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Alterations in steroid hormone production by porcine ovarian granulosa cells caused by bisphenol A and bisphenol A dimethacrylate

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Abstract

We have investigated the effects of bisphenol A (BPA) and BPA-dimethacrylate (BPA-DMA), endocrine disruptors used as plasticizers, on steroid hormone production by porcine ovarian granulosa cells after 72 h incubation. BPA at 10^{-8} M to 10^{-5} M increased basal progesterone levels, while the same concentration range of BPA-DMA did not cause any changes. After FSH-stimulation of the cells, BPA-DMA showed a tendency to inhibit progesterone production. BPA, however, at 10^{-7} M and 10^{-6} M concentrations was even able to amplify FSH-stimulated progesterone synthesis. BPA as well as BPA-DMA inhibited FSH-induced estradiol production in the whole concentration range. LH-stimulated progesterone production was not altered by BPA in 10^{-8} M to 10^{-5} M, while BPA-DMA decreased progesterone levels in the cultured media. Significant inhibitory effect of both tested agents at 10^{-4} M concentrations was observed specifically on progesterone production, basal as well as gonadotropin-stimulated. The results indicate that ovarian steroidogenesis might be one of the possible sites afflicted by the endocrine disrupting action of BPA and BPA-DMA.

Keywords: Bisphenol A; Bisphenol A-dimethacrylate; Granulosa cells (porcine ovary); Steroidogenesis

1. Introduction

During the last decades, there has been an increasing concern about endocrine disrupting effects of the chemicals, widely present in the environment as a result of industry improvement, and their impact on reproductive health of wildlife animals and possibly of humans. A specific group of compounds termed plasticizers, such as phthalates or phenol derivatives, are supposed to alter endocrine system functions. Bisphenol A (BPA), a monomer chemical used as a plasticizer in manufacture of polycarbonate plastics and epoxy resins, is present in multitude of products, including interior coating of food cans, milk containers, baby formula bottles, compact discs, dental sealants, etc. Studies have shown that incomplete polymerization in these products during manufacture and depolymerization due to increased temperatures for sterilization or heating purposes (Markey et al., 2003a) causes BPA to leach into foods (Brotons et al., 1994), infant formula (Biles et al., 1997) or saliva (Olea et al., 1996). The ubiquitous use of BPA provides a great potential for exposure of the general population, as it is evidenced by a detection of BPA in human serum and follicular fluid (1–2 ng/ml), as well as in amniotic fluid and fetal serum (Ikezuki et al., 2002). Derivative of BPA, bisphenol A-dimethacrylate (BPA-DMA), is used as a compound of dental restorative materials. BPA-DMA, along with BPA and BPA-diglycidyl methacrylate, were found to be released from a BPA-diglycidyl methacrylate-based dental composite and sealant before and after in vitro polymerization (Pulgar et al., 2000). Under different hydrolytic conditions, including the presence of the whole saliva, more than 80% of BPA-DMA is converted to BPA (Atkinson et al., 2002).

BPA is a diphenyl compound containing two hydroxyl groups, making it structurally similar to synthetic estrogen, diethylstilbestrol. Indeed, both BPA and BPA-DMA have shown estrogenic properties in vitro: they have activated estrogen receptors in reporter gene assay (Hiroi et al., 1999; Tarumi et al., 2000) as well as have stimulated MCF-7 cells proliferation (Schafer et al., 1999). Moreover, after in vivo studies in rodents it has also been established that BPA can induce alterations in the reproductive tract. The disrupting effects of BPA in exposed mice on onset of puberty, regularity of estrus cyclicity (Markey et al., 2003b) and development of polycystic ovaries (Kato et

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al., 2003) have been observed. Various studies have shown that BPA can mimic estradiol action in inducing vaginal cornification (Steinmetz et al., 1998), uterine vascular permeability (Milligan et al., 1998), growth and differentiation of the mammary gland (Colerangle and Roy, 1997) and growth, differentiation and *c-fos* gene expression in the female reproductive tract of rats (Steinmetz et al., 1998). Furthermore, BPA exhibits uterotrophic activity in both rats (Ashby and Tinwell, 1998; Laws et al., 2000) and mice (Papaconstantinou et al., 2000). Treatment of mice with BPA-DMA has been shown to result in a disturbance of the female reproductive endocrine functions as well (Darmani and Al-Hiyasat, 2004).

Several endpoints, from the neuroendocrine axis to the structures of the reproductive tract, are supposed to be possible targets of the endocrine disrupting chemicals in their action on the reproductive processes. Granulosa cells constitute the vast majority of the follicular cells in the mammalian ovary. These cells represent the main source of the female sex hormones, estradiol and progesterone, which are synthesized after gonadotropin stimulation and subsequently they control the estrus–menstrus cycle of reproduction. As the presence of BPA was determined in the follicular fluid, the possible influence of this agent and its derivatives on granulosa cell function should be recognized. In the present study, the effects of BPA and BPA-DMA on steroid hormone production by granulosa cells isolated from porcine antral follicles were investigated.

2. Materials and methods

2.1. Chemicals

Medium 199 with Earl's salt, Hepes, and BPA and BPA-DMA were purchased from Sigma (St. Louis, MO, USA). Antibiotics (penicillin, streptomycine, fungizone) and L-glutamine were from PAA Laboratories (Linz, Austria). Fetal bovine serum was from GIBCO (Paisley, Scotland, UK). Human recombinant follicle-stimulating hormone (hFSH) and human recombinant luteinizing hormone (hLH) were generously supplied by Dr. Parlow, NIDDK, NIH (Bethesda, MD, USA). Antiserum against 11-OH-succinyl-BSA was donated by Dr. Tománek, Research Institute of Animal Reproduction (Prague, Czech Republic). DSL-4300 Estradiol RIA Kit was from DSLabs (Webster, TX, USA). The 24-well dishes used were from SARSTEDT (Nümbrecht, Germany).

2.2. Cell culture

Porcine ovaries were collected in physiological saline plus antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml) immediately after slaughter from a local abattoir and transported to the laboratory. Granulosa cells were aspirated from antral follicles (4–6 mm), washed three times in Medium 199 with Earl's salt and Hepes buffer (250 mmol/l) and dispersed in Medium 199 supplemented with L-glutamine (1 mmol/l), penicillin (100 U/ml), streptomycin (100 μg/ml) and fungizone (0.25 μg/ml). Incubation of granulosa cell suspension was carried out in 24-well dishes (with well diameter of 15 mm) at 37 °C in 5% CO_2–95% air (Kolena and Channing, 1985). The density and cell viability were determined in a haemocytometer by trypan blue exclusion. The cell viability ranged from 80% to 90%. Cells were incubated with or without different concentrations of BPA or BPA-DMA, respectively, during a 72-h period at a density of $1–1.2 \times 10^6$ viable cells/0.5 ml in cultured medium supplemented with 10% fetal calf serum, in the presence or absence of hFSH or hLH (each 1 μg/ml), respectively. BPA was initially dissolved in DMSO and at the time of use it was diluted to the required concentrations with cultured medium. The final DMSO concentration in the cultured media was 0.1% (v/v). The same concentration of DMSO was added to the control group. Similar approach was used for BPA-DMA where ethanol was used as a solvent.

2.3. Progesterone and estradiol assay

At the end of the incubation period, the cultured media were collected for progesterone and estradiol determination. Levels of progesterone in media were determined by the [^{125}I]-progesterone radioimmunoassay without extraction (Kolena et al., 1977) using a specific antiserum against 11-OH-progesterone succinyl-BSA. Estradiol concentrations in cultured media were determined by the DSL-4300 Estradiol RIA Kit.

2.4. Statistical analysis

The results were confirmed in five to eight independent experiments. Data were analysed via a one-way analysis of variance (ANOVA) and Bonferroni post-test. Significance was assumed when $P < 0.05$.

3. Results

In the present study, the effects of selected plasticizers, BPA and BPA-DMA, on steroid hormone production by porcine ovarian granulosa cells were investigated. After 72 h incubation of granulosa cells with different concentrations (10^{-8} M to 10^{-4} M) of the tested agents, levels of progesterone and estradiol were determined in the cultured media.

As shown in Fig. 1A, basal progesterone synthesis by granulosa cells was increased after BPA treatment in concentrations from 10^{-8} M to 10^{-5} M (for 10^{-6} M, $P < 0.05$), while the highest concentration (10^{-4} M) significantly inhibited progesterone production ($P < 0.001$). BPA-DMA in concentrations 10^{-8} M to 10^{-5} M did not cause any changes in progesterone production as compared to control; however, similarly to BPA, the highest tested concentration (10^{-4} M) significantly reduced the levels of produced progesterone ($P < 0.001$) (Fig. 1B).

It is well known that gonadotropin stimulation of granulosa cells results in a marked enhance of steroidogenesis (Kolena and Channing, 1972). In our experiments, we studied the action of BPA and BPA-DMA on progesterone and estradiol synthesis by granulosa cells induced by hFSH (1 μg/ml). BPA at 10^{-7} M and 10^{-6} M concentrations was able to amplify FSH-stimulated progesterone release to the cultured medium (for 10^{-6} M, $P < 0.05$), while 10^{-8} M and 10^{-5} M concentrations did not cause any changes (Fig. 1C). In contrary, BPA-DMA showed a tendency to inhibit FSH-induced progesterone production by granulosa cells in the tested concentrations (Fig. 1D). Presence of both tested agents in 10^{-4} M concentrations significantly inhibited FSH-stimulated progesterone synthesis ($P < 0.001$) (Fig. 1C and D).

Inhibitory action of BPA and BPA-DMA on FSH-stimulated estradiol production by the granulosa cells was also observed. Both tested agents in the whole concentration range used (10^{-8} M to 10^{-4} M) decreased FSH-induced estradiol synthesis to control values (for 10^{-6} M to 10^{-4} M, $P < 0.05$) (Fig. 2A and B). Interestingly, concentration of 10^{-4} M did not cause stronger inhibition of estradiol production when compared to remaining concentrations, unlike the effects of the highest concentration of the tested chemicals on progesterone production.