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Short communication

Modeling of gene expression pattern alteration by *p*,*p*′-DDE and dieldrin in largemouth bass

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Abstract

In this study, largemouth bass (LMB) were subchronically exposed to *p*,*p*′-DDE or dieldrin in their diet to evaluate the effect of exposure on expression of genes involved in reproduction and steroid homeostasis. Using real-time PCR, we detected a different gene expression pattern for each OCP, suggesting that they each affect LMB in a different way. We also detected a different expression pattern among sexes, suggesting that sexes are affected differently by OCPs perhaps reflecting the different adaptive responses of each sex to dysregulation caused by OCP exposure.

Keywords: *p*,*p*′-DDE; Dieldrin; Endocrine disruptors; Largemouth bass; Real-time PCR

1. Introduction

Normal reproduction is controlled by the hypothalamic–pituitary gonadal (HPG) axis through a cascade of hormones whose concentrations are tightly regulated by hormonal action and feedback control mechanisms. Any chemical that alters the concentration of endogenous hormones or mimics their action by binding or interfering with the binding of hormones with their natural receptors can disrupt this process.

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The focus of this research is to begin to understand the molecular mechanisms underlying endocrine disruption of fish exposed to organochlorine pesticides (OCPs). Largemouth bass (*Micropterus salmoides*, LMB) were chosen for this study, as they exhibit reproductive failure in sites contaminated with OCPs. The OCPs chosen for this study were *p*,*p*′-DDE and dieldrin, which are both found in high concentrations and may be linked to reproductive failure. *p*,*p*′-DDE is a product of the degradation of the pesticide DDT and is reported to have both weak estrogenic and anti-androgenic activity (Kelce et al., 1995). Dieldrin has also been described as an estrogenic compound (Okoumassoun et al., 2002), and is considered one of the most toxic OCPs. The OCPs were selected because they may alter the endocrine system at different points in the HPG axis.

The main objective of this study was to evaluate expression of genes linked to steroid receptor activation, steroid synthesis and steroid metabolism as indicators of OCP action on these pathways of endocrine disruption in LMB.

2. Materials and methods

Adult LMB were purchased from American Sport Fish Hatchery (Montgomery, AL) and housed in freshwater ponds at the USGS in Gainesville, FL, under ambient conditions. LMB were exposed subchronically through the diet to *p*,*p*′-DDE (5.3, and 45.9 ppm) and dieldrin (0.4 and 0.81 ppm). Samples were collected after four months of exposure. Total RNA was isolated from LMB liver and gonad with the RNA Stat-60 reagent (Tel-test, Friendswood, TX), as previously described (Sabo-Attwood et al., 2004). Real-time PCR was performed using an iCycler (Bio-Rad, Hercules, CA). The real-time PCR results were calculated using the ΔΔCt method. Differences between the expression levels for the different treatments and sex were determined by two-way analysis of variance (ANOVA) using Sigma STAT software. Significant differences ($p \leqslant 0.05$) between control and treated samples were determined by Student's *t*-test. The expression pattern analysis was made using the TreeView program (http://rana.lbl.gov/EisenSoftware.htm).

3. Results and discussion

To begin to model the action of *p*,*p*′-DDE and dieldrin in LMB, we selected key genes involved in steroid receptor activation, steroid synthesis and steroid metabolism as sensors of reproductive function. For receptor-mediated effects, we quantified ERα, ERβb, ERβa and AR mRNA in liver and gonad, as well as vitellogenin in liver. For steroidogenesis, we quantified StAR, CYP19 and SF-1 mRNA in gonad; and for metabolism, we quantified CYP1A and two forms of CYP3A mRNA in liver.

The graph obtained with the TreeView program (Fig. 1) quantifies changes in expression for our tested genes as a result of exposure to the pesticides. In both females and males, *p*,*p*′-DDE up-regulated Vtg and ERα in liver, suggesting that it was acting as a weak estrogen mimic as both genes are under the control of ER (Sabo-Attwood et al., 2004). For dieldrin, although Vtg expression was up-regulated in females, we did not detect changes in ERα in liver, suggesting that the effects were not strictly through activation of ERs.