Ecotoxicology and Environmental Safety 147 (2018) 423–429

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Bisphenol A regulates rare minnow testicular vitellogenin expression via reducing its promoter Er recruitment

Yingying Zhang[a,b], Yongjing Guan[a], Xiaoteng Fan[a], Lang Wu[a], Zaizhao Wang[a,*]

[a] College of Animal Science and Technology, Northwest A & F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi 712100, China
[b] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China

ARTICLE INFO

Keywords:
Vitellogenin
Testis
Rare minnow
Bisphenol A
Estrogen receptors

ABSTRACT

Vitellogenins (Vtgs) are major precursor of the egg-yolk proteins. They are synthesized in liver of adult female ovipara, but normally silent in males. For their sensitive response to estrogen, Vtgs are usually used as biomarkers for environmental estrogenic compounds. In the present study, three *vtg* subtypes (*vtg1, vtg2* and *vtg3*) were proved to present in the testis of rare minnow *Gobiocypris rarus* for the first time. Immunohistochemistry result showed that Vtg proteins mainly deposit in spermatogonium and spermatocytes. Following 225 μg/L bisphenol A (BPA) exposure 1, 3 and 9 weeks, testicular *vtg* mRNAs were mostly significantly decreased. The further chromatin immunoprecipitation showed that BPA could decrease estrogen receptor (Er) recruitment in *vtg* promoter, which possibly reduced Er's transcription activation effect on *vtgs*. However, different from the continuously decreased *vtg* mRNA levels, testicular Vtg protein levels were recovered at week 9. Considering the induced hepatic Vtg expression, testicular Vtgs may be replenished by the induced hepatic Vtgs under BPA exposure.

1. Introduction

Vitellogenins (Vtgs) are major precursor of the egg-yolk proteins, which provide energy for embryonic development in adult female ovipara (Arukwe and Goksøyr, 2003). In mature females, Vtgs are generally synthesized in liver and then cleaved and stored in developing oocytes (Arukwe and Goksøyr, 2003). In males and immature females, *vtgs* are normally silence. However, the transcription of hepatic *vtg* genes could be activated in response to exogenous estrogen. The increased *vtg* mRNAs and proteins are useful biomarkers to assess environmental estrogens (Heppell et al., 1995; Sumpter and Jobling, 1995; Denslow et al., 1999; Marin and Matozzo, 2004).

Bisphenol A (BPA) is a well known weak estrogen (Kuiper et al., 1998). It could be detected in most of the rivers and lakes worldwide (Crain et al., 2007). In China, BPA was up to 0.19 μg/L in the surface seawaters, 3.92 μg/L in rivers/lakes/ ponds, and 370 μg/L in waste waters (Huang et al., 2012). Our previous studies showed that BPA at these environmental relevant concentrations significantly induced the hepatic *vtg* mRNA and protein levels in female rare minnow *Gobiocypris rarus* (Zhang et al., 2014, 2015). Similar results were reported in other ovipara, such as chicken and carp (Ma et al., 2015; Virk et al., 2014). *vtgs* were regarded as hepatic specific genes, and few studies focused on extra-hepatic *vtgs*. Unexpectedly, in our RNA-seq data, three *vtg* subtypes were found in rare minnow testis. Moreover, mRNA expression of *vtgs* was decreased in a concentration dependent manner following 1, 15 and 225 μg/L BPA exposure, which was contrary to hepatic *vtgs* (Zhang et al., 2016). But the distribution of testicular Vtgs and the mechanism of its response to BPA were unknown.

In the present study, quantitative real-time PCR (qPCR), western blot and immunohistochemistry (IHC) were performed to investigate the expression and distribution of rare minnow testis Vtg. Furthermore, male rare minnow was exposed to 225 μg/L BPA for 1, 3 and 9 weeks, and chromatin immunoprecipitation (ChIP) was performed to reveal the mechanism of BPA's effect on testicular *vtgs*/Vtg.

2. Materials and methods

2.1. Ethics statement

This study has been carried out in accordance with the regulations on experimental animals of Management methods of Laboratory Animals in Shaanxi Province, China (No. 150, 2011). During the whole experiment, fish were humanly treated. Before sacrificed, all fish were anesthetized using tricaine methane sulphonate (MS-222) (Changzhou josenchem Pharmaceutical Chemical Material, Changzhou, China), and every effort was made to minimize suffering. All experimental

* Correspondence to: College of Animal Science and Technology, Northwest A & F University, 22 Xinong Road, Yangling, Shaanxi 712100, China.
E-mail address: zzwang@nwsuaf.edu.cn (Z. Wang).

http://dx.doi.org/10.1016/j.ecoenv.2017.09.001
Received 23 May 2017; Received in revised form 27 August 2017; Accepted 1 September 2017

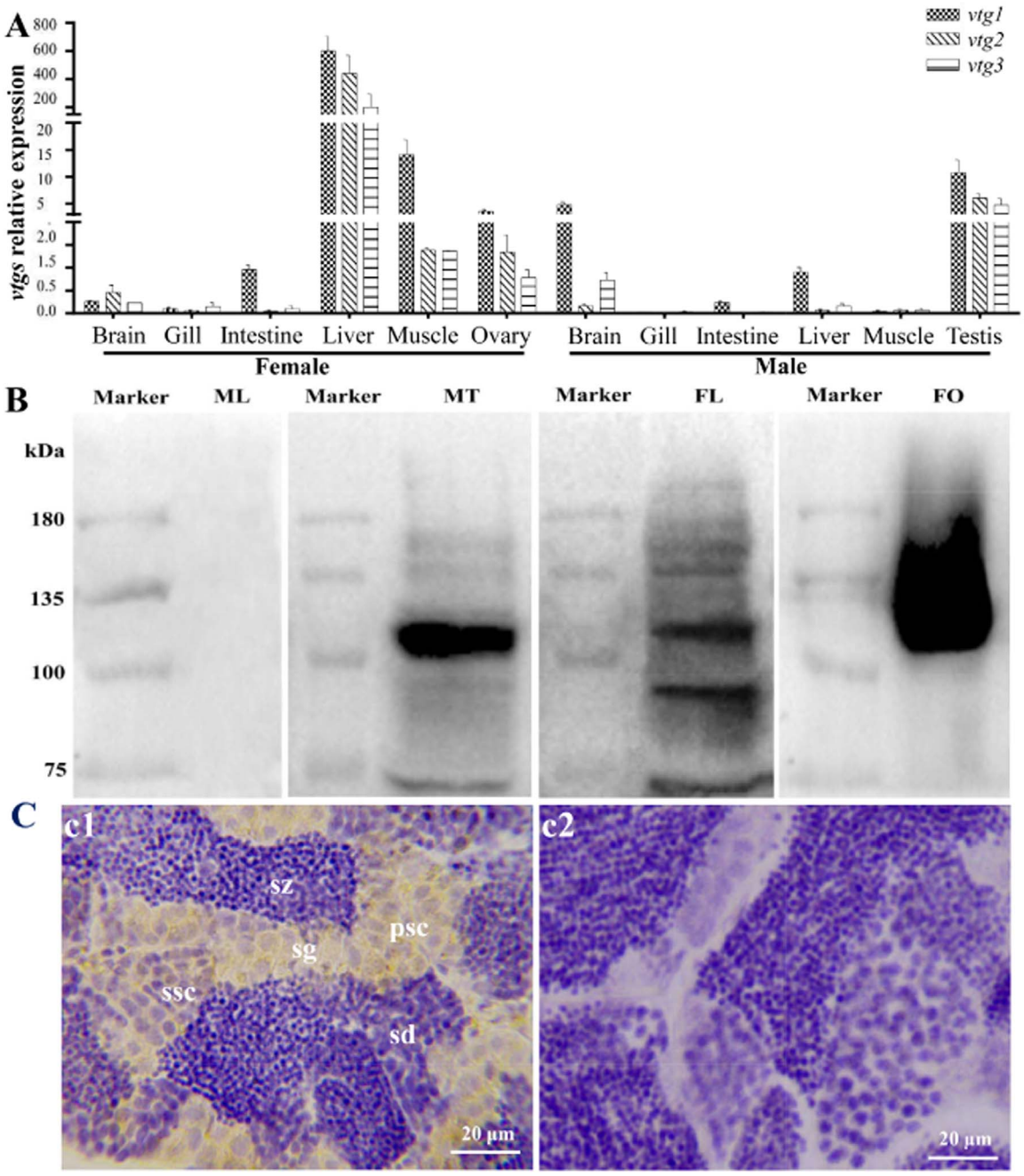

Fig. 1. *vtg* mRNAs and proteins in rare minnow testis. (A) Tissues distribution analysis of *vtg1*, *vtg2* and *vtg3* mRNA in normal male and female rare minnow (n = 8); Data were normalized to the average expression value of the tissues analyzed. All data were expressed as mean ± SEM. (B) Comparation of testicular Vtg protein among other tissues (n = 3). ML: male liver; MT: male testis; FL: female fish liver; FO: female fish ovary. (C) Distribution of Vtg protein in normal rare minnow testis. C1: Vtg immunostaining from testis, the claybank was Vtg positive immunostaining; (c2) Testicular negative control for Vtg immunostaining; sg: spermatogonia; psc: primary spermatocyte; ssc: secondary spermatocyte; sd: spermatid; sz: spermatozoon.

procedures were approved by the Animal Ethics Committee of Northwest A & F University.

2.2. Animals and BPA exposure

Rare minnow was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, Hubei Province, China). They were raised in glass tanks with dechlorinated tap water with a 14 h/10 h light/dark cycle and fed with chironomid larvae twice a day. After 2-weeks acclimation, 5-months old male fish were randomly selected and exposed to 225 μg L^{-1} BPA (Sigma, St. Louis, MO, USA) or the solvent control with 0.001% DMSO (Sigma, St. Louis, MO, USA) in glass tanks for 1, 3 and 9 weeks (in triplicate). There were 81 fish in each replication per treatment, and 27 fish was sacrificed at each time point. 486 fish were used in BPA exposure experiment. Half of the water in each tank was replaced daily with fresh dechlorinated tap water dosed with the appropriate amount of BPA. After each sampling, the exposure water was decreased accordingly to keep the same breeding density (about 1 L water for 1 g fish). The actual concentrations of BPA in water samples was detected by high-performance liquid chromatography (HPLC) once a week according to the previous studies (Mohammad et al., 2009). BPA concentrations were varied between 171.50 ± 8.06 and 212.78 ± 10.61 μg L^{-1} during the exposure duration, and was not detected in the control groups.