Thyroid Hormone Action Is Disrupted by Bisphenol A as an Antagonist

KENJI MORIYAMA, TETSUYA TAGAMI, TAKASHI AKAMIZU, TAKEI USUI, MISA SAJO, NAO TETSU KANAMOTO, YUJI HATAYA, AKIRA SHIMATSU, HIDESHISHU KUZUYA, AND KAZUWA NAKAO

Department of Medicine and Clinical Science, Graduate School of Medicine, Kyoto University (K.M., T.A., M.S., N.K., Y.H., K.N.), Kyoto 606-8507, Japan; and Clinical Research Institute, Center for Endocrine and Metabolic Diseases, Kyoto National Hospital (K.M., T.T., T.U., A.S., H.K.), Kyoto 612-8555, Japan

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 (T3) in a dose-dependent manner. The inhibitory effects were observed in the presence of physiological concentrations of T3. In contrast, in the case of negatively regulated TSHα promoter, BPA activated the gene transcription that is suppressed by T3. To elucidate possible mechanisms of the antagonistic action of BPA, the effects on T3 binding and cofactor interaction with TR were examined. The Kd value for BPA was 200 μM when assessed by inhibition of [125I]T3 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 T3 from the TR and recruit a transcriptional repressor, resulting in gene suppression. This is the first report that BPA can antagonize T3 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.

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 superfamilial of ligand-inducible transcriptional factors, which include receptors for steroid hormones, thyroid hormones, vitamin D, 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 ERα binding and cofactor interaction with TR were examined. The Kd value for BPA was 200 μM when assessed by inhibition of [125I]T3 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 T3 from the TR and recruit a transcriptional repressor, resulting in gene suppression. This is the first report that BPA can antagonize T3 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)
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 T3 binding to the nuclear TRs and recruited nuclear receptor corepressors (N-CoRs) to the TR, resulting in transcriptional inhibition.

Materials and Methods

T3 binding studies

Nuclear TRs were prepared from the Sprague Dawley rat liver as previously described (24). A tracer dose of [125I]T3 (122 MBq/μg; 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 [125I]T3 were separated by adding 1 ml 2% Dowex resin (Supelco, Bellefonte, PA) suspension. The nonspecific binding obtained in the presence of an excess of T3 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-TRβ2 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-N-CoR (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) 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 a palindromic TRE and the ME-TRE, respectively, upstream 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 T3. 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 T3 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 (T3). BPA has two hydroxyl groups, and T3 has a hydroxyl and an alanine group. BPA displaced [125I]T3 from endogenous TR, which is prepared from the rat liver, with an inhibition constant (Ki) of 200 μM (Fig. 1C). Scatchard analysis revealed that BPA decreased the value for the association constant (K0) from 0.44 to 0.28 × 106 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 T3-induced TR activation. In the presence of 3 nM T3, dose-dependent inhibition of transcription mediated by Gal4-TRα1 (Fig. 2A) and Gal4-TRβ (Fig. 2B) was observed. BPA
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 T3. In the presence of 10 nM T3, increasing amounts of BPA suppressed the activity mediated by Gal4-TRα1 to about 50% of the respective control level. Similar results were obtained using Gal4-TRβ1 (Fig. 3B).

The inhibitory effects of BPA were also examined in the context of native receptors. A T3-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 T3 and was repressed in response to T3 (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 T3. 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 T3 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 T3 alone.
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 T3 decreased the interaction between these proteins (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 neurodevelopmental growth 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 T3 has been attributed to increased excretion of free T3 due to competitive binding of PCBs with thyroid hormone transport proteins (10, 39), amplified biliary excretion of T3 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 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 T3 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 T3 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.

Acknowledgments

We thank Ms. Keiko Matsuda, Ms. Maki Kouchi, and Ms. Hitomi Hiratani for their excellent secretarial assistance. We are also grateful to Dr. J. Larry Jameson for comments and suggestions on the manuscript.

Received February 12, 2002. Accepted August 14, 2002.

Address all correspondence and requests for reprints to: Tetsuya Tagami, M.D., Ph.D., Clinical Research Institute, Center for Endocrine and Metabolic Diseases, Kyoto National Hospital, 1-1 Mukaihata-cho, Fukakusa, Fushimi-ku, Kyoto 612-8555, Japan. E-mail: tttagami@kyotolan.hosp.go.jp.

This work was supported by grants from Asahi Brewers Foundation and the Foundation for Total Health Promotion.

References

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, 1998; p 39


23. Bradley DJ, Towle HC, Youn 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


25. Úmesono 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. Sadowski I, Ma J, Tiezenberg S, Ptashne M 1990 Expression of erbA c-myc, and erbB thyroid hormone receptor mRNAs, including the β2-subtype, in the developing mammalian nervous system. J Neurosci 12:2288–2302

27. Tagami T, Madison LD, Nagaya T, Jameson JL 1991 Nuclear receptor corepressors activate rather than suppress basal transcription of genes that are negatively regulated by thyroid hormone. Mol Cell Biol 17:2642–2648


34. Sakurai K, Mori C 2000 Fetal exposure to endocrine disruptors. Nippon Rinsho 58:2308–2313


40. Barter RA, Klaassen CD 1992 UDP-gluconuronyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. Toxicol Appl Pharmacol 113:36–42


43. Nagel SC, vom Saal FS, Thayer KA, Dhar MG, Boehler M, Welschows WH 1997 Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. Environ Health Perspect 105:70–76


47. Sheeler CQ, Dudley MW, Khan SA 2000 Environmental estrogens induce transcriptionally active estrogen receptor dimers in yeast: activity potentiated by the coactivator RAP1. Environ Health Perspect 108:97–103
