Comparative Biochemistry and Physiology, Part C 215 (2019) 25–32

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Joint effects of chronic exposure to environmentally relevant levels of nonylphenol and cadmium on the reproductive functions in male rockfish *Sebastiscus marmoratus*

Ronghui Zheng, Yusheng Zhang, Chao Fang, Mengyun Chen, Fukun Hong, Jun Bo*

Third Institute of Oceanography, State Oceanic Administration, Xiamen, Fujian Province, PR China

A R T I C L E I N F O

Keywords:
Nonylphenol
Cadmium
Aromatase
Testosterone
17β-estradiol
Vitellogenin

A B S T R A C T

Nonylphenol (NP) and Cadmium (Cd) are two common contaminants that can be detected in aquatic environments. Nevertheless, the combined toxicity of NP and Cd at environmentally relevant concentrations in aquatic organisms has not been thoroughly characterized to date. In the present study, the interactions between NP and Cd on male *Sebastiscus marmoratus* were studied. After 21 days of exposure, the brain aromatase activity was observed to be significantly induced by 100 ng/L NP and 40 μg/L Cd, whereas all of the concentrations of co-treatment resulted in an increase in brain aromatase activity. Additionally, NP could also reduce plasma testosterone concentration, while NP, Cd and their mixture could induce plasma 17β-estradiol (E2) concentration and VTG concentration. The interactions between NP and Cd on the reproductive physiology were antagonism. Our results also support the notion of using these indicators as biomarkers for exposure to EDCs and further extend the boundary of biomonitoring to environmental levels.

1. Introduction

Aromatase, a steroidogenic enzyme encoded by the CYP19 gene, is a member of the superfamily of cytochrome P450 enzymes. In contrast, as with most vertebrates, fish have multiple structurally and functionally distinct P450 aromatase (P450arom) isoforms which are derived from separate gene loci (*cyp19a* and *b*), are differentially expressed in brain (P450aromB ≫ P450aromA) and ovary (P450aromA ≫ P450aromB), and have a different constitutive expression (P450aromB ≫ P450aromA) and developmental program (Blázquez et al., 2008; Thomas and Rahman, 2012). Aromatization of testosterone into estrogen locally in the brain has been shown to activate male sexual behaviors in a variety of vertebrate species (Balthazart et al., 2006). In vivo effects are consistent with the presence of estrogen receptor (ER) binding motifs (estrogen responsive elements, ERE) in the *cyp19b* promoter, implying that the gene is the primary target of a subset of endocrine disrupting chemicals (EDCs) (Kishida et al., 2001). However, P450aromA is expressed in the ovary and involved in sexual differentiation and oocyte growth (Kishida et al., 2001). Thus, without prior knowledge of bioavailability, accumulation, metabolism or receptor binding characteristics, P450aromB is a convenient biomarker of the effect of EDCs and provides an entry point for uncovering EDC-mediated perturbations of reproduction and development.

Vitellogenin (VTG) is normally synthesized in response to endogenous estrogen and is the main precursor of vitellins (Vn), which provide the energy reserves for embryonic development (Matozzo et al., 2008). VTG is usually present only in the plasma of female fish. Although male fish also possess the VTG gene but have little circulating estrogen and do not normally produce VTG. However, if exposed to exogenous estrogens, males are capable of synthesizing amounts of VTG equivalent to maturing female organisms (Sumpter and Jobling, 1995). Both laboratory and field studies have shown that abnormal VTG contents in male and juvenile fish, as well as modifications in the sex hormone balance, have been advocated as a reliable and complementary biomarker of the effect of EDCs (Komesli et al., 2015; Sanchez and Porcher, 2009).

Xenoestrogens and metalloestrogens are included in EDCs. Xenoestrogens, such as NP, bisphenol A, and the polychlorinated biphenyls (PCBs) are synthetic chemicals that activate the ER. In contrast to xenoestrogens, metalloestrogens are small ionic metals and metalloids that also activate the ER in the absence of estradiol (Byrne et al., 2013). The metalloestrogens fall into two separate subclasses, oxyanions that include arsenite, antimony, nitrite, selenite, and vanadate and bivalent cations that include Cd, calcium (Ca), cobalt (Co),

* Corresponding author at: Department of Marine Biology and Ecology, Third Institute of Oceanography, State Oceanic Administration, No. 178, Daxue Road, Xiamen, Fujian Province, PR China.

E-mail address: bojun@tio.org.cn (J. Bo).

https://doi.org/10.1016/j.cbpc.2018.09.006
Received 8 September 2018; Accepted 12 September 2018
Available online 11 October 2018
1532-0456/

copper (Cu), nickel (Ni), chromium (Cr), lead (Pb), mercury (Hg), and tin (Sn) (Divekar et al., 2011; Martin et al., 2003; Veselik et al., 2008).

As the representatives of xenoestrogens and metalloestrogens, NP and Cd are two widespread representative contaminants. Over the past decade, NP and Cd were frequently detected in aquatic environments, such as rivers, lakes, and marine environments (Islam et al., 2015; Xu et al., 2016; Wu et al., 2017). NP is a raw material and biodegradation product of nonylphenol polyethoxylates (NPEs) (Rice et al., 2003). NP is widely used in industrial, agricultural, and household applications, such as detergents, emulsifiers, dispersing and antistatic agents, and solubilizers (Langford and Lester, 2002). Accordingly, considerable amounts of NP have been detected in major estuaries and coastal waters around the world, ranging from undetected to 2.6 μg/L (Blackburn et al., 1999; Kim et al., 2005; Li et al., 2007; Liu et al., 2009; Shen et al., 2005; Xu et al., 2015; Xu et al., 2016). Cd is widely used in industry principally in galvanizing and electroplating, nickel–cadmium batteries, electrical conductors, the manufacture of alloys, synthetic pigments, plastics, the stabilization of phosphate fertilizers, and the melting of metals (Byrne et al., 2009; Caride et al., 2010). Thus, a rapid increase in the level of cadmium in aquatic ecosystems has aroused wide public concerns in recent years (Wu et al., 2017). In field studies, during 1996 to 2005, dissolved Cd in the seawaters of the Bohai Bay, China were investigated. The maximum concentration recorded was 0.89 μg/L (Dai et al., 2009). The dissolved Cd values in many coastal waters around the world varied from 1.5 ng/L to 50 μg/L in recent years (Achary et al., 2017; Bat et al., 2012; Jitar et al., 2015; Juma and Al-Madany, 2008; Yigiterhan et al., 2011).

The potential endocrine disrupting effects of NP and Cd on aquatic organisms have raised great concerns (Liu et al., 2016; Nair et al., 2013). Recently, estrogenic effects of NP have been well documented by numerous in vitro and in vivo studies (Bandiera, 2006; Das and Mukherjee, 2013; Tollefsen et al., 2002). NP has been shown to have estrogenic potential in vertebrates, such as fish, birds, and mammals, in which NP displace E2 from its receptor (White et al., 1994). Cd is the best studied metalloestrogen. Most of the published studies address the ability of Cd and the bivalent cationic metals to activate the genomic and nongenomic pathways of estrogen receptor alpha (ERα) and show that, similar to estradiol, they increases the transcription and expression of estrogen regulated genes such as the progesterone receptor (PR) (Brama et al., 2007; Martin et al., 2003; Kluxen et al., 2012), activates ERα in transfection assays (Martin et al., 2003; Martínez-Campa et al., 2006), and increases signaling through the ERK1/2 and Akt pathways (Brama et al., 2007; Zang et al., 2009). Previous studies also demonstrate that the endocrine disrupting effects of NP and Cd depends on the species, age and hormonal status of the animals, the dose and route of exposure, and the target tissue. (Foran et al., 2002; Kortner et al., 2009). Additionally, there is limited data on the potential role of low, environmentally relevant concentrations exposure of NP or Cd in fish in relation to endocrine disruption (Gerbron et al., 2015; Naderi et al., 2015; Watanabe et al., 2017). Recently, the increasing attention has been drawn on the interaction between endocrine disruptor and heavy metals (Lu et al., 2015; Wang et al., 2018). However, research on the combined toxicity of NP and Cd on the reproductive physiology of marine fish is rare. In addition, it should be clarified whether the reproductive physiology influenced by the pollutants was closely linked to environmental levels.

This rockfish *Sebastiscus marmoratus* is indigenous to the nearshore waters of the West Pacific Ocean, including the East China Sea and South China Sea, as well as waters from southern Japan to eastern Korea (Shen, 1993), and the rockfish is a good sentinel organism to monitor marine pollutants (Bo et al., 2017). In the present study, an assessment of the influence of NP, Cd and their mixture on the reproductive endocrinology of the male *S. marmoratus* was performed, and the significance of the observations reported in this study may be further highlighted by the fact that the NP and Cd levels that were used range within environmentally relevant concentrations.

2. Material and methods

2.1. Fish

Adult male *S. marmoratus* were obtained from Dongshan fish hatchery, Fujian Province, China. All animal procedures were conducted in accordance with the animal care and use guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals approved by Decree No. 2 of the State Science and Technology Commission on November 14, 1988). The mean weight and length of fish were 27.56 ± 0.51 g and 11.53 ± 0.05 cm (mean ± S.E), respectively. The fish were acclimatized in a 3-ton cement tank containing sand-filtrated seawater in the aquarium with a temperature of 20 ± 1 °C, salinity of 28 ± 1‰, pH 8.0 ± 0.1 and a natural daylight cycle, and fed with commercial fish bait at 1% of body weight daily for 2 weeks prior to the laboratory exposure.

2.2. Chemicals and materials

Nonylphenol (CAS # 84852-15-3), cadmium chloride (CAS # 10108-64-2), Ethyl 3-aminobenzoate methanesulfonate (MS-222, CAS # 886-86-2) and the protease inhibitor aprotinin (3–7 TIU/ml, from bovine lung; CAS # 9087-70-1) were purchased from Sigma-Aldrich, USA. For microsome preparation, Trizma® base (CAS # 77-86-1) and sucrose (CAS # 57-50-1) were purchased from Sigma-Aldrich, USA. For aromatase assays, 4-(2-hydroxyethyl)-1- piperazineethane sulfonic acid (HEPES, CAS # 7365-45-9), activated charcoal (CAS # 7740-44-0), reduced nicotinamide adenine dinucleotide phosphate (NADPH, CAS # 2646-71-1) were obtained from Sigma-Aldrich, USA. Ultima Gold scintillation fluid (catalog # 6013326), 1β-^{3}H(N)-Androst-4-ene-3,17-dione (catalog # NET 926) with a specific activity of 24.0 Ci/mmol and 7 mL pico glass vials were procured from PerkinElmer, USA. For plasma hormone analyses, estradiol ELISA kit (catalog # 582251) and testosterone ELISA kit (catalog # 582701) were purchased from Cayman Chemical, USA. For VTG analysis, mouse monoclonal antibody to vitellogenin [BN-5] (product # ab36797) was purchased from Abcam, UK, mouse monoclonal antibody to β-Actin [7G6] (catalog # AT0001) and Goat Anti- Mouse IgG (H + L)-HRP (catalog # AT0098) were purchased from CMCTAG, USA. All other chemicals were of analytical grade and were obtained from Sinopharm Chemical Reagent Company, China.

2.3. Treatments

Glass tanks (75 cm × 40 cm × 35 cm) were used and kept in the aquarium at 20 °C. Each tank contained 60 l of sand-filtrated seawater with the given concentration of xenobiotics alone or in mixture.

There were three treatments (NP alone, Cd alone, or NP and Cd) and two controls (blank control and solvent control) in the experiment. The experimental concentrations were chosen with reference to the ambient concentrations in seawater (Achary et al., 2017; Bat et al., 2012; Blackburn et al., 1999; Dai et al., 2009; Jitar et al., 2015; Juma and Al-Madany, 2008; Kim et al., 2005; Li et al., 2007; Liu et al., 2009; Shen et al., 2005;Xu et al., 2015; Xu et al., 2016; Yigiterhan et al., 2011). In the NP treatment, the fish were exposed to 10, 100 and 1000 ng/L NP, respectively, with a solvent control (0.003% ethanol). In the Cd treatment, the fish were exposed to 0.4, 4 and 40 μg/L as Cd^{2+}, respectively, and a blank control (healthy control) was maintained in the same volume of sea water. In the NP and Cd co-exposure treatment, the fish were exposed to the above doses in combination, respectively, with a blank control and a solvent control. In each treatment, 8 adult male fish were kept in a tank and aerated using an electric pump. All treatments had triplicates.

During the exposure experiments, the water containing different concentrations of xenobiotics was renewed every other day. The fish