Available online at www.sciencedirect.com
ScienceDirect
General and Comparative Endocrinology 148 (2006) 273–281
GENERAL AND COMPARATIVE ENDOCRINOLOGY
www.elsevier.com/locate/ygcen

Effects of xenobiotics and steroids on renal and hepatic estrogen metabolism in lake trout

Gail F. Jurgella [a], Ashok Marwah [b], Jeffrey A. Malison [a], Richard Peterson [c], Terence P. Barry [a,*]

[a] *Department of Animal Sciences, University of Wisconsin-Madison, 1675 Observatory Drive, Madison, WI 53706, USA*
[b] *Department of Biochemistry—Enzyme Institute, University of Wisconsin-Madison, 1710 University Avenue, Madison, WI 53726, USA*
[c] *School of Pharmacy, University of Wisconsin-Madison, 777 Highland Avenue, Madison, WI 53705, USA*

Received 15 December 2005; revised 13 March 2006; accepted 23 March 2006
Available online 4 May 2006

Abstract

Experiments were conducted to (1) elucidate the biochemical pathways of E2 metabolism in the lake trout (*Salvelinus namaycush*) kidney and liver, and (2) test the hypothesis that specific xenobiotics and endogenous steroids inhibit E2 metabolism by these tissues. Kidney and liver tissue fragments from immature lake trout were incubated in vitro in the presence of radiolabelled E2 plus various xenobiotics or steroids. E2 metabolites were identified by liquid chromatography/mass spectroscopy, and quantified by liquid scintillation spectroscopy. A major metabolite produced by both tissues was an unidentified hydroxylated estrogen metabolite (E2-OH) with a molecular mass of 288 that was not estriol (16-OH-E2), but possibly 7α-OH-E2 or 2-OH-E2 (catecholestrogen). Both tissues also produced estradiol-17-glucuronide (E2-17-G), estradiol-17-sulfate (E2-17-S), and estradiol-3-glucuronide (E2-3-G). Compared to the kidney, the liver produced half the amount of conjugated metabolites, but twofold more E2-OH. The following xenobiotics (at a concentration of 100 μM) inhibited the production of water-soluble (i.e., conjugated) E2 metabolites by both the kidney and liver: 4,4′-$(OH)_2$-3,3′,5,5′- tetrachlorobiphenyl (4,4′-OH-TCB), bisphenol A (BPA), tetrabromobisphenol A (TB-BPA), tetrachlorobisphenol A (TC-BPA), tribromophenol (TBP), trichlorophenol (TCP), and pentachlorophenol (PCP). The alkylphenols, 4-*n*-nonylphenol (NP), and 4-octylphenol (OP), and 2,2′,4,4′-tetrabromodiphenyl ether (TBDE) had no significant effect on E2 metabolism by either tissue. Testosterone and 17α,20β-dihydroxy-4-pregnen-3-one inhibited the production of conjugated E2 metabolites by both the kidney and liver. Cortisol and 11-ketotestosterone inhibited E2 metabolism by the liver only. The median inhibitory concentrations (IC_{50}) for 4,4′-OH-TCB ranged from 7–32 μM in the kidney and 0.6–1.6 μM in the liver. For BPA, IC_{50}'s ranged from 40–108 μM in the kidney and 11–18 μM in the liver. Low doses (0.1 μM) of 4,4′-OH-TCB and BPA significantly increased estrogen metabolism in the kidney. The results suggest that certain estrogenic xenobiotics and endogenous steroids may inhibit the phase II conjugation of E2 by the kidney and liver of lake trout, and some of the known biological effects of these compounds are likely mediated, at least partially, by this mechanism of action.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Lake trout; Endocrine disruption; Steroidogenic enzymes; Estrogen; Xenobiotic; Steroid hormone; Estradiol-17β; Steroid; Steroid metabolism

1. Introduction

The lake trout (*Salvelinus namaycush*) is the top natural predator in the Great Lakes food web. During the twentieth century, lake trout populations dropped dramatically in Lake Ontario, and it was thought that this decline was the result of sea lamprey predation, declining water quality, and over harvesting. Attempts to reestablish naturally reproducing lake trout populations focused primarily on improving these problems, largely without success (Gilbertson, 1992). Later studies suggested that xenobiotic chemicals present in the water might be the principal cause of lake trout recruitment failure. For example, Walker and Peterson (1994) demonstrated that lake trout larvae are highly sensitive to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

* Corresponding author. Fax: +1 608 262 0454.
E-mail address: tpbarry@wisc.edu (T.P. Barry).

0016-6480/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2006.03.011

(TCDD), and even very low-level exposure to TCDD causes early life stage mortality. Further studies demonstrated that the concentrations of TCDD and related chemicals in Lake Ontario during the 1960s were high enough to explain the observed decline in lake trout populations (Cook et al., 2003). Despite bans on the use of TCDD and other xenobiotics such as polychlorinated biphenyls (PCBs), these lipophilic compounds continue to persist in lake sedimentation basins, and in the tissues of contaminated fish, particularly the top trophic-level predators (Humphrey et al., 2000; Safe, 1994). Moreover, the levels of other xenobiotics that are potentially harmful to lake trout, including polybrominated diphenyl ethers (PBDEs), are currently increasing in the Great Lakes due to their continued production and use by industry (deWit, 2002; Hale et al., 2003). Although the levels of most xenobiotics in the Great Lakes and in Great Lakes fishes are currently too low to cause outright early life stage mortality, they may still reduce lake trout recruitment by acting as endocrine disruptors. Certain PCBs and hydroxylated PCB metabolites are known to modify physiological functions in estrogen-responsive targets such as the reproductive organs, liver, and neuroendocrine centers (Jobling et al., 1995; McLachland and Arnold, 1996; White et al., 1994). Even weakly active compounds may disturb the differentiation or function of estrogen-responsive tissues if exposure occurs during critical developmental periods (Colborn et al., 1993). Developing fish embryos and larvae are particularly at risk because many xenobiotics can be transferred from the mother to the developing eggs (Peterson et al., 1993; Walker and Peterson, 1994).

The mechanisms by which xenoestrogens exert their endocrine-disrupting effects are not well established. Many potent hydroxylated PCB metabolites (PCB-OHs), for example, show little or no affinity for the α- or β-estrogen receptor (ER) subtypes, and thus may not act directly as estrogen receptor agonists (Korach et al., 1988; Kuiper et al., 1998). In humans, Kester et al. (2000) showed that numerous PCB-OHs are powerful inhibitors of the phase II conjugating enzyme, estrogen sulfotransferase (SULT1E1), and indirectly induce estrogenic activity by reducing the metabolism of endogenous estrogen. Indeed, the inhibition of SULT1E1 is the most potent biological effect described to date regarding the endocrine-disrupting activity of PCBs or their metabolites (Kester et al., 2000). The most potent PCB-OHs, with IC_{50} values in the subnanomolar range, were compounds with a 4-OH-3,5-dichloro substitution pattern (Kester et al., 2000). In subsequent studies, other xenobiotic compounds with known estrogenic activity, including halogenated bisphenol A (BPA) metabolites, were also shown to be potent inhibitors of SULT1E1 (Kester et al., 2002).

Xenobiotic inhibition of phase II conjugating enzymes may also be an important mechanism of action of endocrine disruption in fish. For example, Ohkimoto et al. (2003) reported that BPA, 4-*n*-nonylphenol (NP), and 4-*n*-octylphenol (OP) inhibited the sulfonation of estradiol-17β (E2) in zebrafish (*Brachydanio rerio*), and van den Hurk et al. (2002) found that several PCB-OHs inhibited both the sulfonation and glucuronidation of benzo[*a*] pyrene in channel catfish (*Ictalurus punctatus*), indicating that both sulfotransferase (SULT), and glucuronosyltransferase (UGT) are targets for inhibition by xenobiotics in fish.

The goals of the present investigation were to (1) begin characterizing the biochemical pathways of E2 metabolism in the kidney and liver of lake trout, and (2) evaluate the effects of various estrogenic xenobiotics, as well as several key fish steroid hormones, on the production of conjugated, water-soluble estrogen metabolites normally produced by the lake trout kidney and liver.

2. Materials and methods

2.1. Chemicals

Steroids were obtained from Sigma Chemical Co. (St. Louis, MO) or Steraloids (Newport, RI). [6,7-^{3}H]-E2 (40 to 60 Ci/mmol) was purchased from Perkin-Elmer Life Sciences (Boston, MA). 4,4′-OH-TCB was obtained from Ultra Scientific (North Kingstown, RI). BPA, OP, and NP were obtained from Sigma (St. Louis, MO). Tetrachlorobisphenol A (TC-BPA), tetrabromobisphenol A (TB-BPA), PCP, 2,4,6-trichlorophenol (TCP), 2,4,6-tribromophenol (TBP), and 2,2′,4,4′-tetrabromodiphenyl ether (TBDE) were gifts from AccuStandard (New Haven, CT). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Atlanta, GA). HPLC grade methanol, ethanol, hexane, and acetonitrile were purchased from Sigma–Aldrich (Milwaukee, WI, USA) and used as such. HPLC grade acetic acid was purchased from Fisher Scientific (Pittsburgh, PA, USA). Formic acid was purchased from Fluka (Milwaukee, WI, USA). Distilled water was deionized and purified (18.25 ± 0.05 MΩ-cm) using a Nanopure water system from Barnstead International (Dubuque, IA, USA). Solid phase extraction (SPE) cartridges (Oasis-HLB, 3 cc) were obtained from Waters Corporation (Milford, MA, USA).

2.2. Fish

Lake trout were obtained from the Minnesota Department of Natural Resources, Crystal Springs State Fish Hatchery (Altura, MN). Three-year-old, sexually immature fish, 30–50 cm in total length, were used in all experiments. The fish were maintained in 750 L circular fiberglass tanks supplied with flow-through carbon-filtered city water at 10–14 °C. Photoperiod was kept constant at 12 h light/12 h dark. The fish were fed once daily to satiation using Silvercup trout feed (Murray, UT). Food was withheld 24 h prior to sacrifice. Care and treatment of the fish were in accordance with the guidelines of the University of Wisconsin Research Animal Care Committee.

2.3. In vitro cultures

Physiological saline and Hepes-buffered culture medium, developed for culturing salmonid tissues, were prepared and sterilized by ultrafiltration at 0.2 μm (Barry et al., 1997). Estrogen metabolism was quantified by measuring the formation of E2 metabolites after incubation of tissue fragments in 1 ml of culture medium in the presence of [^{3}H]-E2, according to the method of Barry et al. (1997). In brief, the kidney and liver were uniformly fragmented using a Polytron (Brinkmann Instruments, Westbury, NY). The tissue fragments were washed 2–3 times in physiological saline, and 50 μl aliquots of tissue (1.16 ± 0.03 mg protein) were added to 150 × 20 mm test tubes and incubated at 15 °C for 1–24 h. Controls were no-tissue and time 0 incubates. The cultures were terminated and extracted as described below. Experiments used tissues from one fish cultured in duplicate incubations, and each experiment was conducted two to four times ($N = 2$ to 4).

Download English Version:

https://daneshyari.com/en/article/2802417

Download Persian Version:

https://daneshyari.com/article/2802417

Daneshyari.com