Available online at www.sciencedirect.com
SCIENCE DIRECT®
Marine Environmental Research 62 (2006) S215–S218
MARINE ENVIRONMENTAL RESEARCH
www.elsevier.com/locate/marenvrev

Short communication

Organ-specific patterns of P450arom gene isoforms are modulated by *p,p′*-DDE in adult male European common frog, *Rana temporaria*

Augustine Arukwe *

Department of Biology, Norwegian University of Science and Technology (NTNU), Høgskoleringen 5, 7491 Trondheim, Norway

Abstract

The organ-specific gene expression patterns of P450arom isoforms have been studied in European common frog, *Rana temporaria* after exposure to DDE, using real-time PCR. Four groups of frogs were subcutaneously injected with DDE at 0.01, 0.1, 1 and 10 mg/kg body weight and one group, serving as the control was injected with pure corn oil. Brain, kidney, testis and liver P450aromA and P450aromB gene expressions were evaluated at day 14 after exposure. P450aromB data show that 0.1 and 10 mg DDE/kg doses caused 76% and 63% (testis) and 79% and 80% reductions, respectively, of mRNA levels. Brain P450aromB mRNA decreased 10% and 34%, respectively, after exposure to 0.01 and 0.1 mg DDE/kg. Thereafter, a 185% and 177% induction response of brain P450aromB was observed in the groups treated with 1 and 10 mg DDE/kg, respectively. In the kidney, 0.01, 0.1, 1 and 10 mg DDE/kg induced a 516%, 178%, 466% and 247% induction of P450aronB mRNA, respectively. P450aromA expression was not quantified in any of the organs. The relative abundance of P450aromB gene expression in different organs is in the order: kidney > brain > liver > testis. The present data suggest potential detrimental effect of organochlorine pesticides (OCs) and their metabolites on the European frog steroidogenic pathways. Given the high persistency in the environment and continued use in developing countries coupled with the tendency for global atmospheric transport, OCs and their metabolites such as DDE will remain a focus of concern both for scientific and societal reasons.

* Tel.: +47 73 596265; fax: +47 73 591309.
E-mail address: arukwe@bio.ntnu.no.

0141-1136/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2006.04.034

Organochlorine pesticides (OCs), such as DDT (1,1,1-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 comes 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). 1,1′-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 shown that OCs may impair reproductive success and cause 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). In rats, DDE was shown to induce hepatic P450arom expression (You et al., 2001). Receptor binding affinity based experiments and receptor-mediated transcriptional activation have identified DDE as an androgen receptor antagonist (Kelce et al., 1995). Aromatase is a member of the CYP family of synthetic and metabolic enzymes and is encoded by the CYP19 gene (P450arom) that catalyzes the conversion of C19 steroids to estrogens, a reaction that essentially involves the removal of the C19 carbon and aromatization of the A ring of the steroid (Callard et al., 2001). In vertebrates, there are two structurally distinct CYP19 isoforms, namely, P450aromA and P450aromB. P450aromA is predominantly expressed in the ovary and plays important roles in sex differentiation and oocyte growth, while P450aromB is expressed in neural tissues such as brain and retina and is involved in the developing central nervous system (Kishida and Callard, 2001) and sex behaviours (Callard et al., 2001). Natural estrogens and endocrine disrupting chemicals such as DDE are shown to modulate CYP19 genes and thereby alter E2 production (Mosconi et al., 2002).

Therefore, the aim of this study was to investigate the expression of P450arom isotype genes in different organs of common frog after subcutaneous exposure to DDE. We hypothesized that P450arom gene isotypes will show differential organ-specific expression patterns after exposure to DDE doses. Four groups of frogs were subcutaneously injected with DDE at 0.01, 0.1, 1 and 10 mg DDE/kg body weight, respectively. In addition, one group, serving as the control group, was injected with pure corn oil. The frogs were sacrificed after 2 weeks exposure. Liver, brain, testis and kidney samples were collected, weighed, snap-frozen in liquid nitrogen in cryogenic vials kept at −80 °C until used for total RNA isolation using the Trizol reagent (Invitrogen). Complementary DNA (cDNA) was synthesized from total organ RNA to RevertAid^{TM} cDNA Synthesis Kit (Fermentas). P450arom isotypes gene expressions were studied using quantitative (real-time) reverse-transcriptase polymerase chain reaction (qPCR) with 600 pM each of the following primers pairs in 5′-3′ directions; P450aromA (104 bp): GGGCACTGTCTGATGATGTC (forward) GGGCTTGAGGAAGAACTCTG (reverse), P450aromB (97 bp): CTGACCCC-TCTGGACACG (forward), TCTCGTTGAGAGGCACCC (reverse). Gene expression patterns were evaluated using Mx3000P Real-Time PCR System (Stratagene, La Jolla, CA, USA). The real-time PCR program included an enzyme activation step at 95 °C (10 min) and 40 cycles of 95 °C (30 s), 55 °C (1 min), and 72 °C (30 s). We included controls lacking cDNA template or Taq DNA polymerase to determine the specificity of target cDNA amplification. Cycle threshold (C_t) values obtained were converted into copy number using standard plots of C_t-values versus log copy number. The standard plots