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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.

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.

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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 $\Delta\Delta C_t$ 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 ($p \leq 0.05$) 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 ER α , ER β b, ER β a 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 ER α 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 ER α in liver, suggesting that the effects were not strictly through activation of ERs.

Both isoforms of CYP3A were up-regulated by *p,p'*-DDE and dieldrin, likely due to interaction of *p,p'*-DDE and dieldrin with the pregnane-X-receptor (PXR). It has been demonstrated that both *p,p'*-DDE (Wyde et al., 2003) and dieldrin (Coumoul et al., 2002) can act directly as ligands for PXR, increasing CYP3A expression in human cell culture and rat.

CYP1A expression was either decreased or not affected by treatment with either *p,p'*-DDE or dieldrin, clearly demonstrating that these compounds are not activating AhR in LMB. The mechanism responsible for CYP1A down regulation is not clear, however the observed decrease in CYP1A expression may be due to crosstalk between the ER and AhR (Coumoul et al., 2001).

Studies have shown that dieldrin (Coumoul et al., 2001) and *o,p'*-DDT (Jeong and Kim, 2002) can down regulate CYP1A in MCF-7 cells and murine Hepa-1c1c7 cells, respectively, after over expression of CYP1A due to exposure to TCDD, suggesting that this mechanism is operational.

p,p'-DDE decreased the expression of aromatase in both sexes. StAR was up-regulated mostly in males. Dieldrin increased the expression of StAR in both sexes. Dieldrin also increased the expression of CYP19 in females, and decreased it for the lower dose in males.

Both pesticides were similar in the way they alter expression patterns for Vtg, ER β b, ER β a, CYP1A, CYP3A68 and CYP3A69 in the liver; and for StAR, ER α and AR in the gonad. But they were different in their effects for ER α , AR and SF-1 in the liver; and for CYP19, ER β b,

ER β a and AR in the gonad (Fig. 2). In the liver, ER α was highly up-regulated in fish treated with *p,p'*-DDE while this gene was almost not affected by dieldrin. Also, both AR and SF1 were down-regulated by *p,p'*-DDE in females and not affected in males; while for dieldrin they were up-regulated (except for AR in males, that did not change). In the gonad, CYP19 and SF-1 were down-regulated by *p,p'*-DDE, while in the dieldrin treatment, CYP19 was up-regulated in females and slightly down-regulated in males, and SF-1 did not change. For both ER β s, the main difference was in males, showing up-regulation with *p,p'*-DDE and down-regulation with dieldrin.

We conclude that *p,p'*-DDE affects expression of genes involved in the three proposed pathways of endocrine disruption, with significant changes observed in genes involved in hormone synthesis, metabolism and ER activation. Dieldrin affects mainly genes involved in metabolism and hormone synthesis, and does not appear to be acting as an estrogen mimic. Our results suggest that the effects of both *p,p'*-DDE and dieldrin differ for each sex, as many genes are differentially affected in males and females. In support of this conclusion there are gene induction studies with both chemicals in HeLa Cells (Mumtaz et al., 2002) which show distinguishable patterns. Experiments with microarrays are in progress to more globally define pathways affected by these compounds.

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Fig. 1.

Fold changes in mRNA expression are plotted using the TreeView program. Red color shows up-regulation of the gene expression versus the control fish. Down-regulation is shown in green. Black color means no change. The levels for *p,p'*-DDE are 5.3 ppm for the lower dose and 45.9 ppm for the higher dose. The levels for dieldrin are 0.4 ppm for the lower dose and 0.81 ppm for the higher dose.

Fig. 2.

Comparison of gene expression changes in response to *p,p'*-DDE and dieldrin. Fold changes in mRNA expression are plotted according to the $\Delta\Delta C_t$ method. Only the genes showing a different expression pattern for *p,p'*-DDE and dieldrin are shown. In the liver, these genes are ER α , AR and SF1, while in the gonad these genes are CYP19, ER β b, ER β a and SF1.