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17β-Estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FT-IR spectroscopy: A comparative study with nonylphenol

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Abstract

Steroidal hormones produced by humans and animals are constantly being excreted into the environment. It has been demonstrated that sewage effluent discharged to surface water contains natural estrogens and synthetic estrogenic chemicals. As estrogen levels continuously increase in the aquatic environment, it is very important to have a detailed understanding of estrogens' effects on fish. In the present study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to 17β-estradiol (E2) for 3 weeks and the effects of E2 on rainbow trout livers were investigated at the molecular level using Fourier transform infrared spectroscopy. The results revealed that E2 induced significant alterations in the liver tissues. A decrease in glycogen levels and protein concentration, and an increase in both the population of hepatic lipids, especially triglycerides, as well as the relative content of nucleic acids was observed in the E2 treated liver. In addition, a decrease in the membrane fluidity and an increase in lipid order were found in the cells of treated samples. In order to compare the effect of E2 with that of NP at molecular level, the fish were also treated with an estrogenic compound, nonylphenol (NP). The NP-treated fish liver spectra were found to be quite similar to those of E2-treated fish confirming that NP mimics the effect of E2 in immature rainbow trout.

Keywords: Estradiol; Nonylphenol; Rainbow trout; FT-IR spectroscopy; Liver tissue

1. Introduction

Sewage effluent discharged to surface water has been shown to contain human hormones, particularly estrogens, and synthetic estrogenic chemicals. In recent years, the role of estrogens in the regulation of different reproductive events in animal biology has become an area of intensive research. The interest in this field is related to the "estrogen hypothesis" claiming that "environmental estrogens" have the potential to induce severe effects on reproductive performance in wildlife and humans (Colborn and Clement, 1992; Sharpe and Skakkebaek, 1993).

Natural estrogens play a major role in controlling reproduction in females and, to a lesser extent, in males. Additionally, physiological concentrations of estrogen are essential for the maintenance of cell growth and several other biological activities. For example, normal physiological levels of estrogen are known to be involved in the control of cell proliferation, transcription, and DNA synthesis. In addition to estrogen-responsive organs (such as the uterus, breasts, and pituitary), estrogens also exert an effect at a number of other sites (kidney, liver, skeletal tissues, etc.). Imbalance of the steady-state concentrations of estrogens is known to produce adverse effects (Roy et al., 1997).

Estrogen induces hepatic vitellogenin production in oviparous female vertebrates. Vitellogenin is a large lipoglycophosphoprotein, which is specifically synthesized, lipidated, phosphorylated, and glycosylated in the liver of the animals under the control of 17β-estradiol (E2). E2 is a natural estrogen that is secreted into the blood stream, and finally taken up by oocytes via receptor-mediated endocytosis. Normally, vitellogenin is undetectable in male and immature female fish; however, its synthesis can be induced by exogenous stimulation of estrogen and estrogenic compounds (Jobling and Sumpter, 1993; Islinger et al., 1999). It is well established that some of the biochemical and physiological changes that occur in the sexually mature female fish during vitellogenesis in nature can be induced by administration of estrogen to immature females

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as well as males (Korsgaard et al., 1983). It has been shown that E2 treatment affects carbohydrate metabolism by inducing a marked reduction in liver glycogen of fish (Korsgaard and Mommsen, 1993). Bun Ng et al. (1984), reported that E2 treatment increases hepatic lipid content and serum concentrations of protein. It has been also demonstrated that treatment with E2 showed an increase in cell volume, in nuclear size (Patrick et al., 1983) and total liver RNA (Emmersen et al., 1979; Soverchia et al., in press). In addition, Zaroogian et al. (2001) have reported that E2 caused histopathological changes in the liver of immature summer flounder.

In aquatic environments, the source of estrogens and estrogenic endocrine disrupting compounds is mainly human sewage and industrial activities (Atkinson et al., 2003; Tashiro et al., 2003). The source of natural steroid estrogens in the environment is predominantly due to human activity, where women generally produce 10 μg/day and pregnant women can produce up to 30 mg/day (Aldercruetz et al., 1994). Other sources of steroid estrogens are from the use of estrogens in the treatment of cancer, osteoporosis, menopause and hypogonadism (Arcand-Hoy et al., 1998), and from agriculture (Shore et al., 1993). Both natural and synthetic estrogens leave the human body by excretion via urine or feces (Ying et al., 2002). The estrogens then reach the sewage treatment waters from where they are released into the environment with effluent water (Purdom et al., 1994). Analyses of effluent water revealed significant levels of different estrogenic substances. Concentrations of E2 in effluent from sewage treatment works, in various countries, range from the low nanograms up to hundreds of nanograms per liter. For instance, Desbrow et al. (1998) measured concentration of E2 ∼48 ng/L in British rivers. Similar results have also been reported in rivers downstream of sewage treatment waters in the USA (Tabak et al., 1981; Aherne and Briggs, 1989), Sweden (Larsson et al., 1999), and Israel (Shore et al., 1993).

The level of sterodiol estrogens and estrogenic endocrine disrupting compounds continuously increase in the aquatic environment. It is known that they are able to disturb the normal physiology and endocrinology of aquatic organisms. Therefore, it is very important to describe the effects of E2 on fish at the molecular level. Despite several biochemical and physiological studies, some of which were reported above, the studies reporting the effects of E2 on tissues and membranes at molecular level are very limited. These studies investigated the effects of estrogen on phase transition behavior, lipid order, dynamics of model membrane formed from dipalmitoyl phoshatidylcholine (DPPC) (Boyar and Severcan, 1997), and rat brain homogenate membrane (Dicko et al., 1999).

Rainbow trout is one of the most commonly cultured and consumed fresh-water fish in the world. The liver is not only the target tissue for estrogens, where estrogens stimulate vitellogenin synthesis, but is also the site of deactivation in which the estrogens are catabolized. For this reason, the current study was conducted in order to determine the effects of E2 on rainbow trout liver. The effects of E2 on the compositional, structural, and functional changes of macromolecules in rainbow trout liver were investigated at molecular level using Fourier transform infrared (FT-IR) spectroscopy. A powerful technique coupled with experimental convenience, FT-IR spectroscopy, together with infrared microscopy, is an important technique to study the cellular changes at molecular level in various biological samples (Lewis et al., 1989; Takahashi et al., 1991; Liu et al., 1996, 2002; Yano et al., 1996; Fung et al., 1997; Ci et al., 1999; Kidder et al., 1999; Melin et al., 2000; Toyran et al., 2004; Severcan et al., 2005a).

Many anthropogenic chemicals, which are present in the environment as pollutants, interact with the endocrine systems of animals and interfere with estrogen receptor-mediated physiological responses. These so called “endocrine disrupter” chemicals either evoke estrogenic responses by mimicking, or by inhibiting the action of E2 in the animals. In the present study, the action of NP, an estrogenic endocrine disrupter, was compared to the effects of E2 on Rainbow trout liver. Nonylphenol (NP) is an alkylphenol (AP) which is a degradation product of alkylphenol polyethoxylates (APEs). APEs are non-ionic surfactants used in detergents, herbicides, pesticides, and paints (Pedersen et al., 1999), and they are found in rather high concentrations in the aquatic environment (Ahel et al., 1994; Blackburn and Waldock, 1995). NP has been reported to be estrogenic both in vivo (Jobling et al., 1996; Coldham et al., 1998; Schwaiger et al., 2000) and in vitro (Jobling and Sumpter, 1993; White et al., 1994) assay systems in fish.

2. Materials and methods

2.1. Chemicals

17β-Estradiol (E2) and nonylphenol (NP) were purchased from Sigma and Aldrich Company, respectively. Potassium bromide (KBr) was purchased from Merck.

2.2. Animal model

Eight-month-old juvenile rainbow trout were supplied by the ER-SU fish farm (Kesikköprü, Ankara, Turkey). The fish were transported to the laboratory in aerated plastic sacks, and held in the laboratory in 35 L glass aquariums. The fish were acclimated, in exposure conditions, for 4 days prior to starting the experiment. For the entire duration of the experiment the fish were fed with trout chow purchased from Cagatay-Yem Company (İzmir, Turkey).

2.3. Aquarium experiments

A semi static aquaria system was used in the aquarium experiments. Aquarium water came from the municipal water supply and was charcoal filtered to eliminate chlorine, suspended solids, and metals. Aeration with an air pump was continued throughout the experiment to maintain the dissolved oxygen level at 7 ± 1 ppm. The pH of the aquarium water was maintained at 7.2 ± 0.5. The photoperiod was 8 h of light and 16 h of darkness. Prior to use, these media were let to rest for 24 h with an aerator. The fish were treated with 0 (control) ($n = 8$) and 22 μg/L E2 ($n = 6$) for 3 weeks in the aquarium. A group of fish was also exposed to 220 μg/L NP ($n = 6$) for 3 weeks. E2 and