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Low concentrations of bisphenol a suppress thyroid hormone receptor transcription through a nongenomic mechanism

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ABSTRACT

Bisphenol (BPA) is one of the highest-volume chemicals produced worldwide, and human exposure to BPA is thought to be ubiquitous. Various rodent and *in vitro* studies have shown that thyroid hormone (TH) function can be impaired by BPA. However, it is still unknown if low concentrations of BPA can suppress the thyroid hormone receptor (TR) transcription. The present study aims to investigate the possible suppressing effects of low concentrations of BPA on TR transcription and the involved mechanism(s) in CV-1 cells derived from cercopithecus aethiops monkey kidneys. Using gene reporter assays, BPA at concentrations as low as 10^{-9} M suppresses TR or steroid receptor coactivator-1(SRC-1)-enhanced TR transcription, but not reducing TR/SRC-1 interaction in mammalian two-hybrid and glutathione S-transferase pull-down studies. It has been further shown that both nuclear receptor co-repressor (N-CoR) and silencing mediator for retinoid and thyroid hormone receptors (SMRT) are recruited to the TR-β1 by BPA in the presence of physiologic concentrations of T3 or T4. However, the overexpression of β3 integrin or c-Src significantly reduces BPA-induced recruitment of N-CoR/SMRT to TR or suppression of TR transcription. Furthermore, BPA inhibits the T3/T4-mediated interassociation of the β3 integrin/c-Src/MAPK/TR-β1 pathways by the co-immunoprecipitation. These results indicate that low concentrations of BPA suppress the TR transcription by disrupting physiologic concentrations of T3/T4-mediated β3 integrin/c-Src/MAPK/TR-β1 pathways, followed by recruiting N-CoR/SMRT to TR-β1, providing a novel insight regarding the TH disruption effects of low concentration BPA.

Introduction

Thyroid hormone (TH), including thyroxine (T4) and triiodothyronine (T3), is essential for normal brain development and its mild or transient insufficiency produces different cognitive deficits in both humans and animals (Bernal, 2002). Thus, environmental chemicals that can produce TH insufficiency or interfere with TH signaling during development are likely to alter important developmental events (Zoeller et al., 2002). Recent epidemiologic studies have indicated the significant relationships between circulating levels of TH and exposure to environmental chemicals (Blount et al., 2006; Longnecker et al., 2003). Thus, understanding the action of environmental chemicals that can interfere with TH signaling is highly important to human health.

BPA, a component of polycarbonate plastics and epoxy resins, is one of the highest-volume chemicals produced worldwide. Hydrolysis of BPA under heat, acidic or basic conditions leads to the release of BPA to the environment and the potential exposure to human beings. Available data from biomonitoring studies clearly indicate that the general population is exposed to BPA and is at risk from internal exposure to unconjugated BPA (Vandenberg et al., 2010). Notably, the relatively high levels of BPA in the serum of pregnant women, umbilical cord blood, and fetal plasma indicate that BPA can cross the maternal–fetal placental barrier (Vandenberg et al., 2007).

BPA is known to have mixed estrogen agonist/antagonist properties. In addition to its estrogenic activity, there is some evidence that BPA binds to TR, acting as a TH antagonist by preventing the binding of T3 (Moriyama et al., 2002). However, other studies have been unable to duplicate these results, finding that BPA does not competitively inhibit the binding of labeled T3 to the TR or induce TH-dependent production of growth hormone (GH) in GH3 cells (Kitamura et al., 2002, 2005a). *In vivo* studies examining the effects of low dose BPA on TH signaling have suggested that perinatally exposed rats have elevated T4 levels on postnatal day (PND) 15 and up-regulation of a TH responsive gene in the brain (Zoeller et al., 2005). Furthermore, perinatal exposure to low dose BPA in rats has been implicated in the abnormal brain development, characterized by hyperactivity and impaired cognition (Carr et al., 2003; Kubo et al., 2001). It was also observed in medaka fish that the acceleration in embryonic development

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and time to hatch induced by BPA are blocked by a TR antagonist, suggesting that BPA is acting through a TH pathway (Ramakrishnan and Wayne, 2008). However, the mechanisms associated with endocrine disrupting effects of low concentration BPA on the TH system are currently ill-defined and warrant further detailed investigation.

The present study will explore the molecular mechanism of the action of low concentration BPA (10^{-9}–10^{-7} M) on TR transcription in the presence of physiologic concentrations of T3/T4 in CV-1 cells.

Materials and methods

Materials. All chemicals were of reagent grade or better and were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Stock solutions of TH were prepared in 0.04 N KOH, 4% propylene glycol. Although their quality was well guaranteed by manufacturers, it was further confirmed by a chemical analysis for the key regents or solutions, such as BPA before the experiments.

Plasmids. Expression vectors of TR-β1, retinoid X receptor (RXR) β, human SRC-1, Gal4-TRβ1-LBD, GST-SRC-1-NBD-1, a 5× upstream activating sequence (UAS)-TK-LUC in the PT109 vector (aa 595–780) and glucocorticoid receptor (GR), mouse mammary tumor virus (MMTV) promoter containing a 2× glucocorticoid response element (GRE) fused to the luciferase promoter (MMTV-LUC) have been constructed in our lab according to the methods described previously (Takeshita et al., 1998; Iwasaki et al., 2001). The luciferase (LUC) reporter constructs, the chick lysozyme(F2)-TH response element (F2-TRE)-thymidine kinase (TK)-LUC, artificial direct repeat TRE, DR4-TK-LUC (DR4-TRE), 2× palindrome (pal)-TK-LUC (Pal-TRE) in the PT109 vector were performed in our lab based on the methods described previously (Koibuchi et al., 1999). An expression vector of Gal4-DNA-binding domain (DBD)-fused SRC-1-nuclear receptor-binding domain (NBD)-1(aa 595–780), the Gal4-blank, Gal4-N-CoR (aa 1579–2454) and Gal4-SMRT (aa 1669-2507) was constructed according to the methods described previously (Takeshita et al., 2002). The VP16-TR-β1, the glutathione S-transferase (GST)-fused TR-β1, chimeric receptors were generated from TR and GR, human β3 and c-Src cDNA (pcDNA3) were a generous gift from Dr. Yuan Tang (College of Pharmacy, Third Military Medical University, P.R. China).

Cell treatments. Both CV-1 cells lacking TR and TR-replete 293T cells were a generous gift from Dr. Yuan Tang (College of Pharmacy, Third Military Medical University, P.R. China) maintained in phenol red-free DMEM, which was supplemented with 10% charcoal-stripped fetal bovine serum (FBS) depleted of TH by the method of Samuels et al. (1979), 1% glutamine, and 1% penicillin/streptomycin. Almost confluent cells were placed for 2 days in medium containing serum and then in serum-free medium for 2 h. Cells were then

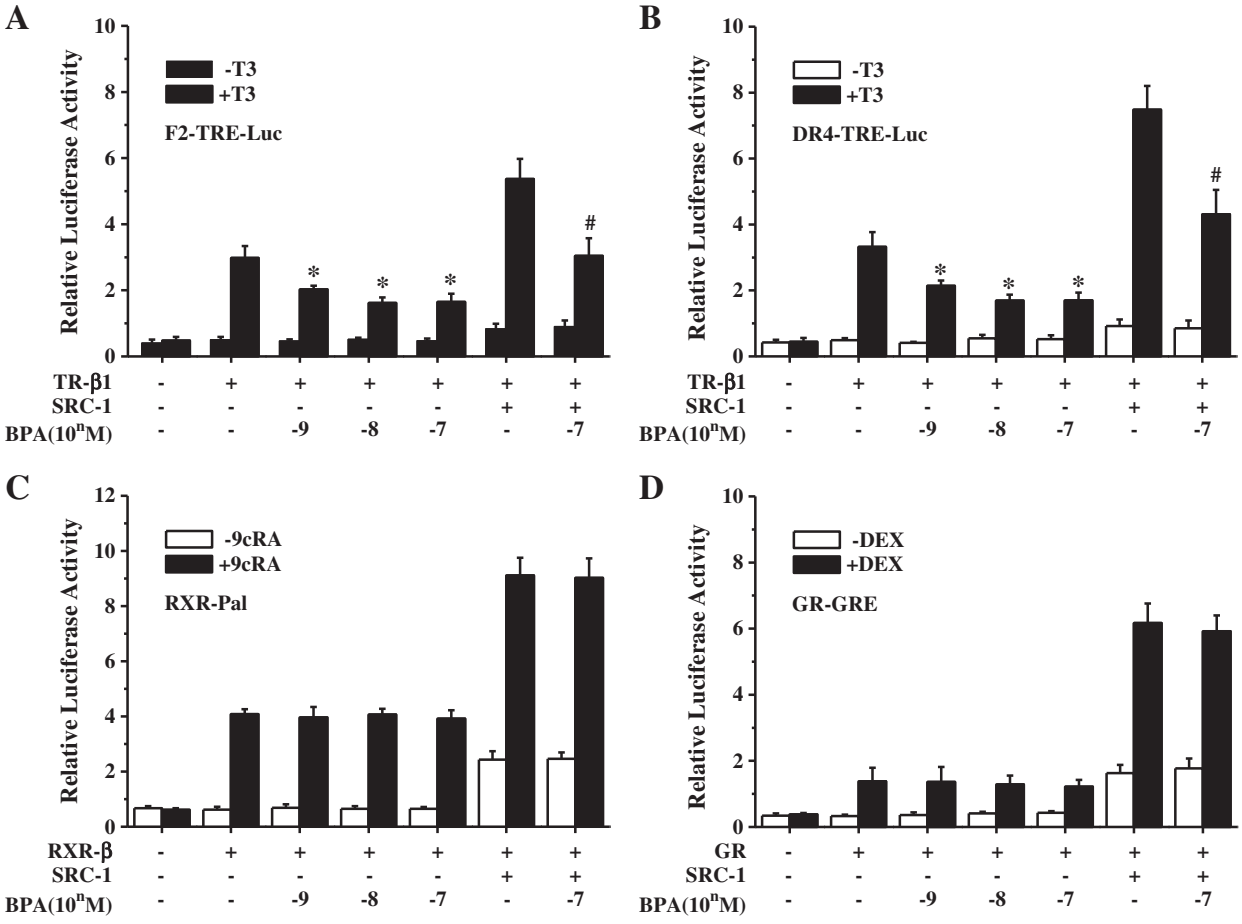

Fig. 1. Low concentrations of BPA suppress TR transcription. Expression plasmids encoding TR-β1 (10 ng) were cotransfected with either F2-TK-LUC (A) or DR4-TK-LUC reporter plasmid (100 ng) (B) and expression vector of SRC-1 (200 ng) into CV-1 cells. Cells were incubated with or without T3 (10^{-10} M) and indicated amounts of BPA. (C), Expression plastids encoding RXRβ (10 ng) were cotransfected with 2× pal-TK-LUC reporter plasmids (100 ng) and expression vector of SRC-1 (200 ng) into CV-1 cells. Cells were incubated with or without 9-*cis*-retinoic acid (*9cRA*) (10^{-7} M) and indicated amounts of BPA. (D), Expression plasmids encoding GR (10 ng) were cotransfected with glucocorticoid response element (*GRE*)-LUC reporter plasmids (100 ng) and expression vector of SRC-1 (200 ng) into CV-1 cells. Cells were grown in the presence or absence of dexamethasone (*DEX*) (10^{-7} M) and indicated amounts of BPA. Total amounts of DNA for each well were balanced by adding vector pcDNA3. Luciferase activity was normalized to β-galactosidase activity and then calculated as fold luciferase activity. Data represent mean ± S.E.M. of three independent experiments performed in triplicate. *, Statistically significant ($p<0.05$ by ANOVA) *versus* TR-β1 (+), T3 (+), and BPA (−). #, Statistically significant ($p<0.05$ by ANOVA) *versus* TR-β1 (+), SRC-1 (+), T3 (+), and BPA (−).