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Thyroid Hormone Action Is Disrupted by Bisphenol A as an Antagonist

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Bisphenol A (BPA), a monomer of polycarbonate plastics, has been shown to possess estrogenic properties and act as an agonist for the estrogen receptors. Although an epidemiologically based investigation has suggested that some chemicals could disrupt thyroid function in animals, the effects on thyroid hormone receptors (TRs) are unknown. We show here that BPA inhibits TR-mediated transcription by acting as an antagonist. In the transient gene expression experiments, BPA suppressed transcriptional activity that is stimulated by thyroid hormone (T_3) in a dose-dependent manner. The inhibitory effects were observed in the presence of physiological concentrations of T_3. In contrast, in the case of negatively regulated TSHα promoter, BPA activated the gene transcription that is suppressed by T_3. To elucidate possible mechanisms of the antagonistic action of BPA, the effects on T_3 binding and cofactor interaction with TR were examined. The K_i value for BPA was 200 μM when assessed by inhibition of [^{125}I]T_3 binding to rat hepatic nuclear TRs. In a mammalian two-hybrid assay, BPA recruited the nuclear corepressor to the TR. These results suggest that BPA could displace T_3 from the TR and recruit a transcriptional repressor, resulting in gene suppression. This is the first report that BPA can antagonize T_3 action at the transcriptional level. BPA may disrupt the function of various types of nuclear hormone receptors and their cofactors to disturb our internal hormonal environment. (*J Clin Endocrinol Metab* 87: 5185–5190, 2002)

ENDOCRINE DISRUPTERS (ED), compounds that modify natural endocrine function, have emerged as a major public health issue. These effects are due to their potentially disruptive effects on physiological processes, particularly through direct interaction with steroid hormone receptors (1). In view of this situation it is important to determine whether a xenobiotic can mimic, block, or modify the effects of these hormones. One of targets of endocrine disrupters is thought to be nuclear hormone receptors, which bind to steroid hormones and regulate target gene transcription. Nuclear hormone receptors constitute a large superfamily of ligand-inducible transcriptional factors, which include receptors for steroid hormones, thyroid hormones, vitamin D_3, retinoids and prostanoids, and a number of proteins with high sequence homology but as yet unidentified ligands (2). Recent public and scientific interest has been mostly focused on environmental chemicals capable of interacting with the estrogen receptors (ERs). The effects of these compounds on the transcriptional activity of other nuclear hormone receptors have not been extensively studied. It is of great interest, therefore, to determine the effects of ED on these receptors to understand the mechanism of ED disruption of endocrine systems at the molecular level.

Bisphenol A (BPA) is a monomer of plastic materials that are widely used in daily life. BPA is detectable in our environment and is present in drinking water, canned goods, and even milk bottles. Recently, it was shown that BPA contaminates not only human plasma, but also fetal tissues (3). Many reports have shown that BPA has a weak effect to stimulate ERs. High doses of BPA may have reproductive toxicity and affect cellular development in rats and mice (4, 5). *In vitro*, BPA competes with estradiol for binding to ERα and introduces the expression of progesterone receptors and proliferation of MCF-7 human breast cancer cells (6–8). Thus, BPA has been shown to mimic estrogen both *in vivo* and *in vitro* as a xenoestrogen. In contrast, polychlorinated biphenyls (PCBs), a class of industrial compounds, are environmentally persistent and bioaccumulative agents that have been shown to affect a number of endocrine targets (9). PCB-induced disruption of thyroid function is thought to be due to their toxicological effects, which may be related to the structural similarities shared by PCBs and thyroid hormone (10, 11). The influences of BPA on the thyroid system are unknown.

The functions of T_3 are mediated by several isoforms of nuclear TRs, TRα1, and TRβ1–3 encoded by two genes, *TRα* and *TRβ*, respectively (12–14). The *TRα* locus generates TRα1 and several related proteins, TRα2, TRα3, and TR$\Delta\alpha$s, which result from alternative splicing of the TRα primary transcript (15, 16). The *TRβ* locus generates TRβ1, TRβ2, TRβ3, and TR$\Delta\beta$s by using different promoters and alternative splicing. TRβ1, TRβ2, and TRβ3 have an identical ligand binding domain (LBD). The expression of TRβ2 is restricted to some specific organs, including the pituitary and hypothalamus, where it appears to play a key role in the regulation of TSH

Abbreviations: BPA, Bisphenol A; CoR, corepressor protein; DBD, DNA binding domain; ED, endocrine disrupters; ER, estrogen receptor; h, human; LBD, ligand binding domain; Luc, luciferase; ME, malic enzyme; N-CoR, nuclear receptor corepressor; PCB, polychlorinated biphenyl; tk, thymidine kinase; TR, thyroid hormone receptor; TRE, thyroid hormone response element; UAS, upstream activation site.

synthesis and secretion. In contrast, the tissue distributions of TRα1, TRβ1, and TRβ3 are relatively ubiquitous (14, 17, 18), and the expression of these proteins begins early in development (19–23).

Here we report that BPA can disturb thyroid hormone action. BPA reduced T_3 binding to the nuclear TRs and recruited nuclear receptor corepressors (N-CoRs) to the TR, resulting in transcriptional inhibition.

Materials and Methods

T_3 binding studies

Nuclear TRs were prepared from the Sprague Dawley rat liver as previously described (24). A tracer dose of [^{125}I]T_3 (122 MBq/μg; NEX-110X, NEN Life Science Products, Boston, MA) and nuclear TRs in 5 mM dithiothreitol were incubated with BPA (Sigma, St. Louis, MO) at 4 C overnight. Bound and free [^{125}I]T_3 were separated by adding 1 ml 2% Dowex resin (Supelco, Bellefonte, PA) suspension. The nonspecific binding obtained in the presence of an excess of T_3 was subtracted from the total binding.

Plasmid constructions

Expression vectors containing wild-type human TRβ1 [pCMX-human (h) TRβ1] and human TRα1 (pCMX-hTRα1) were provided by K. Umesono (The Salk Institute, San Diego, CA) (25). The plasmid pCMX-rTRβ2 contains rat TRβ2 cDNA (26). The LBD of hTRα1 or hTRβ was fused to the DNA binding domain (DBD) of Gal4 in-frame in pSG424 (27). The Gal4-NCoR (residues 1552–2453) construct contains the TR interaction domains of N-CoR (28). The VP16 construct for hTRβ contains the LBD of the receptor downstream of the VP16 activation domain of herpes simplex virus in-frame in pCMX (29). The plasmids, thyroid hormone response element (TRE)-thymidine kinase (tk)-luciferase (Luc) (30) and malic enzyme (ME)-TK-Luc (31) contain two copies of a palindromic TRE and the ME-TRE, respectively, upstream of the tk promoter (tk109) in the pA3 luciferase vector (30), and the Gal4 reporter plasmid, upstream activation site (UAS)-E1BTATA-Luc, contains five copies of the UAS element upstream of E1BTATA in pA3-Luc (28). The pRL-tk vector (Promega Corp., Madison, WI) comprised of the tk promoter and *Renilla* luciferase cDNA was used as an internal control.

Transient expression assays

TSA 201 cells, a clone of human embryonic kidney 293 cells (32), and human hepatoblastoma cells (HepG2) were grown in phenol red-free DMEM (Nikken, Kyoto, Japan) with 10% charcoal-stripped fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) and were transfected using the calcium precipitation method (26) for TSA201 or lipofectin (Lipofectamine Plus, Invitrogen, Carlsbad, CA) for HepG2, according to the manufacturer's instructions. After exposure to the DNA precipitate for 8 h, phenol red-free DMEM with charcoal-stripped FBS was added in the absence or presence of BPA and/or T_3. Cells were harvested for measurements of Luc activity according to the manufacturer's instructions (Dual-Luciferase Reporter Assay System, Promega Corp.). The transfection efficiencies were corrected with the internal control. Results are expressed as the mean ± SE from at least three transfections, each performed in triplicate. Data were analyzed by *t* test to compare with the control.

Results

BPA is a weak ligand for TR

The chemical structures of BPA and T_3 are shown in Fig. 1, A and B, respectively. There is an unexpected resemblance between them. Two benzene cores are linked by carbon (BPA) or oxygen (T_3). BPA has two hydroxyl groups, and T_3 has a hydroxyl and an alanine group. BPA displaced [^{125}I]T_3 from endogenous TR, which is prepared from the rat liver, with an inhibition constant (K_i) of 200 μM (Fig. 1C). Scatchard

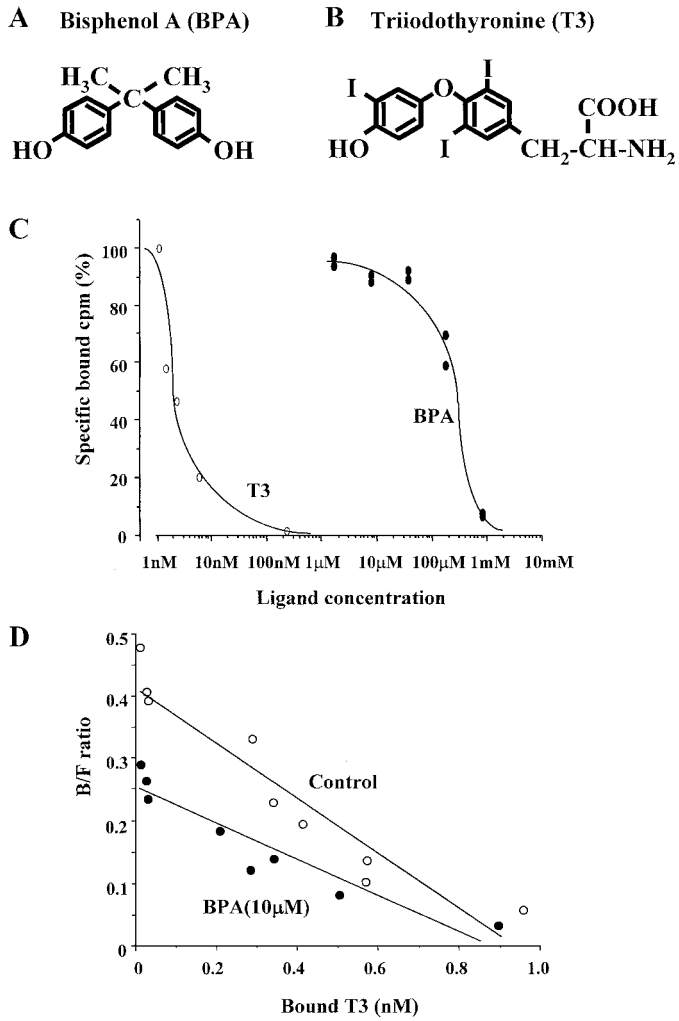

FIG. 1. Binding of BPA to nuclear TR. A and B, Comparison of structures of BPA (A) and T_3 (B). C, Competition binding assay. Rat liver nuclear extract (50 μg protein) was incubated with a tracer dose of [^{125}I]T_3 and increasing amounts of T_3 or BPA. D, Scatchard analysis. Rat liver nuclear extract (50 μg protein) was incubated with various amounts of [^{125}I]T_3 in the presence or absence of 10 μM BPA. B, Bound; F, free.

analysis revealed that BPA decreased the value for the association constant (K_a) from 0.44 to 0.28 $\times$ 10^9 M, whereas little effect was observed on maximum binding capacity (Fig. 1D).

BPA suppressed transcriptional activities mediated by TRα1 and TRβ1

Transient expression experiments were performed using TSA201 cells, which are a derivative of human embryonic kidney 293 cells. The LBD of TRα1 or TRβ was fused to the DBD of the yeast transcription factor, Gal4, and was cotransfected with a Gal4 reporter gene, UAS-E1BTATA-Luc. Although BPA may bind to the TR, BPA did not activate TRs (data not shown). We examined whether BPA antagonizes T_3-induced TR activation. In the presence of 3 nM T_3, dose-dependent inhibition of transcription mediated by Gal4-TRα1 (Fig. 2A) and Gal4-TRβ (Fig. 2B) was observed. BPA

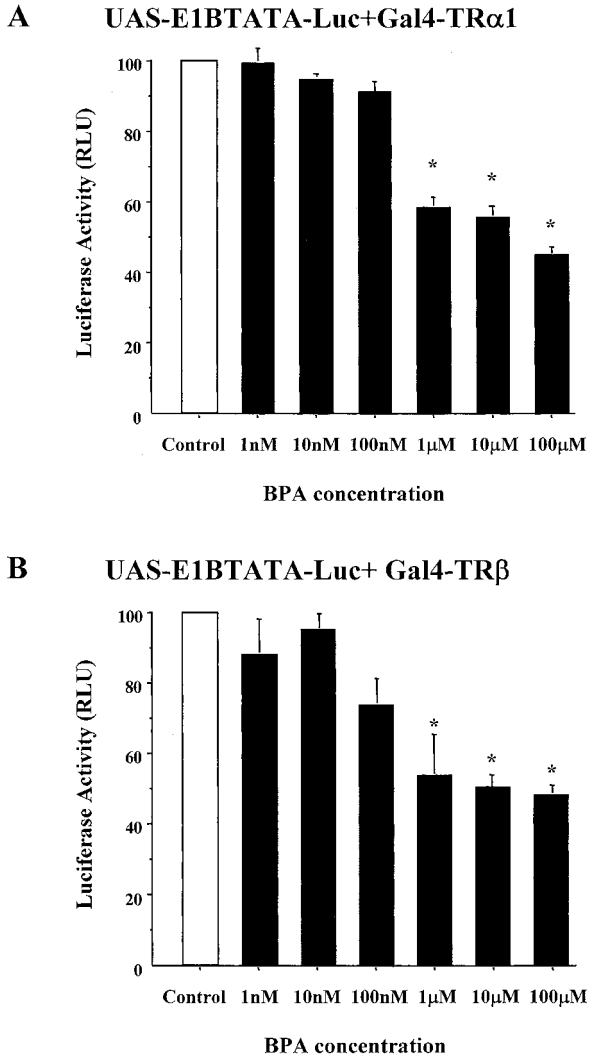

FIG. 2. The inhibitory effects of BPA on the gene transcription mediated by the TR-LBD. Gal4-TRα1 (A) or Gal4-TRβ (B; 50 ng) was cotransfected into TSA-201 cells with 100 ng of the reporter gene, UAS-E1BTATA-Luc, in the presence of 3 nM T_3 and increasing amounts of BPA. RLU, Relative light units. *, $P < 0.01$ *vs.* control.

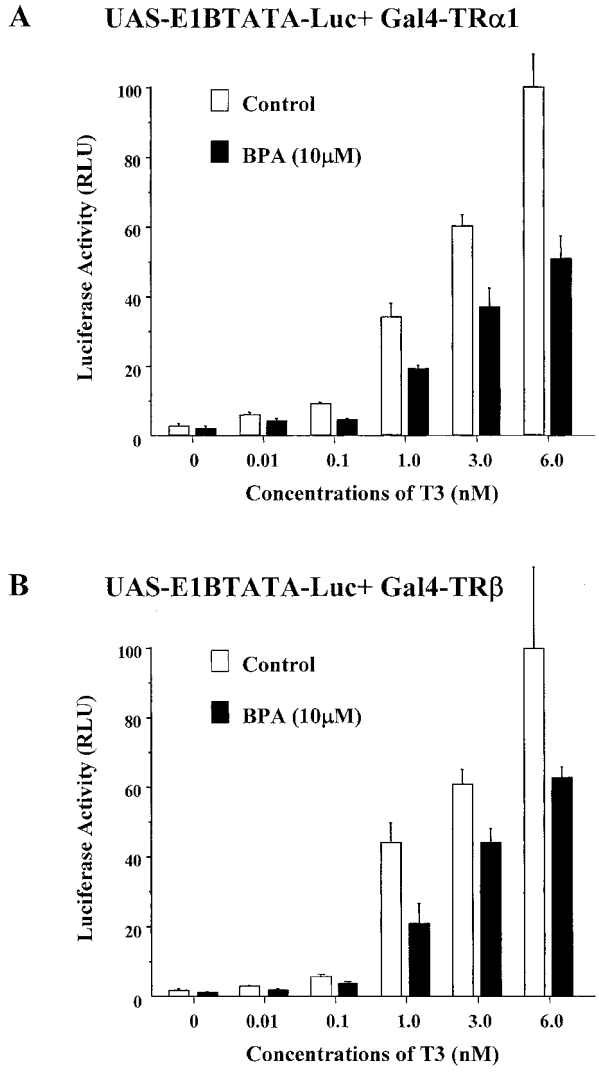

FIG. 3. BPA suppresses TR-mediated transcription in the presence of a physiological range of T_3. Gal4-TRα1 (A) or Gal4-TRβ (B; 50 ng) was cotransfected into TSA-201 cells with 100 ng of the reporter gene, UAS-E1BTATA-Luc, in the absence or presence of 10 μM BPA. RLU, Relative light units.

had no significant effect on the basal transcriptional activity mediated by Gal4-DBD alone (data not shown).

We next determined the effects of BPA on various physiological concentrations of T_3. In the presence of 10 μM BPA, increasing amounts of T_3 were added to the medium, and transcriptional activity was measured. As shown in Fig. 3A, BPA suppressed the activity mediated by Gal4-TRα1 up to about 50% of the respective control level. Similar results were obtained using Gal4-TRβ (Fig. 3B).

The inhibitory effects of BPA were also examined in the context of native receptors. A T_3-responsive reporter gene, TRE-tk-Luc, was cotransfected with full-length TRs. Increasing concentrations of BPA significantly suppressed the transcriptional activities mediated by TRα1 (Fig. 4A) and TRβ1 (Fig. 4B). In a reciprocal manner, another group of negatively regulated genes was stimulated by TRs in the absence of T_3 and was repressed in response to T_3 (26). The effects of BPA on the TSHα promoter were examined as a model of a negatively regulated gene. As shown in Fig. 4C, BPA increased the transcriptional activity, which was already suppressed by 10 nM T_3. The stimulating effects were observed in the presence of TRβ1 as well as TRβ2, which is expressed mainly in the pituitary and hypothalamus.

BPA suppressed transcriptional activities mediated by endogenous TRs

We next studied the effects of BPA using a cell line that contains physiological amounts of endogenous TRs. The reporter gene regulated by the ME-TRE, ME-tk-Luc, was transfected into human hepatoblastoma cells, HepG2. Twenty-four-hour incubation with 10 nM T_3 stimulated the expression of ME-tk-Luc by 1.7-fold (Fig. 5). Addition of 10 μM BPA significantly decreased gene transcription to 78.7% of that with 10 nM T_3 alone.

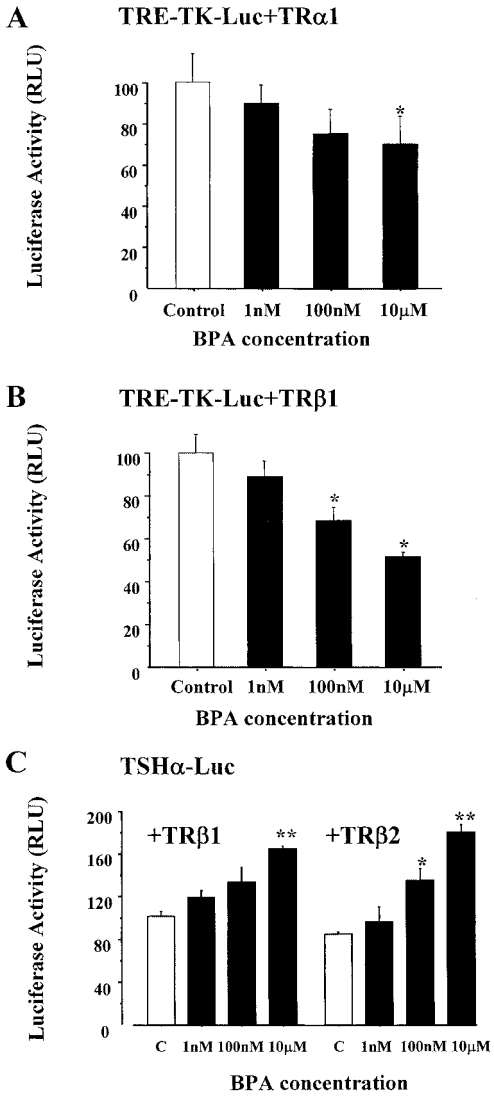

FIG. 4. The inhibitory effects of BPA on the gene transcription mediated by the native TRs. A and B, CMX-TRα1 (A) or CMX-TRβ1 (B; 10 ng) was cotransfected into TSA-201 cells with 100 ng of the reporter gene, TRE-tk-Luc, in the presence of 10 nM T_3 and increasing amounts of BPA. C, CMX-TRβ1 or CMX-TRβ2 (50 ng) was cotransfected into TSA-201 cells with 100 ng of the reporter gene, TSHα-Luc, in the presence of 10 nM T_3 and increasing amounts of BPA. RLU, Relative light units. *, $P < 0.05$; **, $P < 0.01$ (*vs.* control).

BPA recruits N-CoR

Transcriptional repression of the positively regulated genes by unliganded TR is mediated by interacting with corepressor proteins (CoRs). CoRs might also be involved in the basal activation of negatively regulated genes (26). Using a mammalian two-hybrid assay, the effect of BPA on the TR-CoR interaction was examined. The carboxy-terminal half of a CoR, N-CoR, which contains TR interaction domains, was fused to the Gal4-DBD. The LBD of TRβ1 was fused to the transcriptional activation domain of VP16 to allow detection of the interaction between the Gal4-NCoR and VP16-TR. Although increasing concentrations of T_3 decreased the interaction between these proteins

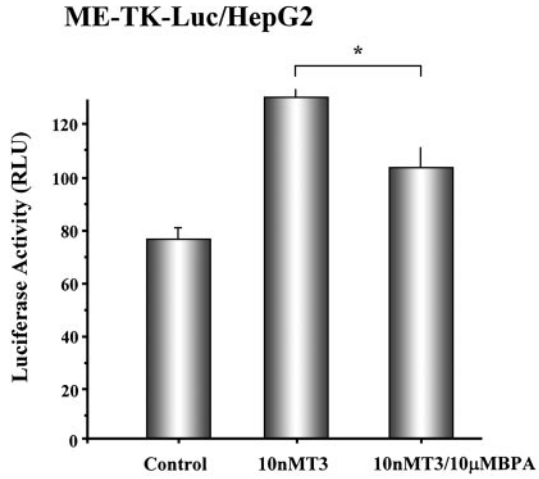

FIG. 5. BPA suppressed transcriptional activities mediated by endogenous TRs. The ME-tk-Luc (100 ng) was transfected into HepG2 cells and incubated with or without 10 nM T_3 and/or 10 μM BPA for 24 h. RLU, Relative light units. *, $P < 0.05$.

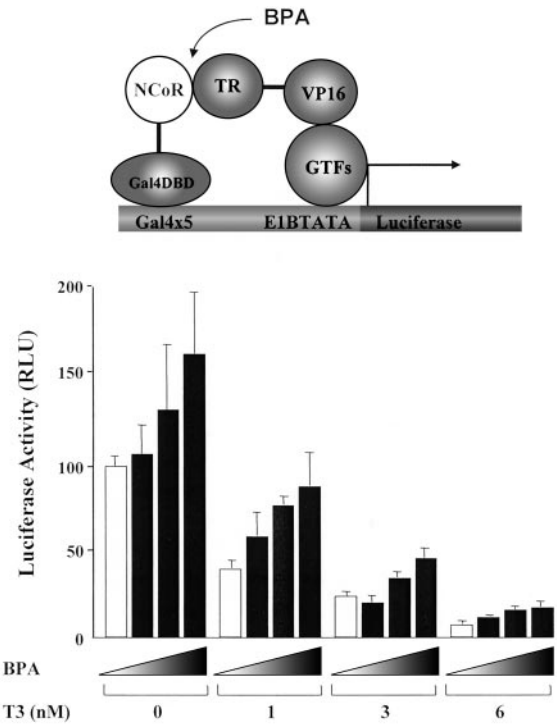

FIG. 6. The effect of BPA on the interaction between TR and N-CoR. The format of the mammalian two-hybrid experiment is shown at the *top* of the figure. Gal4-NCoR (50 ng) were cotransfected into TSA-201 cells with 100 ng VP16-TR together with 100 ng of the reporter gene, UAS-E1BTATA-Luc, in the absence or presence of T_3. Increasing amounts of BPA (1 nM, 100 nM, and 10 μM) were added. RLU, Relative light units.

(indicated by □ in Fig. 6), BPA enhanced those interactions in a dose-dependent manner (■).

Discussion

BPA is detected in human plasma, cord sera, and even fetal tissues (3, 33). The concentration was more than 1 ng/g wet

weight of the umbilical cord. Serum BPA concentrations were reported to be 1.49 ± 0.11 ng/ml in men and 0.64 ± 0.10 ng/ml in women (34). The Japanese Ministry of Health and Welfare has established the standards for regulations against BPA levels in food containers. The upper limit of emission is set to 2.5 ppm (μg/liter), which is more than 90 μM. This level corresponds to world standards. The results in this study indicate that concentrations even below the upper limit can interfere with thyroid hormone action *in vitro*.

Thyroid hormones are essential for normal behavioral, intellectual, and neurological development. Congenital hypothyroidism if left untreated causes irreversible mental retardation. Even mild maternal thyroid deficiency during pregnancy could cause retarded neurological development of the child (35). There is increasing evidence that exposure to certain synthetic compounds, such as dioxins and PCBs, during the perinatal period can impair normal thyroid function. PCBs reduced circulating and tissue thyroid hormone concentrations using animal experiments (36, 37), and dioxins and PCBs were observed to alter thyroid hormone status by epidemiological investigations (38). The PCB-induced reduction in circulating T_4 has been attributed to increased excretion of free T_4 due to competitive binding of PCBs with thyroid hormone transport proteins (10, 39), amplified biliary excretion of T_4 by induction of UDP-glucuronosyltransferase (40), and/or direct damage to the thyroid gland (41). Thus, a given ED can interfere with thyroid hormone functions and homeostasis by inhibiting hormone synthesis, altering serum transport proteins, or increasing catabolism of thyroid hormones. Regarding gene transcription, there are no direct data to support the assertion that certain ED may alter thyroid hormone action.

The effects of BPA on thyroid function have not been elucidated. In contrast, the estrogenicity of BPA has been demonstrated in a number of *in vitro* and *in vivo* assays. *In vitro* assay end points include binding to the ER (42, 43) and activation of ERE-driven reporter gene constructs (44). Upon iv injection of BPA into rats, levels of BPA were determined in serum and various organs (45). BPA was detected predominantly in the lung, followed by kidneys, thyroid, stomach, heart, spleen, testes, liver, and brain. Ratios of the organ to serum BPA concentrations exceeded unity for all organs examined (ratio range, 2.0–5.8), except for brain (ratio, 0.75). Thus, BPA has the potential to interfere with thyroid hormone action in each organ accumulated by BPA. The *in vivo* effects of BPA are under investigation using experimental animals.

In this study we demonstrated that BPA could impair thyroid hormone action by inhibiting T_3 binding to the TR and by suppressing its transcriptional activity. Gene suppression is attributed partly to the recruitment of N-CoR to the TR by BPA. In contrast, some compounds exert their estrogen-like activity through the ER by recruiting coactivators, such as SRC1 and RIP140, in a manner similar to that of estradiol (46, 47). A number of nuclear cofactors have been cloned, but most of their specific functions are unclear (48). Moreover, more than 150 nuclear receptors may exist in mammalian cells as targets for ED. Indeed, BPA activated the transcription mediated by the human orphan receptor, steroid and xenobiotic receptor (49), but not by its mouse ortholog, pregnane X receptor (50). BPA also did not activate the transcription mediated by androgen, progesterone, glucocorticoid, or mineralocorticoid receptors (46). Increasing concerns over the effects of environmental hormones highlight the need for screening of the effects of ED on nuclear receptors to assess potential disruption of the endocrine system.

In summary, our findings demonstrate that BPA, which is one of the most prevalent chemicals for daily use materials, suppresses transcriptional activity by inhibiting T_3 binding to the TR and by recruiting N-CoR on the promoter. Further studies, such as animal experiments and epidemiological investigations, will allow evaluation of the effects of BPA on the human endocrine system.

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References

1. **Colborn T, Dumanoski D, Myers JP** 1996 Our stolen future. New York: Dutton
2. **Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM** 1995 The nuclear receptor superfamily: the second decade. Cell 83:835–839
3. **Mori C** Fetal exposure to endocrine disrupting chemicals (EDCs) and possible effects of EDCs on the male reproductive system in Japan. Proc International Symposium on Environmental Endocrine Disrupters '98, Kyoto, Japan, 1988, p 39
4. **Reel JR, George JD, Lawton AD, Myers CB, Lamb JC** 1985 Bisphenol A: reproduction and fertility assessment in CD-1 mice when administered in feed. NTP/NIEHS Report
5. **Morrissey RE, George JD, Price DJ, Tyl RW, Marr MC, Kimmel CA** 1987 The developmental toxicity of bisphenol A in rats and mice. Fund Appl Toxicol 8:571–582
6. **Krishanan AN, Stathis P, Permuth SF, Tokes L, Feldman D** 1993 Bisphenol-A: an estrogenic substance is released form polycarbonate flasks during autoclaving. Endocrinology 132:2279–2286
7. **Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO** 1995 The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103(Suppl 7): 113–122
8. **Villalobos M, Olea N, Brontons JA, Olea-Serrano F, Ruiz de Almodovar JM, Pedraza V** 1995 The E-screen assay: a comparison of different MCF7 cell stocks. Environ Health Perspect 103:844–850
9. **McKinney JD, Waller CL** 1994 Polychlorinated biphenyls as hormonally active structural analogues. Environ Health Perspect 102:290–297
10. **Rickenbacher U, McKinney JD, Oatley SJ, Blake CCF** 1986 Structurally specific binding of halogenated bisphenyls to thyroxin transport protein. J Med Chem 29:641–648
11. **McKinney JD** 1989 Multifunctional receptor model for dioxin and related compounds toxic action: possible thyroid hormone-responsive effector-linked site. Environ Health Perspect 82:323–336
12. **Sap J, Munoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H, Vennstrom B** 1986 The c-erb-A protein is a high-affinity receptor for thyroid hormone. Nature 324:635–640
13. **Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol DJ, Evans RM** 1986 The c-*erb*-A gene encodes a thyroid hormone receptor. Nature 324:641–646
14. **Williams GR** 2000 Cloning and characterization of two novel thyroid hormone receptor β isoforms. Mol Cell Biol 20:8329–8342
15. **Koenig RJ, Lazar MA, Hodin RA, Brent GA, Larsen PR, Chin WW, Moore DD** 1989 Inhibition of thyroid hormone action by a non-hormone binding c-erbA protein generated by alternative mRNA splicing. Nature 337:659–661

16. **Plateroti M, Gauthier K, Domon-Dell C, Freund JN, Samarut J, Chassande O** 2001 Functional interference between thyroid hormone receptor α (TRα) and natural truncated TRδα isoforms in the control of intestine development. Mol Cell Biol 21:4761–4772
17. **Bradley DJ, Yound III WS, Weinberger C** 1989 Differential expression of α and β thyroid hormone receptor genes in rat brain and pituitary. Proc Natl Acad Sci USA 86:7250–7254
18. **Cook CB, Koenig RJ** 1990 Expression of erbAα and β mRNAs in region of adult rat brain. Mol Cell Endocrinol 70:13–20
19. **Strait KA, Schwartz HL, Perez-Castillo A, Oppenheimer JH** 1990 Relationship of c-erb A mRNA content to tissue triiodothyronine nuclear binding capacity and function in developmental and adult rats. J Biol Chem 265:10514–10521
20. **Forrest D, Sjoberg M, Vennstrom B** 1990 Contrasting developmental and tissue-specific expression of α and β thyroid hormone receptor genes. EMBO J 9:1519–1528
21. **Forrest D, Hallbook F, Persson H, Vennstrom B** 1991 Distinct functions for thyroid hormone receptors α and β in brain development indicated by differential expression of receptor genes. EMBO J 10:269–275
22. **Mellstrom B, Naranjo JR, Santos A, Gonzalez AM, Bernal J** 1991 Independent expression of α and β c-*erb* A genes in developing rat brain. Mol Endocrinol 5:1339–1350
23. **Bradley DJ, Towle HC, Yound III WS** 1992 Spatial and temporal expression of α- and β-thyroid hormone receptor mRNAs, including the β2-subtype, in the developing mammalian nervous system. J Neurosci 12:2288–2302
24. **Tagami T, Nakamura H, Sasaki S, Imura H** 1990 Characterization of interaction between nuclear T_3 receptors and antiserum against cellular-erb A peptide. Endocrinology 126:1105–1111
25. **Umesono K, Murakami KK, Thompson CC, Evans RM** 1990 Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D receptors. Cell 65:1255–1266
26. **Tagami T, Madison LD, Nagaya T., Jameson JL** 1997 Nuclear receptor corepressors activate rather than suppress basal transcription of genes that are negatively regulated by thyroid hormone. Mol Cell Biol 17:2642–2648
27. **Sadowski I, Ma J, Tiezenberg S, Ptashne M** 1988 Gal4-VP16 is an unusually potent transcriptional activator. Nature 335:563–564
28. **Tagami T, Lutz WH, Kumar R, Jameson JL** 1998 The interaction of the vitamin D receptor with nuclear receptor corepressors and coactivators. Biochem Biophys Res Commun 253:353–363
29. **Tagami T, Gu W, Peairs PT, West BL, Jameson JL** 1998 A novel natural mutation in the thyroid hormone receptor defines a dual functional domain that exchanges nuclear receptor corepressors and coactivators. Mol Endocrinol 12:1888–1902
30. **Nagaya T, Jameson JL** 1992 Thyroid hormone receptor mutations that cause resistance to thyroid hormone. J Biol Chem 267:13014–13019
31. **Desvergne B, Petty KJ, Nikodem VM** 1991 Functional characterization and receptor binding studies of the malic enzyme thyroid hormone response element. J Biol Chem 266:1008–1013
32. **Margolskee RF, McHendry-Rinde B, Horn R** 1993 Panning transfected cells for electrophysiological studies. BioTechniques 15:906–11
33. **Sakurai K, Mori C** 2000 Fetal exposure to endocrine disruptors. Nippon Rinsho 58:2508–2513
34. **Takeuchi T, Tsutsumi O** 1991 Serum bisphenol concentrations showed gender differences, possibly linked to androgen levels. Biochem Biophys Res Commun 291:76–78
35. **Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ** 1999 Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341:2015–2017
36. **Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM** 1995 Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. Toxicol Appl Pharmacol 135:77–88
37. **Morse DC, Groen D, Verman M, van Amerongen CJ, Koeter HB, Smits van Prooije AE, Visser TJ, Koeman HJ, Brouwer A** 1993 Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. Toxicol Appl Pharmacol 122:27–33
38. **Koopman-Esseboom C, Morse DC, Weisgalas-Kuperus N, Lutkeschipholt IJ, van der Paauw CG, Tuinstra LG, Brouwer A, Sauer PJ** 1994 Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 36:468–473
39. **Brouwer A, van den Berg KJ** 1986 Binding of a metabolite of 3,4,3′4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol 85:301–312
40. **Barter RA, Klaassen CD** 1992 UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. Toxicol Appl Pharmacol 113:36–42
41. **Ness DK, Schantz SL, Moshtaghian J, Hansen L** 1993 Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol Lett 68:311–323
42. **Dodge A, Glasebrook AL, Magee DE, Phillips DL, Sato M, Short LL, Bryant HU** 1996 Environmental estrogen: effects on cholesterol lowering and bone in the ovariectomized rat. J Steroid Biochem Mol Biol 59:155–161
43. **Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boechler M, Welshons WV** 1997 Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octyphenol. Environ Health Perspect 105:70–76
44. **Gaido KW, Leonard LS, Lovell S, Gould JC, Babai D, Portier CJ, McDonnel DP** 1997 Evaluation of chemicals with endocrine-modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol Appl Pharmacol 143:205–212
45. **Yoo SD, Shin BS, Kwack SJ, Lee BM., Park KL, Han SY, Kim HS** 2000 Pharmacokinetic disposition and tissue distribution of bisphenol A in rats after intravenous administration. Toxicol Environ Health A 61:131–139
46. **Nishikawa J, Saito K, Goto J, Dakeyama F, Matsuo M, Nishihara T** 1999 New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator. Toxicol Appl Pharmacol 154:76–83
47. **Sheeler CQ, Dudley MW, Khan SA** 2000 Environmental estrogens induce transcriptionally active estrogen receptor dimers in yeast: activity potentiated by the coactivator RIP140. Environ Health Perspect 108:97–103
48. **McKenna NJ, Lanz RB, O'Malley BW** 1999 Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321–344
49. **Takeshita A, Koibuchi N, Oka J, Taguchi M, Shishiba Y, Ozawa Y** 2001 Bisphenol-A, an environmental estrogen, activates the human orohan nuclear receptor, steroid and xenobiotic receptor-mediated transcription. Eur J Endocrinol 145:513–517
50. **Masuyama H, Hiramatsu Y, Kunitomi M, Kudo T, MacDonald PN** 2000 Endocrine disrupting chemicals, phthalic acid and nonylphenol, activate pregnane X receptor-mediated transcription. Mol Endocrinol 14:421–428