX7, purinergic receptor P2X, ligand-gated ion channel, 7; PCK2, phosphoenolpyruvate carboxykinase 2 (mitochondrial); PDE7B, phosphodiesterase 7B; POMC, proopiomelanocortin; PRL, prolactin; PROC, protein C (inactivator of coagulation factors Va and VIIIa); PVALB, parvalbumin; SCARB1, scavenger receptor class B, member 1; SCP2, sterol carrier protein 2; SENP1, SUMO1/sentrin specific peptidase 1; SLC10A1, solute carrier family 10 (sodium/bile acid cotransporter family), member 1; SLC12A2, solute carrier family 12 (sodium/potassium/chloride transporters), member 2; STAR, steroidogenic acute regulatory protein; SYT7, synaptotagmin VII; TGFB1, transforming growth factor, beta 1; TH, tyrosine hydroxylase; VEGFA, vascular endothelial growth factor A.

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Satyanarayan et al., 2005) and for fish exposed in the natural environment (Johnson et al., 2007; Blocksom et al., 2010). There are also human health concerns surrounding dieldrin that are based upon epidemiological and neurophysiological evidence. Exposure to dieldrin may be associated with increased risk for the progression of human neurological diseases such as Alzheimer's and Parkinson's disease (Fleming et al., 1994; Kanthasamy et al., 2005, 2008; Weisskopf et al., 2010), although a direct causative effect remains difficult to ascertain.

In the context of neurodegenerative diseases, there exist sex differences within the vertebrate brain that may contribute to increased risks to neurodegeneration (Schultz et al., 1996). Neurotransmitter systems (GABA, dopamine, and serotonin) show differences throughout the male and female brain, and serotonin levels and receptors are higher in abundance in females compared to males (Cosgrove et al., 2007). This is also the case for many brain structures and nuclei. Specifically within the hypothalamus, studies report that the infundibular nucleus (INF), an important regulator of neuroendocrine function in the mediobasal hypothalamus, can exhibit sex dependent neurofibrillary pathology which is more prevalent in men than woman with neurodegenerative diseases (Schultz et al., 1999). Lastly, in the mammalian hypothalamus, there is evidence that gene expression patterns show sexual dimorphism with aging, one of the risk factors for neurodegeneration (Berchtold et al., 2008). Males show more changes in gene expression compared to females in the somatosensory cortex, superior frontal gyrus, and entorhinal cortex which is associated with a down regulation of genes in males that are related to protein processing and energy generation. The molecular basis for sex differences in the vertebrate brain are not completely understood and continued exploration into the relationship between sex, exposure to chemicals in the environment, and neurological disease etiology is warranted.

The primary mechanism of action of dieldrin is antagonism of the gamma-aminobutyric acid $(GABA)_A$ receptor, blocking inhibitory GABAergic synaptic transmission in the vertebrate central nervous system (Lawrence and Casida, 1984; Gant et al., 1987). There is also evidence that dieldrin can act as a weak estrogen by binding estrogen receptors (ERs) in mammals (Lemaire et al., 2006) and activating Esr1 in fish (Weil and Denslow, unpubl. data). Thus, the molecular and physiological responses regulated by dieldrin can be multi-modal, involving both direct modulation of GABAergic synaptic transmission in the central nervous system (CNS) and ER signaling cascades.

Genomic and proteomic responses in the teleostean hypothalamus to dieldrin have been investigated previously in reproductively mature female and male largemouth bass (*Micropterus salmoides*) (LMB). In an acute exposure, female LMB injected with 10 mg dieldrin/kg and sacrificed after seven days showed a 20–30% increase in GABA levels in the hypothalamus and cerebellum after injection, suggestive of a compensatory mechanism for dieldrin-mediated $GABA_A$ receptor antagonism (Martyniuk et al., 2010a). In the same study, functional enrichment analysis revealed that genes with a role in DNA repair and the ubiquitin-proteasome pathway were over-represented in a microarray analysis. In a second sub-chronic study, male LMB were fed 3 mg dieldrin/kg feed for approximately 2 months to achieve an environmentally relevant exposure to dieldrin during mid to late stages of sexual maturation. Gene expression profiling identified genes involved in the biological processes of nucleotide base excision, protein transport, and metabolism as being significantly altered by dieldrin, suggesting protein degradation pathways and DNA repair mechanisms were impacted at the genomic level (Martyniuk et al., 2010b). Proteomics analysis in the hypothalamus also revealed that proteins differentially affected by dieldrin included well characterized biomarkers for human neurodegenerative diseases such as apolipoprotein E (ApoE), microtubule associated tau protein (Mapt), and enolase alpha (Eno1). Thus, the molecular and cellular responses identified in these studies may serve as bio-indicators of adverse effects in the brain due to pesticide exposures.

The major objective of this study was to determine the genomic responses in the female and male hypothalamus to the neuroactive pesticide dieldrin. LMB for this study were in early stages of gonad development (August–October). The aforementioned studies by Martyniuk et al. (2010a,b) focused on reproductive animals (February–April) and it is not known if LMB in earlier stages of gonad development show differences in transcriptomic responses to dieldrin. Studies on non-reproductive (i.e. sexually regressed) adults are important because gene expression profiles are known to vary naturally throughout the reproductive cycle in the fish hypothalamus (Zhang et al., 2009) and it was hypothesized that dieldrin affects LMB differently based on the time of year and reproductive state. The hypothalamus was chosen because this neuroendocrine tissue regulates pituitary hormone release of the gonadotropins, luteinizing and follicle stimulating hormone (LH and FSH). The hypothalamus of teleost fish is also a sensitive target for dieldrin neurotoxicity because of its high concentration of GABAergic cells (Martyniuk et al., 2007a). LMB are semi-synchronous spawners and in Central Florida, LMB are typically pre-vitellogenic in September and October, reaching sexual maturity in early March to late April. LMB in August were used for this study because these animals have significantly less circulating levels of steroids compared to sexually mature LMB (Sabo-Attwood et al., 2004; Doperalski et al., 2011). LMB were fed either control, 3 mg dieldrin/kg feed, or 3 mg dieldrin + 0.7 mg E_2/kg feed over 60 days to test the null hypotheses that (1) males and females do not differ in the genomic response in the hypothalamus after sub-chronic dieldrin exposure and (2) dieldrin + E_2-fed males do not show a reduced transcriptomic response when compared to dieldrin-fed males. Due to its well known neuroprotective role, it was reasoned that co-treatment with E_2 in sexually regressed LMB males would mitigate or reduce the response to dieldrin in male LMB.

2. Materials and methods

2.1. Largemouth bass and experimental feeding regime

Largemouth bass were purchased from the American Sport Fish Hatchery (Montgomery, Alabama) in July 2009 and maintained at the Aquatic Toxicology Laboratory at the Center for Environmental and Human Toxicology (University of Florida). Average weight (±SD) of the LMB was 558 (±103.6) g and the tail length was 33.1 (±1.82) cm. The age of the LMB was approximately 2–3 years. LMB were acclimated in aerated 147- to 220-gallon fiberglass tanks for three weeks before the exposures. The tanks are a flow through system. A week before initiating the feeding study, LMB were treated with a regime of oxytetracycline, nitrofurozine, and Rid-ick for the treatment of parasites and infection.

Dieldrin was incorporated into the feed pellets at a concentration approximating 3 mg dieldrin/kg feed. This dose was chosen based on previous data that demonstrated that treatment with 3 mg dieldrin/kg feed resulted in a whole carcass dieldrin body burden that approximated body burdens of animals placed into mesocosms in the North Shore of Lake Apopka, Florida (Denslow, unpublished data). This region is heavily contaminated with OCPs and other legacy pesticides. Therefore, the experimental dose results in body burdens that are environmentally relevant for wild LMB inhabiting contaminated areas in Florida.

For diet preparation, dieldrin was dissolved in 90% ethanol, mixed with menhaden oil, and coated onto a trout diet (Silver Cup, Odgen Utah) using a cement mixer. Pellets were allowed to air dry

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