General and Comparative Endocrinology 168 (2010) 181–189

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Amphibian metamorphosis as a model for studying endocrine disruption on vertebrate development: Effect of bisphenol A on thyroid hormone action

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ARTICLE INFO

Article history:
Received 13 November 2009
Revised 17 February 2010
Accepted 18 February 2010
Available online 21 February 2010

Keywords:
Thyroid hormone receptor
Bisphenol A
Endocrine disrupting compound
Xenopus laevis
Metamorphosis

ABSTRACT

Thyroid hormone (TH) is essential for proper development in vertebrates. TH deficiency during gestation and early postnatal development produces severe neurological, skeletal, metabolism and growth abnormalities. It is therefore important to consider environmental chemicals that may interfere with TH signaling. Exposure to environmental contaminants that disrupt TH action may underlie the increasing incidence of human developmental disorders worldwide. One contaminant of concern is the xenoestrogen bisphenol A (BPA), a chemical widely used to manufacture polycarbonate plastics and epoxy resins. The difficulty in studying uterus-enclosed mammalian embryos has hampered the analysis on the direct effects of BPA during vertebrate development. As TH action at the cellular level is highly conserved across vertebrate species, amphibian metamorphosis serves as an important TH-dependent *in vivo* vertebrate model for studying potential contributions of BPA toward human developmental disorders. Using *Xenopus laevis* as a model, we and others have demonstrated the inhibitory effects of BPA exposure on metamorphosis. Genome-wide gene expression analysis revealed that surprisingly, BPA primarily targets the TH-signaling pathway essential for metamorphosis in *Xenopus laevis*. Given the importance of the genomic effects of TH during metamorphosis and the conservation in its regulation in higher vertebrates, these observations suggest that the effect of BPA in human embryogenesis is through the inhibition of the TH pathway and warrants further investigation. Our findings further argue for the critical need to use *in vivo* animal models coupled with systematic molecular analysis to determine the developmental effects of endocrine disrupting compounds.

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1. Introduction

The high incidence of multiple cancers, diabetes, heart disease, obesity and reproductive and developmental problems in humans worldwide have led to speculations that environmental change and chronic exposure to excess environmental chemical contaminants is negatively affecting human populations. Exposure to such exogenous hormonally active substances may also have epigenetic effects (e.g., via histone modification) that can influence human diseases across subsequent generations (Wade and Archer, 2006). Therefore, endocrine disruptors represent a significant area of environmental research with important implications for human health, particularly the health of children.

The steroid nuclear receptor systems have by far attracted the most attention with reports that environmental chemicals (referred to as endocrine disrupting compounds; EDCs) act either as estrogenic and androgenic or anti-estrogenic and anti-androgenic agents and subsequently affect the regulation and function of the reproductive system and sexual development (Maffini et al., 2006). Specifically, EDCs have been suggested to be responsible for the increased appearance of reproductive human health problems including breast and testicular cancers and sterility (Jensen et al., 1995; Skakkebaek et al., 1998; Wolff and Weston, 1997). In wildlife, decreased species populations and increased animal malformations, including feminization and hermaphroditism, have been reported globally (Hayes et al., 2006; Houlahan et al., 2000; Ouellet et al., 1997). Emerging evidence now shows that EDCs can also alter the function of other members of the nuclear receptor super family, as disorders associated with non-reproductive tissues such as the brain are also on the rise (Zoeller, 2007). Thyroid hormone (TH) is essential for vertebrate development, including brain maturation (Atkinson, 1994; Denver, 1996; Lazar, 1993;

Abbreviations: TH, thyroid hormone; TR, thyroid hormone receptor; BPA, bisphenol A; EDC, endocrine disrupting compound.

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0016-6480/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.ygcen.2010.02.016

Shi, 1999; Yen, 2001), hence it is important to consider the impact of environmental chemicals on the actions of the TH nuclear receptor system during development. Our intent here is to highlight the importance of combining morphological and molecular studies in exploring the impact of environmental chemicals that interfere with TH signaling by focusing on our recent studies on the EDC bisphenol A (BPA).

2. Thyroid hormone and development

The regulation of the TH system is very complex and involves both central and peripheral regulation via a hormonal feedback system (Denver, 1996; Lazar, 1993; Shi, 1999; Yen, 2001). The thyroid gland synthesizes and secretes the prohormone, thyroxin (T4), as well as the more active form of the hormone, 3,5,3′-triiodothyronine (T3). Both forms are generally referred to here as thyroid hormone. TH action at the cellular level is highly conserved across vertebrate species and is primarily mediated by mechanisms involving TH binding to specific nuclear TH receptors (TRs) that regulate gene expression during development. TRα and TRβ are the two TR isoforms that possess dual functions as transcription repressors and activators in the absence and presence of TH, respectively (Fig. 1A) (Buchholz et al., 2006; Glass and Rosenfeld, 2000; Ranjan et al., 1994; Wong and Shi, 1995; Yen, 2001). Many different TR coactivators, whose actions are mediated in part through covalent modification of histone proteins, have been identified to modify the chromatin structure, and their roles in regulating cellular processes such as cell transformation, differentiation, and apoptosis have been extensively studied (Burke and Baniahmad, 2000; Chen and Archer, 2005; Chen and Li, 1998; Ito and Roeder, 2001; Jepsen and Rosenfeld, 2002; Jones and Shi, 2003; Lazar, 1993; Mangelsdorf et al., 1995; McKenna and O'Malley, 2001; Rachez and Freedman, 2000, 2001; Shi, 1999; Thompson and Potter, 2000; Tsai and O'Malley, 1994; Yen, 2001; Zhang and Lazar, 2000).

Disruption of TH-signaling pathways have profound effects on development, growth and metabolism and are therefore also a disease risk to all vertebrates (Yen, 2001). In humans, inadequate levels of TH during development, both *in utero* and in the early *postpartum* period, lead to a complex of deficits termed cretinism, a condition where the brain and skeleton fail to develop properly, resulting in severely stunted physical and mental growth (DeLange, 2005, 1996; Haddow et al., 1999; Hetzel, 1989). Functional deficits include mental retardation, ataxia, spasticity and deafness (DeLange, 1996; Román, 2007; Sadamatsu et al., 2006). The influence of environmental factors, such as dietary iodine deficiency during pregnancy, is well recognized as the common cause of insufficient TH supply for fetus development (DeLange, 2005; DeLong et al., 1985). An important implication of these studies is that environmental chemicals that produce TH insufficiency or interfere with TH signaling during development may alter important developmental events. Recent studies show that human neurological diseases such as autism and attention deficit disorders (ADD) have increased within the past two decades and there is now a growing concern that the prevalence of such neural disorders may be related to human exposure to persistent environmental anti-thyroid agents that interfere with TH regulation of development (Román, 2007; Sadamatsu et al., 2006). These studies and others highlight the significance of TH regulation, and therefore any interference in TH levels or signaling by EDCs may cause developmental disorders (Boas et al., 2006; Colborn, 2002; Crump et al., 2002; Howde-

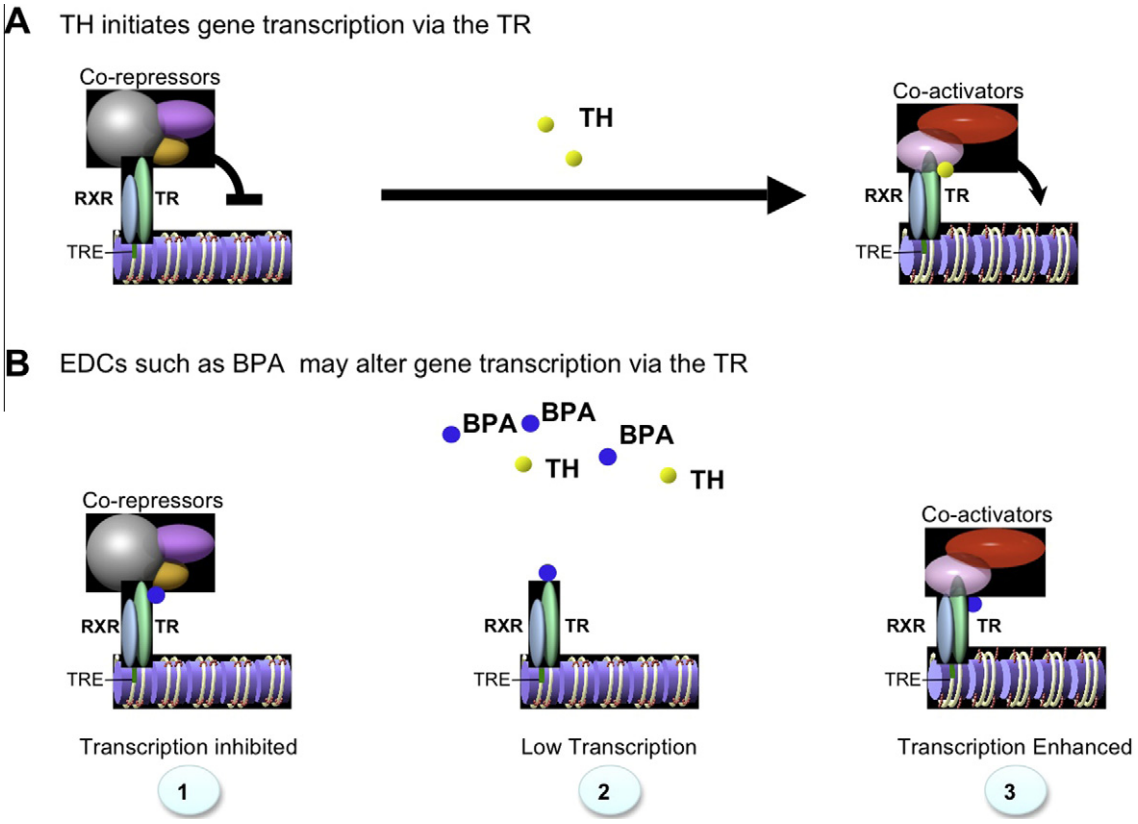

Fig. 1. Mechanisms of transcriptional regulation by TR (A) and potential modes of BPA (B) interference on TH action. Molecular studies have revealed that TR heterodimers with RXR (9-cis retinoic acid receptors) and binds to thyroid response elements (TREs, green line) in the promoters or enhancers of TH-regulated genes. In the absence of TH, TR binds to corepressor complexes and puts chromatin in a "closed" state for transcription. In the presence of TH, a conformational change of TR allows coactivator complexes to bind, which in turn initiates transcription. BPA may disrupt TR transcription simply by (1) binding to TR and directly inhibiting the action of endogenous TH. Transcription may be inhibited by the recruitment of nuclear corepressors. (2) BPA may bind to TR and lower transcription of target TH-response genes by inhibiting coactivator binding. (3) BPA may bind to TR and directly activate TH-response genes via the recruitment of nuclear coactivators.