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Bisphenol A accumulation in eggs disrupts the endocrine regulation of growth in rainbow trout larvae

Oana Birceanu, Mark R. Servos, Mathilakath M. Vijayan*

Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

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ABSTRACT

Bisphenol A (BPA), a monomer used in the production of plastics and epoxy resins, is ubiquitously present in the aquatic environment. BPA is considered a weak estrogen in fish, but the effects of this chemical on early developmental events are far from clear. We tested the hypothesis that BPA accumulation in eggs, mimicking maternal transfer, disrupts growth hormone/insulin-like growth factor (GH/IGF) axis function, leading to defects in larval growth in rainbow trout. Trout oocytes were exposed to 0 (control), 0.3, 3, and 30 μg ml^{-1} BPA for 3 h, which led to an accumulation of around 0, 1, 4 and 40 ng BPA per egg, respectively. All treatment groups were fertilized with clean milt and reared in clean water for the rest of the experiment. The embryo BPA content declined over time in all groups and was completely eliminated by 42 days post-fertilization (dpf). Hatchlings from BPA accumulated eggs had higher water content and reduced total energy levels prior to first feed. There was an overall reduction in the specific growth rate and food conversion ratio in larvae reared from BPA-laden eggs. BPA accumulation disrupted the mRNA abundance of genes involved in GH/IGF axis function, including GH isoforms and their receptors, IGF-1 and -2 and IGF receptors, in a life stage-dependent manner. Also, there was a temporal disruption in the mRNA levels of thyroid hormone receptors in the larvae raised from BPA-laden eggs. Altogether, BPA accumulation in eggs, mimicking maternal transfer, affects larval growth and the mode of action involves disruption of genes involved in the GH/IGF and thyroid axes function in trout.

1. Introduction

Bisphenol A (2,2-bis[4-hydroxyphenyl]propane; BPA), a monomer used in the production of common plastics and epoxy resins, is ubiquitously present in the environment (Staples et al., 1998; Sajiki and Yonekubo, 2004; Cao et al., 2008, 2009). BPA levels ranging from 0.45 to 8000 ng l^{-1} have been reported in freshwater bodies across North America (Staples et al., 2000; Chen et al., 2006), while tissue levels of BPA as high as 10.4 ng g^{-1} wet weight in wild fish have been detected in the muscle, brain and liver (Wei et al., 2011; Renz et al., 2013). BPA has been labeled as an endocrine disrupting compound, mainly based on its ability to weakly mimic the effects of the female hormone 17β-estradiol (E2), including production of the egg yolk protein vitellogenin in fish (Brian et al., 2005; Larsen et al., 2006; Correia et al., 2007; Aluru et al., 2010; Alonso-Magdalena et al., 2012; Hanson et al., 2014). However, recent research has shown that the effects of this chemical in fish extend beyond its xenoestrogenic role, with BPA toxicity being pleiotropic and life stage-dependent (Vandenberg et al., 2009; Aluru et al., 2010; Vandenberg, 2013). Chronic exposures to waterborne BPA during early development led to embryonic deformities and mortality (Honkanen et al., 2004; McCormick et al., 2010), while exposure of adults impacted reproduction and growth (Crain et al., 2007; Mandich et al., 2007; Lam et al., 2011; Hanson et al., 2012, 2014). Recently, accumulation of BPA in eggs, mimicking maternal transfer, had long-term effects on growth hormone/insulin-like growth factor (GH/IGF) axis function in rainbow trout (*Oncorhynchus mykiss*), but whether this translated to altered growth rate was not known (Aluru et al., 2010).

GH and IGFs are key mediators of somatic growth in teleosts (Björnsson et al., 2002; Shepherd et al., 2007). In fish, GH targets a multitude of organs, leading to activation of intracellular pathways by binding to the two GH receptors, GH-1r and GH-2r (Reinecke et al., 2005; Shepherd et al., 2007). The growth promoting effect of GH is mediated by its binding to GH-r and activating, in part, the synthesis and release of IGF-1 and IGF-2 from the liver (Reinecke et al., 2005; Reinecke, 2010). Together with GH, IGFs are involved in metabolism, osmoregulation, growth and development (Reinecke, 2010). In addition to the GH/IGF axis, thyroid hormones (THs) also

* Corresponding author at: Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada, T2N 1N4. Tel.: +1 403 220 3094.
E-mail address: matt.vijayan@ucalgary.ca (M.M. Vijayan).

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play an important role in development, growth and metabolism (Terrien and Prunet, 2013). Thyroid hormone action is mediated via the activation of the thyroid hormone receptors (TRs), encoded by two genes, TRα and TRβ (Raine et al., 2004; Terrien and Prunet, 2013). A role for GH, IGFs and THs in growth regulation during early development is well established (for detailed reviews, see Power et al., 2001; Reinecke, 2010; Terrien and Prunet, 2013).

Recent studies have identified the GH/IGF and TH axes as targets of xenobiotic toxicity. For instance, environmental estrogens impact growth, salinity adaptation and GH and IGF sensitivity in trout post-hatch (Hanson et al., 2012; Hanson et al., 2014). Also, BPA acts as a TR antagonist in *Xenopus laevis* (Moriyama et al., 2002; Zoeller, 2005) and disrupts thyroid function in zebrafish (*Danio rerio*; Terrien et al., 2011). While exposure of contaminants through the water column is of main concern for fish, maternal accumulation of contaminants in lipid depots and the subsequent transfer of these chemicals to the offspring through eggs have recently gained attention (Takao et al., 2008; Ostrach et al., 2008; Aluru et al., 2010). Indeed, exposure of early developing embryos to contaminants led to long-term abnormalities in growth and development (Westerlund and Billsson, 2000; Nye et al., 2007; McCormick et al., 2010; Aluru et al., 2010; Corrales et al., 2014), but the mechanisms are far from clear.

We tested the hypothesis that BPA accumulation in oocytes, mimicking maternal contaminant transfer, affects larval growth by disrupting the endocrine regulation of growth in fish. To test this, rainbow trout oocytes were exposed to BPA prior to fertilization, to mimic maternal transfer, as described previously (Aluru et al., 2010). The offspring total energy content, food conversion ratio and growth were monitored for 112 days. Temporal changes in mRNA abundance of GH, IGF and their receptors and TRs were determined to assess if GH/IGF and thyroid axes disruption during early development plays a role in the BPA-mediated effect on growth in trout.

2. Materials and methods

2.1. Materials and chemicals

Unless otherwise specified, BPA and all other chemicals and enzymes were purchased from Sigma–Aldrich (St. Louis, MO, USA). D-Glucose was purchased from Bioshop (Burlington, ON, CA), while monobasic and dibasic sodium phosphate, sodium bicarbonate and borosilicate tubes were purchased from Fisher Scientific (Ottawa, ON, CA). The 96-Well plates for quantitative real-time PCR (qRT-PCR), along with the iQ SYBR® green fluorescent dye mix were purchased from Bio-Rad (Mississauga, ON, CA). The TRIZol used for RNA extraction was purchased from Invitrogen (Burlington, ON, CA), while the first strand cDNA synthesis kit and the DNase I for treating the RNA prior to making cDNA were purchased from Fermentas (Burlington, ON, CA). All the solutions used for BPA extraction were HPLC grade and were purchased from Sigma–Aldrich.

2.2. Experimental fish

Experiments were conducted at the Alma Aquaculture Research Station (AARS), Alma, Ontario, Canada. The protocol was approved by the Animal Care and Use Committees at the University of Guelph and the University of Waterloo. Gametes were pooled from reproductive age rainbow trout (3+ year class brood stock) that were born and raised at the AARS. Oocytes from four female and milt from four male rainbow trout were pooled and used for all treatments. Ovarian fluid was collected from all four females (a total of ~200 ml), and it was stored at 6–8 °C prior to the beginning of the experiment.

2.3. Bisphenol a treatments and sampling protocol

The pooled oocytes were distributed among four treatment groups (12 ml of oocytes per treatment for a total of 875 oocytes/treatment). The oocytes were immersed in 50 ml of ovarian fluid containing either vehicle alone (<0.01% ethanol; control group) or BPA dissolved in vehicle at concentrations of 0.3, 3 or 30 μg ml^{-1} for 3 h at 6–8 °C as described previously (Aluru et al., 2010). At the end of the treatment period, the oocytes were fertilized by the addition of 1–2 ml of milt and water (to activate the sperm). The fertilized eggs were rinsed with clean fresh water to remove any trace of BPA and were incubated in a Heath chamber incubator receiving AARS water (8 °C) at a rate of 10 l min^{-1}. Samples were collected at time 0 (zygotes, immediately after fertilization), 7, 14 (organogenesis), 28 (eyed egg stage), 42 (hatching), 65 (just prior to first feed), 85 and 112 days post-fertilization (dpf).

Larvae were maintained in the incubator for a week after first hatch (until 49 dpf), after which they were moved to holding tanks (3 × 200 l tanks per treatment) receiving flow-through water at a rate of 10 l min^{-1}, under a 12hL: 12hD photoperiod. After 65 dpf, larvae were fed to satiation daily (1.5–2% body weight), using mechanical feeders, at a rate proportional to their predicted growth rate that was calculated by AARS personnel on a weekly basis (Aluru et al., 2010). Food was only withheld for 48 h prior to sampling at the post-feeding life stages (85 and 112 dpf). Mortality in control embryos from fertilization to just prior to first feed was 1.6%, while in the 0.3, 3 or 30 μg ml^{-1} BPA groups it was 3.9, 2.2 and 4.8%, respectively.

Sampling protocol involved anesthetizing the embryos and larvae in a lethal dose of tricaine methanosulfonate (MS222; 1.0 g l^{-1}) buffered with sodium bicarbonate (2.0 g l^{-1}). A minimum sample size of six (pool of 5–10 embryos per sample prior to hatch) was collected at each time point during development. All analysis prior to the first feed (42 and 65 dpf) was carried out on whole body after the removal of yolk. At all sampling points post-hatch the length and weight measurements were taken and the samples were stored at −80 °C until further analysis. Condition factor ($CF = (10^5 \times W)/(L^3)$), specific growth rate ($SGR = \mathrm{Ln}[W_2/W_1]/(t_2 - t_1) \times 100\%$) and food conversion ratio [$FCR = W_f/(W_2 - W_1)$] were determined for all groups; W and L are the weight and length, respectively, of individual fish sampled at 85 and 112 dpf; W_1 and W_2 represent the total weight in g of the fish in the tank at the beginning (t_1) and at the end (t_2) of the experiment, respectively, and W_f is the total weight of food consumed over the experimental period.

2.4. Analytical techniques

2.4.1. BPA extraction and quantification

BPA extraction from the embryos and larvae was carried out as described previously (Aluru et al., 2010) with minor modifications. Briefly, fertilized eggs (pool of 5–10 frozen eggs and embryos for 0–28 dpf; $n = 3$) and hatchlings (pool of 4–6 individuals at 42 dpf; $n = 3$) were pulverized on dry ice using a mortar and a pestle, after which they were dissolved in 20 ml of a dichloromethane:methanol (2:1) solution, to which an internal standard of deuterated BPA (BPA-d_{16}) was added. The samples were vortexed for 30 s. A KCl solution (0.9%) was added to the mixture (20% of total volume), and the mixture was vortexed again for 30 s. The homogenate was centrifuged for 10 min at 1000 × *g*, at room temperature. The dichloromethane (organic) phase was removed with a glass pipette and evaporated to dryness under nitrogen gas, on a shaker, at room temperature. The samples were re-dissolved in 1.0 ml methanol:hexane (1:20) by vortexing them for 30 s and sonicat-