ESTROGEN RECEPTOR-MEDIATED EFFECTS OF A XENOESTROGEN, BISPHENOL A, ON DEVELOPMENT OF PREIMPLANTATION MOUSE EMBRYOS

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Introduction

Xenoestrogens are nonsteroidal manmade chemicals that can enter the body by ingestion or absorption and mimic the actions of estrogens [1]. Xenoestrogens have been shown to affect reproduction in wildlife and may have adverse effects on humans given their ubiquitous presence in the environment, resistance to degradation, and potential for accumulation in adipose tissue. Bisphenol A (BPA) is a monomer in polycarbonate plastics and a constituent of epoxy and polystyrene resins that are used extensively in the food-packaging industry and in dentistry. Human exposure to BPA is significant. Microgram amounts of BPA have been detected in liquid from canned vegetables [2] and in the saliva of patients treated with dental sealants [3].

Exposure of mouse fetuses to BPA at a dose typical for environmental exposure in humans recently was found to produce postnatal estrogenic effects: increased prostate gland weight [4] and reduced daily sperm production in males [5], and accelerated growth and puberty in females [6]. We recently identified expression of both estrogen receptor α (ERα) and a novel recently cloned subtype, ERβ, in preimplantation mouse embryos [7]. We suspected that these embryos might be more sensitive to xenoestrogens than fetuses because preimplantation exposure to xenoestrogen that had entered maternal tissues would be direct, with no placental barriers. In the present study, we examined the effects of BPA on cultured preimplantation embryos at the earliest stages of development.

Materials and Methods

All animal studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by University of Tokyo. Two-cell embryos obtained from 5- to 7-week-old B6C3F1 female mice were placed onto 35 mm dishes (Falcon, Oxnard, CA) containing 1 ml of phenol red-free, serum-free media, Brinster's BMOC3 Medium (Gibco BRL, Tokyo, Japan) and cultured for 48 h to obtain blastocysts in vitro in the presence or absence of the xenoestrogen bisphenol A (BPA; 2,2-bis(4-hydroxy-phenyl)propane) (Aldrich, Tokyo, Japan) at concentrations between 100 pM and 100 μM. The antiestrogen tamoxifen (1-*p*-□-dimethylamino-ethoxy-phenyl-*trans*-1,2-diphenyl-1-butene) was purchased from Sigma Chemical Co. (St. Louis, MO).

Trophoblast spreading of cultured blastocysts was quantitatively analyzed as described previously [8]. Expanded blastocysts developed after 72-h culture of 2-cell embryos were transferred to F0-CMRL medium [8] supplemented with charcoal stripped fetal bovine serum at a concentration of 20% (v/v), and cultured in a humidified atmosphere of 95% air and 5% CO2 at 37°C for 48 h. The surface areas of the trophoblast spreads were quantitatively evaluated using a digitizer tablet (Model DT1000, Watanabe Sokki, Tokyo, Japan) connected to a personal computer (Model 9801, NEC, Tokyo, Japan).

Results and Discussion

In a control group not exposed to BPA, the rate of in vitro development of two-cell embryos to eight-cell embryos was approximately 88% (334/378) at 24 hr in culture (Fig. 1). This rate was significantly increased by exposure to BPA at a concentration of 3 nM (94%; 172/182) compared with the rate in the control group (Fig. 1).Similarly, the rates of development to the blastocyst stage in 48-hr cultures of two-cell embryos were significantly increased by exposure to BPA at concentrations of 1 nM (69.0%; 207/300)and 3 nM (69.2%; 126/182) compared with unexposed control embryos (58.7%; 222/378; Fig. 1). On the other hand, the frequency of development to the blastocyst stage at 48 hrwas significantly decreased by exposure to BPA at 100 μM (31.2%; 93/298) comparedwith control group development, although development to eight-cell embryos at 24 hr was not inhibited by 100 μM BPA (Fig. 1). No stimulatory or inhibitory effects on the development to eight-cell embryos at 24 hr or to blastocysts at 48 hr were observed at concentrations between 10 nM and 10 μM (data not shown). Significant differences were observed between development rates of embryos exposed to 1 or 3 nM of BPA and those exposed to 10 nM (Fig. 1).

To examine estrogen receptor involvement in BPA effects on development of preimplantation mouse embryos, two-cell mouse embryos were cultured in the presence of BPA with or without 100 nM tamoxifen, an antiestrogen. Rates of development of BPA-exposed two-cell embryos to eight-cell embryos were not changed by the presence of tamoxifen. On the other hand, the frequency of development to the blastocyst stage from two-cell embryos exposed to BPA at concentrations of 1 nM and 3 nM was significantly decreased by the presence of tamoxifen (49.1% or 55/112 and 52.1% or 76/146, respectively). In contrast, blastocyst formation with exposure to 100 μM BPA was significantly increased by tamoxifen (72.4%; 92/127). Tamoxifen alone did not influence frequency of blastocyst development (59.8%; 73/122).

Blastocysts that developed in the presence of BPAappeared morphologically normal, and could not be distinguished from those not exposed to BPA.In addition, the number of cells per blastocyst did not differ significantly between embryos exposed or unexposed to varying concentrations of BPA, even 100 μM. On the other hand, we examined the viability and quality of blastocysts that developed in the presence of BPA by quantifying trophoblast spread from the cultured blastocysts. Expanded blastocysts resulting from 72-hr culture of two-cell embryos were transferred to medium (F0-CMRL) containing no BPA and cultured for another 48 hr. No significant difference was seen between spread from control blastocysts and those that had developed in the presence of BPA at concentrations of 1 nM and 3 nM. However, blastocysts that developed in the presence of 100 μM BPAexhibited significantly more extensive spread afteran additional 48 hr in culture without BPA than controls although occurrence of development tothe blastocyst stage was significantly decreased (Fig. 2).

To date several studies have indicated that BPA mimics estrogen and 10 to 1000 nM BPA exerts estrogenic effects on various kind of cells in vitro[9-11]. In the present study, however, 10 to 1000 nM BPA did not show any effects on developmental rates of two-cell embryos to blastocysts. Interestingly, it significantly increased the developmental rates at the lowest concentration (1 nM) ever reported (Fig. 1). These observations reflect the exquisite sensitivity of the early preimplantation embryos, as previously shown with a variety of other agents[12].

We examined effects ofBPA on the quality and viability of those embryos that survived and developed into blastocysts. Blastocysts that had developed in the presence of BPA appeared morphologically identical to those without early BPA exposure, and the number of cells in the blastocysts did not differ significantly between these groups. However, a significant increase beyond control observations was noted in surface area of trophoblast spread from blastocysts previously exposed to 100 μM BPA during an additional 48-hr BPA-free period (Fig. 2).It remains to be elucidated whether the viability of blastocysts actually was increased by 100

μM BPA or better intrinsic viability was needed for survival under exposure to 100 μM BPA. Even though all embryos that had survived to form blastocysts in the presence or absence of BPA appeared identical morphologically, BPA may exert some late effecton subsequent development of these blastocysts. These possible effects of BPA on preimplantation embryos are now being studied by transferring them to the uterus of an unexposed recipient and examining their fetal and postnatal development, including reproductive function in mature rodents.

Humans are exposed to significant amounts of BPA in canned food[2] and dental sealants[3], but the biologic influences of this substance remain to be clarified. Our presentstudy suggests that early embryos are highly sensitive, and it offers avenues for future study of how xenoestrogens affect human health. In particular, more information is needed concerning exposure, pharmacokinetics, synergistic interactions, as well as long-term effects of preimplantation exposure.

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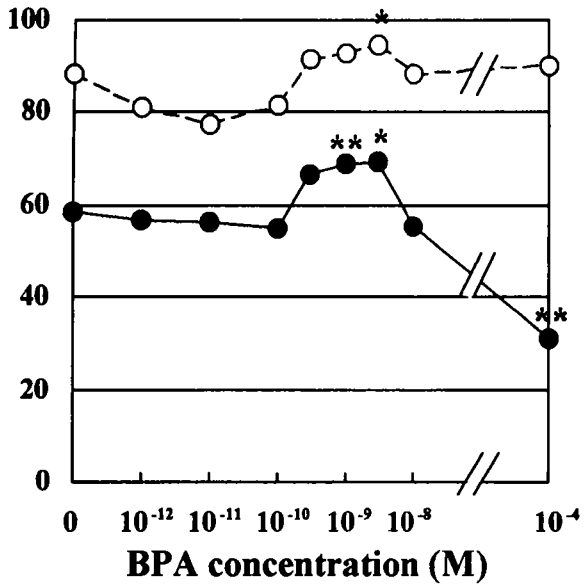

Figure 1. Effects of bisphenol A (BPA) on development of two-cell mouse embryos cultured in the presence of the indicated concentrations of BPA and evaluated at 24-hr intervals for development to eight-cell embryos (O) and blastocysts (●). The symbols * and ** indicate $p<0.05$ and $p<0.01$, respectively, compared with development of control embryos incubated with vehicle (0.1% ethanol) alone. Results are the summation of at least three independent analyses; each group consisted of 100 to 400 embryos.

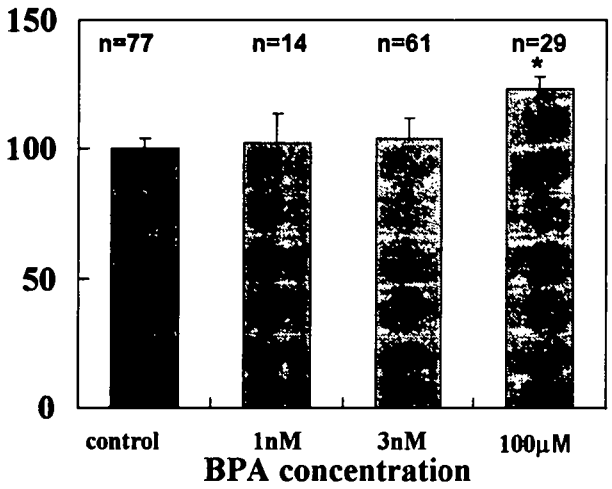

Figure 2. Comparison of the surface areas of the trophoblast spreading after an additional BPA-free culture of blastocysts that previously developed in the presence or absence of BPA. Values are mean±SEM % of the mean trophoblast spreading of control blastocysts that developed with vehicle (0.1% ethanol) alone. Number of the samples was indicated above each column. * indicates $p<0.05$ compared with control