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Differential organ expression patterns of thyroid hormone receptor isoform genes in *p,p′*-DDE-treated adult male common frog, *Rana temporaria*

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Abstract

Using the European common frog, *Rana temporaria*, as a model, we have studied the organ-specific gene expression patterns of thyroid hormone receptor isoforms after exposure to an organochlorine (OC) compound, *p,p′*-DDE. Four groups of frogs were subcutaneously injected with *p,p′*-DDE at 0.01, 0.1, 1 and 10 mg/kg body weight, respectively. In addition, one group, serving as the control group, was injected with pure corn oil. TH receptor isoforms (TRα and TRβ) gene expressions were evaluated in the brain, kidney, testis and liver using real-time PCR with gene-specific primers. Our results show that *p,p′*-DDE doses induced slight elevations of TRα and TRβ mRNA in the brain. In the testis, *p,p′*-DDE induced an initial significant 3-fold increase of TRα mRNA at 0.01 mg/kg and thereafter clear dose-dependent decreases of TRα mRNA levels were observed. For testicular TRβ mRNA levels, *p,p′*-DDE induced a slight elevation at 0.01 mg/kg and thereafter significant decreases in TRβ mRNA levels were observed. *p,p′*-DDE induced significant 2–4-fold elevations of both TR isoforms in frog kidney. The strongest transcriptional effect of *p,p′*-DDE on TR isoforms was observed in the kidney. While TRα mRNA was not measurable in the liver, *p,p′*-DDE induced an initial 1.7-fold increase at 0.01 mg/kg of TRβ mRNA and thereafter an apparent dose-dependent decrease was observed. The relative abundance of TRα and TRβ gene expression in different organs are in the order: kidney > testis > brain > liver. While the induction TRα and TRβ might result to hypersensitivity and subsequent gain of biological functions, the inhibition might result to loss of biological function. Given the high persistency in the environment and continued use in developing countries coupled with the tendency for global atmospheric transport, DDT and its metabolites such as *p,p′*-DDE will remain a focus of concern both for scientific and societal reasons.

Keywords: Common frog; DDE; Thyroid hormone receptors; Hormone mimic; Multiple organs

1. Introduction

Thyroid hormones (THs: triiodothyronine, T_3 and thyroxine, T_4) are required for their roles in various aspects of vertebrate development, metabolism, homeostasis, cellular proliferation and differentiation (Oppenheimer and Schwartz, 1997). The pleiotropic effects of T_3 and T_4 are mediated through two thyroid hormone receptor proteins, TRα and TRβ (Gauthier et al., 1999). TRs function as hormone-inducible transcription factors that regulate the expression of target genes and are members of the hormone and orphan nuclear receptor superfamily. The TRα and TRβ isoforms are the products of distinct genes residing on separate chromosomes (Wurtz et al., 1996). For example, there are two TRβs resulting from an alternative ex on use within the same TRβ locus (Koenig et al., 1989).

The receptors are entirely homologous, with the exception of distinct N-terminals as a result of alternative splicing or alternative promoter usage (Koenig et al., 1989). TRs bind to DNA sequences known as thyroid hormone response elements (TREs) found in the regulatory regions of target genes, and depending to the nature of the TREs, gene expression may be enhanced or inhibited (Wu et al., 2001). In humans, various

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pathological states and developmental abnormalities result from inadequate or excessive levels of circulating thyroid hormones. These include mental retardation, stunted growth, hearing loss, and alterations in thermogenic homeostasis and heart rate (Oppenheimer, 1999). Considering the functions of thyroid hormones, it is notable that the essential components making up the thyroid axis have largely been conserved across vertebrates with species-specific features (Power et al., 2001).

A large number of chemicals released into the environment disrupt endocrine homeostasis in humans and animals by interfering with developmental processes and endocrine systems (Colborn et al., 1993). By acting as agonists or antagonists to and disturbing the organismal steroid system, some endocrine disrupting chemicals (EDCs) have elicited toxicological effects at concentrations previously shown to be safe (Hayes et al., 2002; Lans et al., 1993). Several EDCs interfere with TH homeostasis by binding to the plasma proteins (e.g. transthyretin, TTR) responsible for the distribution of endogenous THs (Lans et al., 1993). For example, polychlorinated biphenyls (PCBs), hydroxylated PCBs (OH-PCBs), dibenzo-*p*-dioxins and dibenzofurans have been shown to interact strongly with mammalian TTRs, inducing a rise in the plasma clearance rates of THs, and resulting in hypothyroxinemia in rat, seal and human (Brouwer et al., 1998, 1999; Lans et al., 1993). These results suggest that other chemicals may interact strongly with TTR. Furthermore, it remains to be clarified whether EDCs modulate transcriptional patterns of TRs in adult target tissues. When compared with studies examining chemical effects on steroid hormone receptors, only few studies have characterized the transcriptional modulation of TRs by EDCs during anuran metamorphosis (Crump et al., 2002; Veldhoen and Helbing, 2001).

Organochlorine pesticides (OCs), such as DDT (l,l,l-trichloro-2,2-bis[*p*-chlorophenyl]ethane), have been progressively banned in much of North America and Europe owing to their long-term persistence and high toxicity. Presently, exposure to such pesticides come both from accumulation in the sediments, where they were deposited from past usage, and from aerial dispersal from other areas of the world in which they are still in use (Turusov et al., 2002). l,l′-Dichloro-2,2-bis[*p*-chlorophenyl]ethylene (*p*,*p*′-DDE) is a persistent metabolite of DDT (Chiba et al., 2002; Schafer and Kegley, 2002). Laboratory and field data have implicated OCs in impaired reproductive success and abnormal sexual development in wildlife species. For example, DDT and its metabolites have altered population structure by causing eggshell thinning and endocrine and reproductive toxicity in wild birds (Forsyth et al., 1994). The sexual abnormalities reported in Florida alligators are assumed to be a result of the demasculinizing effects of DDT metabolites, including *p*,*p*′-DDE (Guillette and Gunderson, 2001). DDT and its metabolites have been shown to have variable effects on steroid hormone receptors (Kelce et al., 1995b; Sohoni and Sumpter, 1998). Several other reports have discussed the role of OCs exposure on the endocrine disruption of aquatic species (Gunderson et al., 2004; Pickford and Morris, 2003). For example, two DDT metabolites were observed in surface water at Sierra Nevada Mountain sites in California at 0.31 and 0.46 ng/l for *p*,*p*′-DDE and *o*,*p*′-DDT, respectively, and *p*,*p*′-DDE was found at significantly greater concentrations in frog tissue at some sites (Fellers et al., 2004). DDE was heavily sprayed in the 1960s to kill insects and pests like mosquitoes. Although it is no longer legally used, traces of DDE continue to be found in the leopard frog, which harms its development.

Anuran species are important aquatic vertebrates with a large number of scientific reports describing the toxicological effects of chemicals on their metamorphosis, development, general fitness, viability and reproductive performance (Mosconi et al., 2002) and therefore offer an excellent model to study endocrine disruption (Veldhoen and Helbing, 2001; Crump et al., 2002). The phenomenon of frogs showing signs of feminization due to exposure to endocrine disruptors is well described (Levy et al., 2004). Presently, the vast majority of the knowledge of endocrine effects on amphibians is limited to experiments on the effects on metamorphosis and sexual differentiation under laboratory conditions.

As a result, the present study was designed to investigate the organ-specific gene expression patterns of thyroid hormone receptor isoforms after exposure to an OC compound, *p*,*p*′-DDE, using the European common frog, *Rana temporaria*, as a model test organism. Despite being widespread in Europe and as such has a potential as a model for environmental monitoring of endocrine disruptors, this species are less studied compared to the xenopus species. We hypothesize that TR gene isoforms will show different organ-specific patterns, reflecting biological functions, after exposure to the OC, *p*,*p*′-DDE.

2. Materials and methods

2.1. Chemicals and reagents

l,l-Dichloro-2,2-bis(4-chlorophenyl)ethane (*p*,*p*′-DDE or DDE) was purchased from Sigma–Aldrich Co. (St. Louis MO, USA). Trizol reagent and oligo(dT)$_{18}$ primer and Superscript II RNase H-reverse transcriptase were purchased from Invitrogen Corporation (Carlbad, CA, USA). DNA ladder, RevertAid^{TM} First Strand cDNA Synthesis Kit (FERMENTAS GMBH, Germany), deoxynucleotide triphosphates (dNTPs), Brilliant® SYBR® Green QPCR Master Mix were purchased from Stratagene (La Jolla, CA, USA).

2.2. Frog and treatment

Adult males of European common frog (*R. temporaria*) were purchased from the Frog Farm (Kells, Ireland). Frogs were housed individually in small aquaria with a pool and