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Environmental estrogens interact with and modulate the properties of plasma sex steroid-binding proteins in juvenile Atlantic salmon (*Salmo salar*)

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Abstract

Environmental chemicals may modulate the endocrine system through interaction with plasma sex steroid-binding proteins (SBP) and SBP-regulated processes. Some of these chemicals, which are known to interact with the estrogen receptor (ER), were found to bind competitively to the Atlantic salmon (*Salmo salar*) SBP and potentially disrupt the endocrine function of these proteins. Furthermore, both weakly acting (di-*n*-butyl phthalate) and potent estrogen mimics (ethynylestradiol), were able to induce a substantial up-regulation of circulating levels of SBP in vivo. Interestingly, modulation of SBP-levels was found to be a more sensitive endpoint than chemically induced interference with classical ER-mediated mechanisms for weakly acting estrogen mimics like di-(*n*-butyl) phthalate. Interference with the endocrine function of SBPs may thus introduce a novel mechanism for endocrine disruption, and give additional answers to the question why some weakly acting xenoestrogens are causing "estrogen-like" reproductive disturbances in developing males.

Keywords: Endocrine disrupters; Sex steroid-binding proteins; Ethynylestradiol; Di-(*n*-butyl) phthalate; Atlantic salmon

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In most vertebrate species, sex steroid hormones circulate in complex with a specific high affinity sex steroid-binding protein (SBP) and low affinity proteins such as corticosteroid-binding proteins and albumin (Westphal, 1986). In the blood, typically 97–99% of the estrogens and androgens are bound to these proteins, whereof binding to the SBP accounts for 40–70%. The functions of the SBP, also known as sex hormone-binding globulin (SHBG) or testosterone/estradiol-binding globulin (TeBG), are not fully understood. It is, however, generally believed that the SBP protects steroids from rapid metabolic degradation and plays a role in regulating the amount of steroid that is available to target tissues (Rosner, 1990). In addition to the role as a sex steroid carrier, it has been proposed that SBP itself is involved in cellular signalling to nuclear steroid receptors through a specific SBP membrane receptor (Fortunati, 1999). As in other vertebrates documented to have SBP, the fish liver seems to be the main organ for SBP synthesis, although testicular production of an androgen-binding protein with similar primary structure as the SBP has been reported (Foucher, Niu, Mourot, Vaillant, & Le Gac, 1991).

The SBP, which has been identified in plasma from several fish species, exhibits a broad capability for binding endogenous hormones. Despite an increasing focus on the endocrine modulating effects of environmental pollutants in fish, the possible interference of these compounds with the function of the piscine SBP is largely unknown. The aim of this study was therefore to determine if compounds that bind to and modulate the properties of the estrogen receptor (ER) could also interfere with the function of the Atlantic salmon (*Salmo salar*) plasma SBP (asSBP).

In this work, estradiol binding sites were characterised in plasma from untreated juvenile Atlantic salmon by radio-ligand saturation analysis with 1,2,4,6,7-[^{3}H]-estradiol ([^{3}H]-E2) as described by Øvrevik, Stenersen, Nilssen, and Tollefsen (2001). In subsequent assays, 17β-estradiol (E2), ethynylestradiol (EE2) and di-(*n*-butyl) phthalate (DBP) were assayed for the ability to compete with [^{3}H]-E2 for the estrogen binding site at the asSBP as described by Tollefsen (2002). Total E2-binding capacity was assayed by a one-point saturation analysis of diluted dextran-charcoal stripped plasma from juvenile Atlantic salmon after 2 weeks flow through exposure to the vehicle 2-propanol (C), EE2 and DBP.

The main concern has traditionally been that environmental pollutants mimic endogenous estrogens and exert direct effects via the estrogen receptor. Our results do, however, suggest that these chemicals may modulate the endocrine system through the interaction with the plasma SBP and SBP-regulated processes. As seen with the potent estrogenic pharmaceutical EE2 and the weakly estrogenic DBP, both chemicals were able to bind to the high affinity E2-binding sites in Atlantic salmon plasma (Fig. 1). Interestingly, EE2 display a strikingly high affinity for the asSBP (approx. 100 times less than E2) whereas DBP only display weak binding activity. Subsequent saturation analysis in presence and absence of the estrogen mimics show that both chemicals inhibit the binding of [^{3}H]-E2 without altering the maximum amount of binding sites (B_{max}), thus suggesting that the binding is competitive (results not shown). These observations agree with similar studies in rainbow trout (Tollefsen, 2002), which indicate that the SBP resemble the estrogen receptor in its accommodation of a variety of non-steroidal ligands.

The SBP is primarily a plasma transport system, and compounds binding to this protein may influence the metabolism and tissue availability of the compounds themselves or the native hormones carried by the SBP. As demonstrated by Déchaud, Raward, Claustrat, de la Perriére, and Pugeat (1999) presence of high concentrations of SBP-bound environmental chemicals may potentially displace endogenous sex steroids from their plasma binder and transiently induce an increase in the bioavailable levels of these potent steroids. Since the binding of the SBP ligands is reversible, SBP-bound xenobiotics may also potentially become displaced by high concentrations of locally produced endogenous estrogens and androgens. This shuttle effect of SBP could consequently lead to more specific targeting of environmental chemicals to tissues producing estrogens (ovaries) and androgens (testis).

In vivo exposure to EE2 and DBP induces a dose-dependent increase in the circulating levels of SBP as revealed by an increased E2 binding capacity in juvenile Atlantic salmon plasma (Fig. 2). The exact mechanism for SBP modulation by environmental chemicals is still not known, but results presented by Foucher et al. (1991) suggest that hepatic SBP synthesis is regulated by direct exposure to estrogens as well as modulation of circulating levels of non-steroidal hormones like

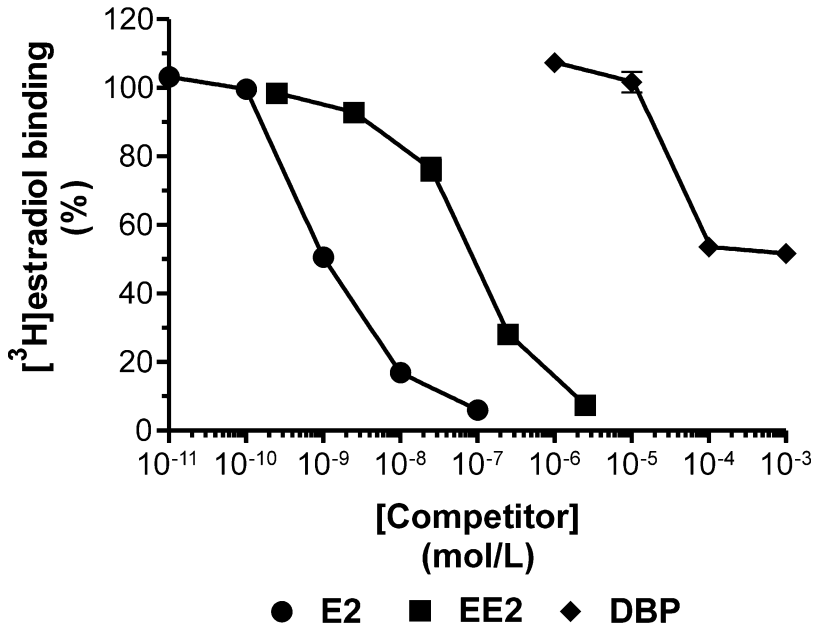

Fig. 1. Displacement of [^{3}H]estradiol from the high affinity estradiol binding site in diluted Atlantic salmon plasma (0.5 mg protein/ml) by inert 17β-estradiol (E2), ethynylestradiol (EE2) and di-(*n*-butyl) phthalate (DBP). The results (mean ± S.E.M.) depict the percentage of bound [^{3}H]estradiol (1 nM) for a given concentration of competitor from 3 individual assays.

growth hormone, insulin and insulin-like growth factors. Interestingly, the increase in SBP-levels occurs in Atlantic salmon at substantially lower concentrations of DBP than is required for the activation of ER-mediated vitellogenin production in vitro and in vivo (Tollefsen, unpublished). Although not investigated in this study, modulation may possibly cause a decrease in the free and therefore bioavailable levels of sex steroids. Exposure to chemicals like EE2 and DBP could thus lead to anti-androgenic effects in males and anti-estrogenic effect in females through the indirect action of these proteins. The fact that several environmental chemicals, including some phthalates, are more potent anti-androgens than estrogens introduces the possibility that reproductive disturbances could be caused by blocking of natural androgen action rather than exposure to environmental estrogens (Sohoni & Sumpter, 1998). Interference with the endocrine functions of SBPs may thus represent a novel mechanism for endocrine disruption, and explain why chemicals like the phthalates are causing "estrogen-like" reproductive disturbances in developing males without exerting their action through the estrogen receptor as reported by Gray, Ostby, and Kelce (1998).

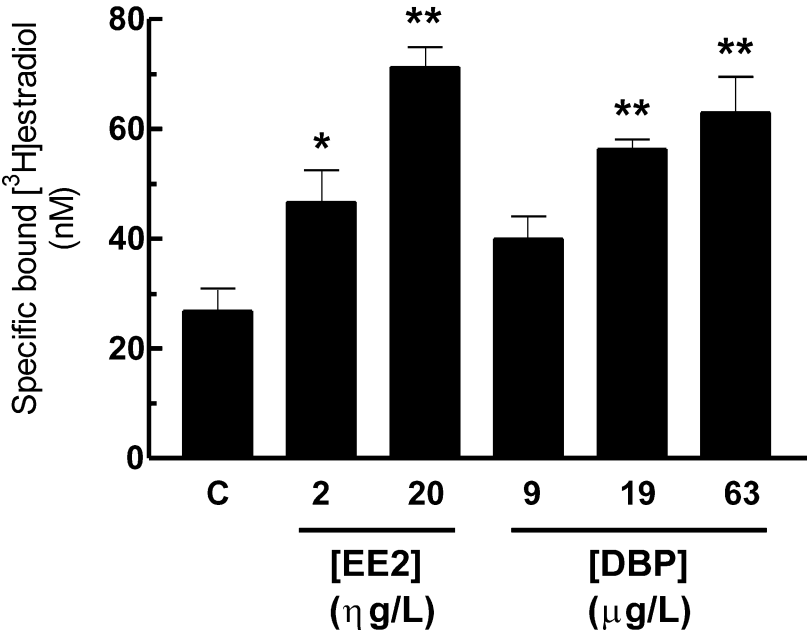

Fig. 2. Modulation of the high affinity estrogen-binding proteins in plasma of juvenile Atlantic salmon exposed to ethynylestradiol (EE2), di-(*n*-butyl) phthalate (DBP) and the vehicle 2-propanol (C). The results (mean ± S.E.M.) are based on the specific binding of [^{3}H]estradiol (10 nM) to diluted DCC-stripped plasma from groups of 6–8 individual fish after 2 weeks of flow-through water exposure. * ($P < 0.05$) and ** ($P < 0.01$) depict level of significance compared to the control group when analysed by a one-way ANOVA test followed by a Dunnett's post test.

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