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Endocrine disrupting compounds: Can they target the immune system of fish?

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ABSTRACT

Endocrine disruption, in particular disruption by estrogen-active compounds, has been identified as an important ecotoxicological hazard in the aquatic environment. Research on the impact of endocrine disrupting compounds (EDCs) on wildlife has focused on disturbances of the reproductive system. However, there is increasing evidence that EDCs affect a variety of physiological systems other than the reproductive system. Here, we discuss if EDCs may be able to affect the immune system of fish, as this would have direct implications for individual fitness and population growth. Evidence suggesting an immunomodulatory role of estrogens in fish comes from the following findings: (a) estrogen receptors are expressed in piscine immune organs, (b) immune gene expression is modulated by estrogen exposure, and (c) pathogen susceptibility of fish increases under estrogen exposure.

1. Introduction

Endocrine disruption has been identified as an important toxicological hazard in the aquatic environment (Colborn et al., 1993; Tyler et al., 1998; Segner, 2005; Sumpter, 2005). Organisms have evolved sensitivity to endogenous and exogenous chemical signals as a means to adaptively respond to physical, chemical or biological stimuli and to maintain internal homeostasis. At the same time, however, this sensitivity makes organisms vulnerable to inadvertent signals in their environment (Cheek et al., 1998). Environmental compounds that act as such inadvertent exogenous signals interfering with endogenous hormone signalling are designated as endocrine disrupting compounds (EDCs). They exert their biological activity either by interacting with endogenous hormone receptors, or by disturbing endogenous hormone metabolism.

Research on the effects of EDCs on fish has given most attention to estrogen-active compounds, i.e. substances that bind as agonists to estrogen receptors. A key physiological role of estrogens is the regulation of sexual differentiation and reproduction. Therefore, the vast majority of studies on the impact of estrogen-active EDCs on fish focused on reproductive parameters, i.e., sexual differentiation, sexual behaviour, gonad maturation and vitellogenesis, fecundity, fertility, or offspring survival (Mills and Chichester, 2005; Ankley et al., 2009; Segner, 2011). However, estrogen actions are not restricted to reproductive physiology, but they have pleiotropic actions targeting a variety of physiological functions beyond the reproductive system. For instance, estrogens play a role in the growth hormone/insulin-like growth factor system (Filby et al., 2006; Shved et al., 2007, 2008), in the stress response (Pottinger et al., 1996), in osmoregulation (Madsen et al., 2004), or in the differentiation of neurosensory systems (Froehlicher et al., 2009). Thus, the potential impact of environmental estrogens on functions other than reproduction must not be neglected.

Here, we discuss the possibility that estrogen-active EDCs may target the immune system and, as a consequence, may modulate the immunocompetence of fish. For mammals, a role of estrogens in immune functioning is well known, both under physiological and pathological conditions (Olsen and Kovacs, 1996; Martin, 2000; Beagley and Gockel, 2003; Straub, 2007). For fish, the currently available information on an immunomodulatory role of estrogens is scant and by no way conclusive (Rice, 2001; Yada and Nakanishi, 2002; Segner et al., 2006). In order to evaluate the possible impact of estrogens and estrogen-active EDCs on immune parameters and immunocompetence of teleostean fish, we will address in the following three questions: (i) Is there evidence that fish immune cells express estrogen receptors, indicating the possibility of direct estrogen signalling in the immune system (instead of or in addition to indirect effects, e.g., via neuroendocrine axes)? (ii) Are estrogens able to modulate the expression of immune genes in fish? (iii) Are estrogens able to modulate immunocompetence and pathogen resistance of fish? These questions will be discussed for (physiological levels of) 17β-estradiol (E2) as prototypic estrogen; future studies will then have to evaluate if and

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under what conditions immunomodulatory effects of E2 are relevant for environmental exposure of fish to estrogen-active substances. To prepare the scene for the discussion of estrogen actions in the piscine immune system, initially short reference is given to the existing knowledge on the role of estrogens in the immune system of mammals.

1.1. Estrogen actions in the mammalian immune system

Epidemiological, clinical and experimental data clearly indicate a sexual dimorphism in the differentiation and functioning of the mammalian immune system. Estrogens have a well documented immunomodulatory action in the mammalian immune system, but the role is complex, with effects on diverse processes such as thymus development and involution, lympho-haematopoiesis, antibody production, or the expression of pro- and anti-inflammatory cytokines and chemokines. The estrogen effects depend on a variety of factors including estrogen concentration, age and actual physiological context, immune stimulus and cellular environment (Olsen and Kovacs, 1996; Straub, 2007). For instance, peri-ovulatory serum levels of 17β-estradiol (E2) stimulate the secretion of pro-inflammatory cytokines in human and rat monocytes, whereas high E2 levels as they occur at pregnancy inhibit pro-inflammatory cytokines and lead to an anti-inflammatory effect. Such dichotomic actions of estrogens bear biological significance, as the pro-inflammatory activity of low E2 levels would support the defence against invading pathogens, whereas during pregnancy, inflammatory processes may compromise fetal survival and therefore it is instrumental that the high E2 levels have an anti-inflammatory effect (Beagley and Gockel, 2003; Schaefer et al., 2005). Another factor that critically influences the biological outcome of estrogen actions on the immune system is the cellular distribution of the ERs and the preponderance of one ER subtype over the other (Lambert et al., 2005; Tiwari-Woodruff et al., 2007; Cvoro et al., 2008). Research over recent years has established that mammalian immune cells show cell type- as well as differentiation status-dependent expression of ER subtypes, co-activators and co-repressors (Phiel et al., 2005; Stygar et al., 2007), and that this distribution has direct implications for the immunomodulatory action of estrogens (Medina et al., 2000; Maret et al., 2003). For instance, while ERβ mediates the E2-effect on thymic cortex atrophy (Erlandsson et al., 2001), it is ERα that signals estrogenic effects on thymic organogenesis (Selvaraj et al., 2005). Similarly, while the E2 action on dendritic cell proliferation is mediated through ERα (Douin-Echinard et al., 2008), the E2 regulation of apoptosis in lymphohaematopoietic cells depends on the stage-specific expression of either ERα or ERβ (Igarashi et al., 2001; Mor et al., 2003). Given the prominent and complex role of estrogens in the mammalian immune system, it is not surprising that environmental estrogen-active compounds are able to exert immune disrupting effects in mammals (e.g., Ahmed, 2000; Golub et al., 2004; Hung et al., 2010).

1.2. Is the fish immune system sensitive to estrogens? Evidence for the presence of estrogen receptors in immune organs

The classical mechanism of estrogen action is the ligand-dependent activation of intracellular estrogen receptors (ERs). In mammals, the expression of ERs in specific immune populations is well established (see above) so that direct signalling of natural as well as environmental estrogens in immune cells is possible (e.g., Medina et al., 2000; Maret et al., 2003). In order to explore if estrogen signalling would be possible in the immune system of fish as well, we performed real time RT-PCR analysis of the expression of estrogen receptors (ERs) in two immune organs of rainbow trout, *Oncorhynchus mykiss*, the spleen and the head kidney. All four ER isoforms, ERα1, ERα2, ERβ1 and ERβ2 (Nagler et al., 2007) of rainbow trout were studied. Both in head kidney and spleen of rainbow trout, mRNA signals of the four ER isoforms were detected (Table 1). Presence of the four isoforms in the spleen of rainbow trout was also observed by Nagler et al. (2007). ER expression levels in the spleen are higher than in the head kidney, and, with the exception of ERα2, hepatic ER expression levels are higher than those in both immune organs. The difference between liver and immune organs is particularly strong for ERα1, but less pronounced for ERβ. The demonstration of ER mRNA expression in immune organs points to the possibility that ER signalling can take place in the immune system of rainbow trout. However, this information has been obtained with extracts form whole tissues which contain not only immune cells but also stromal cells; thus the observation of ER mRNA expression in a tissue extract does not yet inform if the ER expression associates with immune cells, and which immune cell types carry which ER subtypes and at what ratios. As discussed above for mammals, to understand the role of estrogens in the fish immune system, and to assess the risk environmental estrogens impose on fish immunocompetence, future studies will have to reveal which immune cell types express which ER isoforms, what the cell type-specific expression ratios of ERα and ERβ are, and whether their expression changes with the physiological status of the fish (e.g., during the reproductive cycle, during disease or during environmental (xeno-)estrogen exposure).

1.3. Is the fish immune system sensitive to estrogens? Evidence for estrogen effects on immune gene expression

The presence of ERs in immune cells does not necessarily implicate an estrogenic regulation of immune genes, but the ER function in the immune cells may be directed primarily towards genes involved in cellular metabolism, growth and differentiation, e.g., genes of the IGF system (Shved et al., 2009), while the expression of immune-specific genes may be affected only indirectly, for instance, as consequence of E2-promoted immune cell maturation. From mammals, it is known that a number of immune genes, e.g., complement factor C3, are indeed under direct estrogenic regulation as they possess functional estrogen-responsive elements in their promoter regions (Fan et al., 1996), but corresponding knowledge for fish is not available to date.

In order to obtain preliminary information into whether estrogens are able to regulate immune gene expression of fish, we examined the hepatic transcriptome of E2-exposed rainbow trout. During a 14-day-period, the fishes were fed once per day at 1% body weight with a diet containing 20 mg 17β-estradiol (E2)/kg dry food. Table 2 shows examples from Gene Ontology (GO)-based analysis of immune gene categories that responded significantly to E2 treatment. The results pinpoint to the potential of E2 to

Table 1
Relative estrogen receptor mRNA expression measured by TaqMan®-based real-time RT-PCR in the head kidney, spleen and liver of rainbow trout.

	Head kidney	Spleen	Liver
ERα1	1 ± 0.22	8.48 ± 5.50	77.1 ± 85.6
ERα2	1 ± 0.90	1.58 ± 2.11	0.05 ± 0.03
ERβ1	1 ± 0.40	3.67 ± 2.83	15.8 ± 10.4
ERβ2	1 ± 0.25	2.60 ± 1.85	12.4 ± 5.07

ER expression was measured in juvenile rainbow trout (average body weight 50 g, $n = 10$) by means of real time RT-PCR using Applied Biosystems 7500 Fast Real-Time PCR System. The primers and probes were designed using Primer Express Software ver. 3.0 (Applied Biosystems), based on the sequence information published by Nagler et al. (2007). Expression of each ER isoform was calculated by the $2^{(-\Delta\Delta CT)}$ method (Bookout and Mangelsdorf 2003), and 18S rRNA was used as endogenous reference. Expression levels of the ER forms in the head kidney were set to be 1, and the expression levels in the other organs are expressed relative to that in the head kidney.