Endocrine Journal 1999, **46** (6), 773–778

Differential Interactions of Bisphenol A and 17β-estradiol with Estrogen Receptor α (ERα) and ERβ

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Abstract. Bisphenol A (BPA), a monomer of plastic used in consumer products, is abundant in the environment and enters the body by ingestion or adsorption. We developed a cell based transcription assay system using a reporter gene under the transcriptional control of estrogen receptor α (ERα) as well as ERβ and performed chloramphenicol acetyltransferase (CAT) assay on HeLa cells transfected with either human ERα cDNA or ERβ cDNA to characterize the estrogenic effect of BPA. Estrogenic activity of BPA was detectable at a concentration of 10^{-9} M and the activity increased in a dose dependent manner between concentrations of 10^{-9} M and 10^{-6} M of BPA for both ERα and ERβ. The estrogenic activity of 17β-estradiol at a concentration of 10^{-8} M was almost compatible with that of BPA at the concentration of 10^{-6} M of BPA for ERα as well as ERβ. CAT activity was significantly decreased when cells expressing ERα were incubated with 10^{-6} M of BPA and 10^{-8} M of 17β-estradiol while the activity was essentially the same for ERβ in the same condition, indicating that BPA exhibits only agonistic action for ERβ whereas it has dual actions as an agonist and antagonist of estrogen for ERα. These results indicates that BPA exerts its effects in ER subtype specific way, thus suggesting that the mode of action of endocrine disruptors are more complex than thought.

Key words: Bisphenol A, Estradiol, Estrogen receptor α, Estrogen receptor β, Endocrine disruptor
(*Endocrine Journal* **46**: 773–778, 1999)

ENVIRONMENTAL estrogens (xenoestrogens) are nonsteroidal, man-made chemicals that enter the body by ingestion or adsorption, bind to estrogen receptors and mimic estrogen actions [1-2]. Bisphenol A (BPA), one of the best known environmental estrogens, has two unsaturated phenol rings and has no structural homology with estradiol (Fig. 1). However, the structure of BPA is similar to diethylstilbestrol (DES), a potent synthetic estrogen, prenatal exposure to which in utero is known to cause genital abnormalities and carcinomas [3]. BPA is a monomer of polycarbonate plastics and BPA-based epoxy and polystyrene resins are used in many products, such as inner coating of food cans, dental composites and drug delivery systems. BPA can be liberated from polycarbonate plastics subjected to high temperature [4] or from incomplete polymerized epoxy resins. Indeed, a significant amount of BPA was detected in liquid from canned vegetables (20 μg/can) that are exposed to high temperature during autoclaving [5] and in saliva (20–30 μg/ml) of dental patients fitted with restorative materials [6]. Recently, it was shown that BPA contaminates not only human plasma but fetal tissues as well [7]. Estrogen is considered to play a crucial role from the early stage of fetal development, and estrogen receptors are expressed even in the preimplantation embryos [8]. Thus far, several lines of evidence implicated the influence of environmental disruptors if exposed during fetal stage. For instance, development of early stages of embryos was affected by dioxins, another well-known endocrine disruptor [9].

Received: January 13, 1999
Accepted: August 30, 1999
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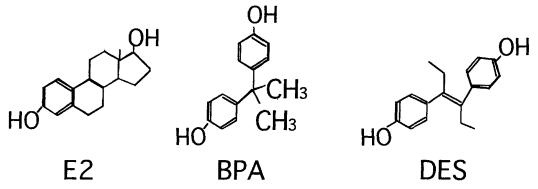

Fig. 1. Comparison of chemical structures of 17b-estradiol (E2), bisphenol A (BPA) and diethylstilbestrol (DES).

It was further reported that fetal exposure of low dose BPA or DES led to increased prostate weight in adulthood [10].

Estrogen receptor (ER), a ligand dependent transcription factor, specifically binds with estrogen, and regulates gene transcription via estrogen responsive element (ERE). ER had been assumed to exist as a single species, until a novel estrogen receptor (ERβ) was recently isolated in rats [11], humans [12, 13], and mice [14]. ERβ has a high degree of sequence homology with the classical estrogen receptor (ERα). ERβ mRNA was detected predominantly in rat ovary, prostate, lung, brain, bladder, uterus and bone [11, 15–17], and in human breast cancer tissues [15]. Although ERβ has a slightly lower binding affinity for 17β-estradiol than ERα [11, 14, 15], its transactivating manner via ERE is similar to ERα [19, 20]. On the other hand, some of the transcription activating functions of ERβ are different from that of ERα, which depend on the ligand and their responsive element [14, 21, 22]. It is of interest to note that relative transactivation activity of DES through ERα was higher than that through ERβ , although binding activities of DES for ERα and ERβ were not different [2].

Estrogenic activity of BPA has been shown by the experiment that BPA induced the expression of estrogen responsive genes and promoted cell proliferation in MCF-7, a breast cancer cell line [4]. In another experiment using rats, BPA also induced prolactin gene expression and its release both in vivo and in vitro [23]. Furthermore, BPA treatment induced the growth differentiation and c-fos gene expression in rat uterus and vagina, with nearly similar effects to estradiol [24]. In this study, we have evaluated the estrogenic and antiestrogenic activity of BPA with ERα and ERβ and the interaction of BPA and estradiol with ERs, employing chloramphenicol acetyltransferase (CAT) assay.

Materials and Methods

Plasmid Construction

The ERα cDNA originated from HEG0 [25] was cloned into pCXN2 expression vector [26], which can also express the neomycin resistance gene, to construct pCXN2-hERα [13]. The reverse transcription polymerase chain reaction (RT-PCR) product of ERβ cDNA was cloned into pCXN2 to construct pCXN2-hERβ [13]. The oligonucleotide containing the wild-type ERE of the *Xenopus* vitellogenin gene A2 enhancer (VitERE) was synthesized and inserted at the upstream position of pGCAT [21] to construct ERE-GCAT [21].

Cell Culture and Chloramphenicol Acetyltransferase (CAT) Assay

17β-estradiol was obtained from Sigma Chemical Company and dissolved with ethanol at 10^{-5} M in a 1.5 ml microreaction tube and diluted with cell culture medium to the final concentration (10^{-8} M). BPA (more than 99% pure) was obtained from Aldrich Chemical Company and dissolved with ethanol in a 1.5 ml microreaction tube at 10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M. They were diluted with cell culture medium to 1 : 1000 (10^{-9} M, 10^{-8} M, 10^{-7} M and 10^{-6} M, respectively).

HeLa cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. CAT assay was performed as described previously [27]. Briefly, 0.5×10^6 HeLa cells were transfected with 0.05 μg of pCXN2-hERα or pCXN2-hERβ, 1 μg of VitERE-tk-CAT and 1 μg of PCH110 β-galactosidase expression vector (Pharmacia). One h before transfection, cell culture medium was replaced with phenol red free Dulbecco's modified Eagle's medium (GIBCO) containing 10% dextran-coated charcoal treated fetal bovine serum (HyClone, Cat. No. SH30068), which was treated at 60°C for 30 min before using. After 12 h of incubation, the cells were cultured further with or without 10^{-8} M 17β-estradiol or with 10^{-9}–10^{-6} M of BPA for 24 h. Cell extracts were prepared and assayed for β-galactosidase and CAT activity. The CAT activity was determined by Luminous Imager system (Aisin Cosmos R & D Co., Tokyo).

Statistical Analysis

Data are presented as means ± standard deviation (SD) of three independent experiments. The differences between each treated value were evaluated by analysis of variance and Student's *t*-test.

Results

We determined estrogenic activity of BPA employing a cell based transcription assay system in which CAT assays were carried out. A significant increase in estrogenic activity was detectable at 10^{-9} M BPA concentration in cells transfected with ERα (Fig. 2A). The activity increased in a dose dependent manner up to a concentration of 10^{-6} M of BPA, indicating that higher doses of BPA produce greater estrogenic activity ($P < 0.01$). The estrogenic activity of 17β-estradiol at a concentration of 10^{-8} M was almost compatible with that of BPA at the concentration of 10^{-6} M (Fig. 2A).

Estrogenic activity was also observed in cells transfected with ERβ (Fig. 2B). The activity was increased in a dose dependent manner between concentrations of 10^{-9} M and 10^{-6} M of BPA. The higher doses of BPA increased greater estrogenic activity ($P < 0.01$). This trend, however, was less pronounced with ERβ compared with ERα (Fig. 2A, B). At a concentration of 10^{-6} M of BPA, estrogenic activity was almost compatible with that of 10^{-8}M of 17β-estradiol (Fig. 2).

The combination of 17β-estradiol and BPA exhibited differential actions depending on which subtype of ERs was expressed. In cells expressing ERα, CAT activities with 10^{-8} M of 17β-estradiol and 10^{-9} M–10^{-7} M of BPA were not different from that of 17β-estradiol alone (Fig. 2A). However, CAT activity was significantly decreased when incubated with 10^{-8} M of 17β-estradiol and 10^{-6} M of BPA. On the other hand, in cells expressing ERβ, CAT activities were essentially the same when incubated with 10^{-8} M of 17β-estradiol and different concentrations of BPA between 10^{-9} M and 10^{-6} M of BPA (Fig. 2B).

Discussion

In the present study, we determined that BPA, a monomer of plastics that is environmentally produced in huge amounts, mimics estradiol action as determined by in vitro transcription stimulation assay. This study particularly provides the first evidence that BPA exhibits only agonistic action for ERβ whereas it has dual actions as both an agonist and antagonist of estrogen for ERα. In previous studies, several lines of evidence indicate that BPA has estrogenic activities [4, 23, 24]. In addition, BPA has been shown to bind both ERα and ERβ despite its relative binding affinity being 10000-fold lower relative to 17β-estradiol [2]. Another noteworthy finding of this study is that BPA at 10^{-8} produced as much as half of the estrogenic activity of that by estradiol at equivalent concentration, an unexpected finding, despite the great difference in binding affinities.

ER is unique among steroid receptor family in that it interacts with a wide variety of compounds. This is true for each ER subtype. Kuiper *et al.* recently examined estrogenic potency of various estrogenic chemicals via both ERα and ERβ, and found that the relative transactivation activities of 10^{-6} M of BPA with ERα and ERβ were 50 and 41, respectively, when the relative transactivation activity of 10^{-6} M of 17β-estradiol was set at 100 [2]. In the present study, 10^{-8} M of BPA exhibited approximately half of estrogenic activity of that produced by 17β-estradiol at an equimolar concentration, via either ERα and ERβ, in agreement with Kuiper *et al.* [2] (Fig. 2A, B). It appears that BPA exhibits as much as half of the estrogenic activities of estradiol in the transactivation assay system via ERα and ERβ as well, despite the more than 100-fold lower binding affinities for the receptors compared with 17β-estradiol. In a previous report, estrogenic activity of BPA was shown at 1 nM [23], which is compatible with our study. It was reported that 10^{-9} M of BPA caused PRL release in a dose dependent manner using primary anterior pituitary cells [23]. In the same primary cell system, the magnitude of release at 10^{-6} M BPA was same at 10^{-9} M estradiol [23].

BPA when added to the medium together with 17β-estradiol did not exert additive effect for both ERα and ERβ. (Fig. 2A, B). It is of interest to note

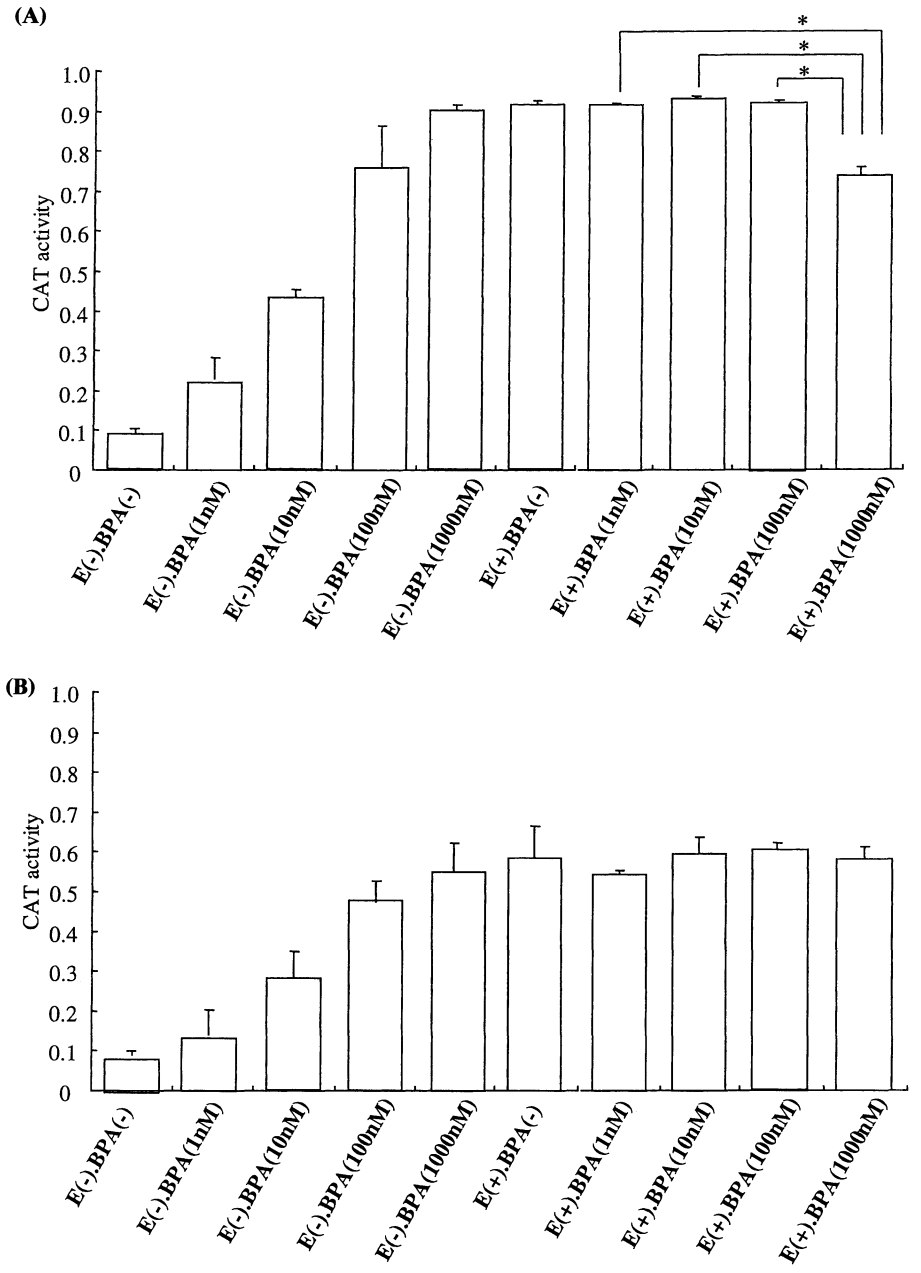

Fig. 2 Transcriptional activity of BPA and/or 17β-estradiol for ERα (A) and ERβ (B). HeLa cells were transfected with the reporter plasmid, ERE-GCAT, and an internal control PCH110 plasmid and either human ERα or ERβ expression plasmid, pCXN2-hERα and pCXN2-hERβ, respectively. After transfection, cells were incubated for 12 h in the absence of 10^{-8} M 17β-estradiol (E2) and incubated for another 24 h in the presence or absence of BPA (10^{-9} to 10^{-6} M) and/or 10^{-8} M 17β-estradiol (E2). Results were indicated as acetylation rate. Each bar and point represent means$\pm$SD (n=3). *, $P<0.05$ by Student's *t*-test.

that a weak antagonistic activity of BPA was observed only for ERα when 10^{-6} M of BPA was added in the presence of 10^{-8} M of 17β-estradiol. We could not detect an antagonistic effect for ERβ in the same setting. The reason for this difference is at this moment unknown. It is speculated that it might reflect the differential expression of transcriptioinal coactivators or differential stability of the receptor protein. It is also possible that an antagonistic effect may vary depending on the concentrations of BPA,

cell lines and ERE-promoter context of reporter gene. It was reported that some of transcription activating functions of ERβ are different from that of ERα, in a ligand specific way [21].

The tissue distribution of ERα and ERβ is different; moderate to high expression for ERα in rat was detected in uterus, testis, pituitary, ovary, kidney, epididymis and adrenal and that moderate to high expression for ERβ in rat was detected in prostate, ovary, lung, bladder, brain, uterus and testis [15]. In view of this, the estrogen-like activity of BPA may vary from tissue to tissue. Although estrogen-like activity of BPA may occur in tissues which express ER, the magnitude of activity is unknown because the distribution and concentration of BPA in tissues are unclear. It is possible that antagonistic activity is detected in tissue expressing ERα and containing high concentration of BPA.

Here we report that BPA has dual actions as an estrogen agonist and antagonist, in an ER subtype specific way, thus highlighting that the modes of actions of environmentally produced estrogen-like substances are more complex than expected. It is known that BPA is detected in human plasma and in fetal tissues as well. At present, the biological influences of the substance remains to be clarified. The present results offer clues to future studies as to how environmental chemicals affect human health.

Acknowledgments

We thank Drs. S. Inoue and S. Ogawa and Prof. M. Muramatsu for kindly providing expression vectors of human estrogen receptors α and β. This study was supported in part from Grants-in-Aid from the Ministry of Education, Science and Culture, from the Ministry of Health and Welfare, and from the Science and Technology Agency, Japan.

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