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Review

Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish

Ksenia Cheshenko [a], Farzad Pakdel [b], Helmut Segner [c], Olivier Kah [b,*], Rik I.L. Eggen [a,*]

[a] *Eawag, Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, Postfach 611, CH 8600 Dübendorf, Switzerland*
[b] *Endocrinologie Moléculaire de la Reproduction, UMR CNRS 6026, Campus de Beaulieu, Université de Rennes 1, 35042 Rennes Cedex, France*
[c] *Centre for Fish and Wildlife Health, University of Bern, Länggass-Strasse 122, Post Box 8466, 3001 Bern, Switzerland*

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Abstract

Many natural and synthetic compounds present in the environment exert a number of adverse effects on the exposed organisms, leading to endocrine disruption, for which they were termed endocrine disrupting chemicals (EDCs). A decrease in reproduction success is one of the most well-documented signs of endocrine disruption in fish. Estrogens are steroid hormones involved in the control of important reproduction-related processes, including sexual differentiation, maturation and a variety of others. Careful spatial and temporal balance of estrogens in the body is crucial for proper functioning. At the final step of estrogen biosynthesis, cytochrome P450 aromatase, encoded by the *cyp19* gene, converts androgens into estrogens. Modulation of aromatase CYP19 expression and function can dramatically alter the rate of estrogen production, disturbing the local and systemic levels of estrogens. In the present review, the current progress in CYP19 characterization in teleost fish is summarized and the potential of several classes of EDCs to interfere with CYP19 expression and activity is discussed. Two *cyp19* genes are present in most teleosts, *cyp19a* and *cyp19b*, primarily expressed in the ovary and brain, respectively. Both aromatase CYP19 isoforms are involved in the sexual differentiation and regulation of the reproductive cycle and male reproductive behavior in diverse teleost species. Alteration of aromatase CYP19 expression and/or activity, be it upregulation or downregulation, may lead to diverse disturbances of the above mentioned processes. Prediction of multiple transcriptional regulatory elements in the promoters of teleost *cyp19* genes suggests the possibility for several EDC classes to affect *cyp19* expression on the transcriptional level. These sites include cAMP responsive elements, a steroidogenic factor 1/adrenal 4 binding protein site, an estrogen-responsive element (ERE), half-EREs, dioxin-responsive elements, and elements related to diverse other nuclear receptors (peroxisome proliferator activated receptor, retinoid X receptor, retinoic acid receptor). Certain compounds including phytoestrogens, xenoestrogens, fungicides and organotins may modulate aromatase CYP19 activity on the post-transcriptional level. As is shown in this review, diverse EDCs may affect the expression and/or activity of aromatase *cyp19* genes through a variety of mechanisms, many of which need further characterization in order to improve the prediction of risks posed by a contaminated environment to teleost fish population.

Keywords: Endocrine disrupters; Aromatase; cyp19a; cyp19b; Brain; Gonad; Estrogens; Xenoestrogens; Dioxin; Reproduction; Fish; Teleost

1. Introduction

Estrogens are steroid hormones found in representatives of all classes of marine and terrestrial vertebrates, including fish, amphibians, reptiles, birds and mammals (Lange et al., 2003), as well as in some invertebrates (Osada et al., 2004; Zhu et al., 2003). Several types of estrogens are naturally present in the organism. However, 17β-estradiol (E2) is the form responsible for most biological activities, while estrone and estriol are less potent than E2. The most commonly referred to function of estrogens is the involvement in the control of sexual differentiation, maturation and

* Corresponding authors. Fax: +33 0 2 23 23 67 94 (O. Kah), +41 0 44 823 5398 (R.I.L. Eggen).
E-mail addresses: olivier.kah@univ-rennes1.fr (O. Kah), eggen@eawag.ch (R.I.L. Eggen).

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reproduction. However, these hormones also exert numerous other effects on the development, differentiation and homeostasis of diverse target organs. Estrogens are also involved in the control of the cell cycle and proliferation. For example, they regulate uterine growth and they have been associated with the control of neuronal growth and differentiation.

Estrogens act *via* two main mechanisms. Well-studied genomic mechanism involves specific nuclear receptors (estrogen receptors, ERs), acting as ligand-dependent transcription factors. This classic pathway involves diffusion of the estrogen into the target cell and binding to ERs, which facilitates their activation, dimerization and binding to the specific DNA sequences called estrogen-responsive elements (EREs) located in the 5′-flanking regions of most E2-responsive genes studied up to date. This event promotes the assembly of the transcription initiation complex and thus triggers transcription. Genomic estrogen actions require a relatively long time (from hours to days) to be accomplished. However, more rapid effects (from seconds to few minutes) also exist, referred to as ‘‘non-genomic signaling’’ (Falkenstein et al., 2000). This pathway involves diverse second messenger systems. Non-genomic effects of estrogens are thought to be mediated through membrane receptors (Acconcia et al., 2005; Kelly and Levin, 2001; Loomis and Thomas, 2000; Razandi et al., 1999). However, the exact structure and identity of these non-genomic receptors are not yet fully characterized (Sak and Everaus, 2004).

Several enzymes are involved in the process of estrogen biosynthesis. At the rate-limiting step, androgens are converted into estrogens *via* aromatization. This reaction is catalyzed by an enzymatic complex formed by the cytochrome P450 aromatase, a heme binding protein encoded by the *cyp19* gene (aromatase CYP19), functioning in combination with the flavoprotein, NADPH-cytochrome P-450 reductase (Simpson, 1994; Thompson and Siiter, 1974). The gonads are considered to be the major source of estrogens in the body. However, local estrogen production in other sites, especially in the brain, is also very important. For example, many effects of androgens in the developing mammalian brain are exerted through their local conversion to estrogens, as was first proposed over two decades ago (MacLusky and Naftolin, 1981). Thus, aromatase CYP19 is the key enzyme regulating local and systemic levels of estrogens in the body.

Many natural and synthetic chemicals present in the environment are suspected of exerting a number of adverse effects on the organism, leading to endocrine disruption. These compounds have been generally termed endocrine-disrupting chemicals (EDCs). Wildlife species from ecosystems contaminated with EDCs display a variety of reproductive alterations (Gray, 1998; Jobling et al., 1998). Many studies have been dedicated to the understanding of the causes of endocrine disruption in fish, not only because these aquatic animals are often exposed to multiple sources of EDCs coming from domestic and industrial effluents, and diverse cases of abnormal development possibly caused by EDC exposure have been reported (Eggen et al., 2003; Goksoyr, 2006; Guillette et al., 1995; Segner et al., 2003; Sumpter, 1998), but also because of the possibility of extrapolation and risk prediction for higher vertebrates, including humans. Aromatase CYP19 is considered to be one of the potential EDC targets because modulation of its expression and function can dramatically alter the rate of estrogen production, disturbing the local and systemic levels of estrogen, and thus may lead to disruption of estrogen-related biological processes. Several indications exist that interference with the aromatase CYP19 system might lead to malfunctioning of the reproductive system, which is the most frequently reported symptom of endocrine disruption in fish.

The goal of the present review is to summarize the current progress in CYP19 characterization in teleost fish and to discuss the potential of several classes of EDCs to interfere with CYP19 expression and function.

2. Aromatase CYP19 in teleost fish

A wealth of data has been obtained describing CYP19 in teleost fish. Characterization of *cyp19* genes in teleosts, localization of expression and presumed function will be described in the following subsections.

2.1. Characterization of *cyp19* genes in teleost fish

The *cyp19* gene that encodes aromatase was first cloned in human (Evans et al., 1986). Since then, full coding sequences of the aromatase *cyp19* gene were obtained from 11 other mammalian species (goat, horse, cow, sheep, pig, marmoset, macaque, mouse, rat, rabbit and white-sided dolphin) (Wilson et al., 2005). Most of the higher vertebrates possess a single *cyp19* gene (Simpson et al., 1994), excluding pig, in which multiple tissue-specific aromatase isoforms encoded by different *cyp19* genes are present (Choi et al., 1997). The control of the tissue-specific expression of *cyp19* in human has been extensively studied and shown to depend on the use of an alternative splicing of promoter and untranslated exon I, resulting in the generation of transcript variants with different tissue-specific 5′-UTRs, but identical coding sequences (Bulun et al., 2004; Harada et al., 1993; Sebastian and Bulun, 2001). In contrast, two different *cyp19* genes are found in many teleosts, including channel catfish *Ictalurus punctatus* (Trant, 1994), Japanese medaka *Oryzias latipes* (Fukada et al., 1996), Nile tilapia *Oreochromis niloticus* (Chang et al., 2005, 1997b; Kwon et al., 2001), goldfish *Carassius auratus* (Gelinas et al., 1998; Tchoudakova and Callard, 1998), Japanese flounder *Paralichthys olivaceus* (Kitano et al., 1999), zebrafish *Danio rerio* (Chiang et al., 2001b; Kishida and Callard, 2001), rainbow trout *Oncorhynchus mykiss* (Dalla Valle et al., 2002b), Eastern mosquitofish *Gambusia holbrooki* (Orlando et al., 2002), sea bass *Dicentrarchus labrax* (Blazquez and Piferrer, 2004; Dalla Valle et al., 2002a),