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Endocrine disruption mechanism of *o,p′*-DDT in mature male tilapia (*Oreochromis niloticus*)

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Abstract

The aim of the present study was to evaluate, in vivo, the potential of *o,p′*-DDT to disrupt the endocrine system of mature male tilapia. In particular, the possibility that *o,p′*-DDT effects were mediated directly via the estrogen receptor (ER). Compounds with known ability to bind to the ER were employed: estradiol to induce and tamoxifen to inhibit the estrogenic effects result of the activation of the ER. In addition, an aromatase inhibitor, 4-hydrxyandrostenedione (4-OHA), was used to assess the ability of *o,p′*-DDT to induce estrogenic effects in a surrounding of low estradiol concentration. The effects of estradiol and *o,p′*-DDT were studied alone or in the presence of tamoxifen or 4-OHA at the end of a 12-day period of exposure. The main endpoints measured were plasma alkaline-labile phosphorous (ALP; an indirect indicator of vitellogenin), estradiol, testosterone and *o,p′*-DDT. It was found that *o,p′*-DDT was able to induce the vitellogenesis (measured as plasma ALP increase) and decrease the circulating levels of estradiol and testosterone. Interestingly, *o,p′*-DDT kept this ability in whole fish with low concentrations of estradiol which would exclude endogenous estradiol as indirect mediator of the estrogenic effects induced by *o,p′*-DDT. In addition, the plasma concentration of *o,p′*-DDT, instead of that of estradiol, was closely related to the plasma ALP increase induced by *o,p′*-DDT. This indicates that *o,p′*-DDT could have directly activated the vitellogenesis. The antiestrogenic action of tamoxifen to inhibit the vitellogenesis and the decrease on plasma estradiol induced by *o,p′*-DDT indicates that *o,p′*-DDT can bind directly to the ER. In conclusion, this in vivo study shows that *o,p′*-DDT has the potential to disrupt the endocrine system and strongly supports that the estrogenic actions of *o,p′*-DDT involve binding to the ER.

Keywords: *o,p′*-DDT; Endocrine disruption; Tamoxifen; Aromatase inhibitor; Vitellogenin; Fish; Antiestrogens; Estradiol; Testosterone

Introduction

Concern has been raised that exposure to some environmental chemicals may be able to disrupt the endocrine system of humans and wild-living animals, resulting in the harmful effects on the reproductive system (Arcand-Hoy and Benson, 1998; Gillesby and Zacharewski, 1998). The DDT pesticide was one of the first well-documented findings correlating wildlife exposure with developmental and reproductive toxicity (Arcand-Hoy and Benson, 1998). A major pesticide spill occurred in Lake Apopka (Florida, USA) in 1980. Later, Guillette et al. (1996) found that male alligators inhabiting Lake Apopka showed significantly smaller penis size and lower plasma testosterone levels than those of males from a control lake, linking these effects with DDT exposure. The study of endocrine disrupters typically includes in vitro and in vivo assays. Both modalities provide insight into how these compounds can disrupt the endocrine and reproductive systems. In vitro tests identify modes of action of endocrine disrupters in a particular environment while in vivo tests take into account the complexity of important mechanisms inherent to in vivo systems, such as endocrine homeostasis and toxicokinetic mechanisms (Ankley et al., 1998; Gillesby and Zacharewski, 1998). Another important consideration about in vivo studies of endocrine disrupters is that many aspects of endocrine function are conserved among species. For example it has been shown that, in vertebrates, including teleost fish, the neuroendocrine regulatory system is constituted by the hypothalamic–pituitary–

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gonadal (HPG) axis (Van Der Kraak et al., 1998). Similarities also exist regarding hormones, their receptors and biochemical pathways. This is the case of the recent discovery of a second estrogen receptor (ER) subtype, named ERβ, in mammals (Kuiper et al., 1996; Mosselman et al., 1996) which has also been cloned in fish (Chang et al., 1999; Menuet et al., 2002; Sabo-Attwood et al., 2004). Among endpoints potentially indicative of exposure to estrogenic chemicals in oviparous species, vitellogenin production has received much attention. Vitellogenin, a complex glycophospholipoprotein, is synthesized in the liver in response to estradiol. Once synthesized, vitellogenin is delivered into the bloodstream to be finally incorporated into the yolk of developing oocytes (Kime et al., 1999; Okoumassoun et al., 2002). This endpoint is highly sensitive in mature male fish in which vitellogenin is not produced in normal conditions but also only after exposure to estrogenic chemicals (Ankley et al., 1998; Kime et al., 1999). In addition, sexual steroid hormones are frequently involved as target of endocrine disruption and often used as endpoints. Estradiol and testosterone play important roles in male fertility (O'Donnell et al., 2001). Particularly, it has been shown in humans, mammals and fish that estradiol can modulate its own production as well as that of testosterone in males (Loomis and Thomas, 2000; Moger, 1980; Raven et al., 2006; Rochira et al., 2002; Taxel et al., 2001; Trudeau et al., 1993). However, in contrast to vitellogenin induction, alterations in plasma estradiol and testosterone concentrations are often less specific with respect to their mechanisms of action, such as effects on steroidogenic enzymes or modifications associated with altered feedback loops (Ankley et al., 1998; Arcand-Hoy and Benson, 1998; Gray et al., 1997).

In vivo and in vitro assays have been developed to study commercial DDT as well as the two pure isomers, *o*,*p*′-DDT and *p*,*p*′-DDT. However, the endocrine disruption mechanism of DDT in whole animals has not been fully established. In vivo studies of DDT exposure in laboratory conditions have found that the *o*,*p*′-DDT isomer has the potential to mimic estrogen effects, such as to produce uterotrophic responses in mammals (Bigsby et al., 1997), and to induce vitellogenin production in fish (Donohoe and Curtis, 1996; Leaños-Castañeda et al., 2002; Mills et al., 2001). Although these studies show that *o*,*p*′-DDT resembles estrogens, the precise mechanism of action has yet to be established. The study of endocrine disruption mechanisms in whole animals is complicated by the fact that multiple ways exist to interfere with the endocrine system. The mechanisms of action of endocrine disrupters in relation to (anti-)estrogenic effects in whole animals can be classified, at molecular level, as either direct or indirect. The direct mechanism implies that the endocrine disrupter interacts with the ER producing a series of events to finally mimic and/or inhibit the estrogen effects. In contrast, the indirect mechanism involves several different signaling pathways in which the endocrine disrupters interact with steroidogenic enzymes, binding globulins, growth factors, and/or different receptor systems (Gillesby and Zacharewski, 1998).

In vitro assays have shown that *o*,*p*′-DDT has the ability to competitively bind to the ER of humans, mammals and fish (Chen et al., 1997; Donohoe and Curtis, 1996; Klotz et al., 1996; Matthews et al., 2000). Although receptor binding is indicative of the ability of a ligand to bind ER, it is not sufficient to determine whether these interactions will result in positive or negative modulation of ER-mediated signal transduction. Lemaire et al. (2006) showed that *o*,*p*′-DDT is able to transactivate both ER subtypes, ERα and ERβ, in stable reporter cell lines (HELN ERα and ERβ). In order to test whether this mechanism of action could also occur in vivo, a compound with known ability to bind to ER and inhibit the ER activation can be used in combination with *o*,*p*′-DDT. Among drugs which antagonize the ER-mediated signal transduction in humans and mammals, it has been shown that tamoxifen binds to fish ER and has the ability to inhibit the estradiol-induced vitellogenesis in cultured hepatocytes of fish (Hawkins and Thomas, 2004; Mori et al., 1998; Pelissero et al., 1993). In a previous work, we tested tamoxifen in mature male tilapia in vivo (Leaños-Castañeda et al., 2002). The results showed that *o*,*p*′-DDT-induced vitellogenesis was inhibited by tamoxifen. Additionally, an increase on plasma estradiol was observed in fish treated with *o*,*p*′-DDT. Based on these results, it has not been possible to establish if *o*,*p*′-DDT induced the vitellogenesis by binding directly to the ER or indirectly by increasing the endogenous estradiol.

The aim of the present study was to evaluate in vivo the potential of *o*,*p*′-DDT to disrupt the endocrine system of mature male tilapia. In particular, the possibility of a direct mechanism of action, via ER, was explored using compounds with known ability to bind to the ER. Estrogenic effects were induced by estradiol and in order to inhibit them tamoxifen was employed. Additionally, an aromatase inhibitor, 4-hydrxyandrostenedione (4-OHA), was used to assess the ability of *o*,*p*′-DDT to induce estrogenic effects in a surrounding of low estradiol concentration.

Materials and methods

Chemicals. 17β-Estradiol 3-benzoate, tamoxifen citrate salt, 4-hydroxyandrostenedione, heparin sodium salt, inorganic phosphorous Kit 670-C, and trichloroacetic acid were purchased from Sigma (Mexico). *o*,*p*-DDT (1-(2-Chlorophenyl)-1-(4phenyl)-2,2,2 trichloroethane) was purchased from Lancaster Synthesis Inc. (Windham, NH). Chromatography standards *o*,*p*′-DDT (1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane) and endosulfan II were purchased from AccuStandard (New Haven, CT).

Fish. Tilapia (*Oreochromis niloticus*) were bred and raised from broodstock kept at the Center for Research and Advanced Studies (CINVESTAV), Mérida, Yucatan, Mexico. The fish were raised outdoors at ambient temperature, with a natural photoperiod. Two series of experiments were conducted with mature male tilapia at two different times. Experimental series number I was conducted from November to December during the tilapia reproductive resting season, ambient temperatures ranged from 16 to 25 °C, and the photoperiod was approximately 10 h:L and 14 h:D. Sexually mature male tilapia weighing from 60 to 150 g were randomly divided into eight experimental groups of fifteen fish each and kept in 1000-l tanks. Experimental series II was conducted from May to June during the tilapia breeding season, ambient temperatures ranged from 25 to 40 °C, and the photoperiod was approximately 14 h:L and 10 h:D. Sexually mature male tilapia weighing from 60 to 200 g were randomly divided into 8 experimental groups of 8–10 fish and kept in 1000-l tanks. The fish were fed a commercial diet ad libitum throughout the experiment including a 5-day acclimation period.