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ULTRASTRUCTURAL CHANGES IN THE OVARIES OF ADULT OFFSPRING FOLLOWING A SINGLE MATERNAL EXPOSURE TO LOW DOSE 2,2', 4, 4',5-PENTABROMODIPHENYL ETHER

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in textiles, electrical housings and foams for furnishings. These additives are not chemically bound to the materials and are released into the environment over time[1]. Indeed, congeners of PBDEs were detected in seven different species representing different trophic levels of the North Sea food web[2] and levels in harbor seals from the San Francisco Bay have risen drastically over the last decade[3]. Correspondingly, human exposure to PBDEs is dramatically increasing as Meironyte *et al.* reported that the total concentration of congeners in human breast milk samples obtained in Sweden has increased from 0.07 to 4.02 ng/g lipids in the period from 1972 to 1997[4]. PBDEs have the potential to interact with the endocrine system in a variety of ways. Using cell line assays based on estrogen receptor dependent luciferase reporter gene expression, some PBDE congeners have been shown to exhibit *in vitro* estrogenicity[5]. It has also been demonstrated that PBDEs can activate the aryl hydrocarbon receptor (AhR)[6]. Furthermore, PBDEs can interfere in thyroid hormone balance in a number of ways. *In vitro* data suggest that hydroxylated metabolites of PBDE can compete with T4 for transthyretin, thereby affecting its transport to target tissues[7]. In addition, metabolites may also be capable of binding to thyroid hormone receptors α and ß leading to altered regulation of thyroid hormone dependent genes[8]. Due to PBDEs broad spectrum of possible activities and the influence of thyroid hormones on several developing systems, we examined possible effects of prenatal and lactational (via milk) exposure to a single congener on the ultrastructure of the adult ovary. The congener, 2,2',4,4',5-pentabromodiphenyl ether (PBDE-99), was chosen because it and 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) have proved to be most abundant in both human and environmental samples[9]. Our group has decided to limit our studies to environmentally relevant doses (low exposure) to better assess possible risks for humans and because the rat is considered to be very sensitive regarding thyroid hormone-related effects.

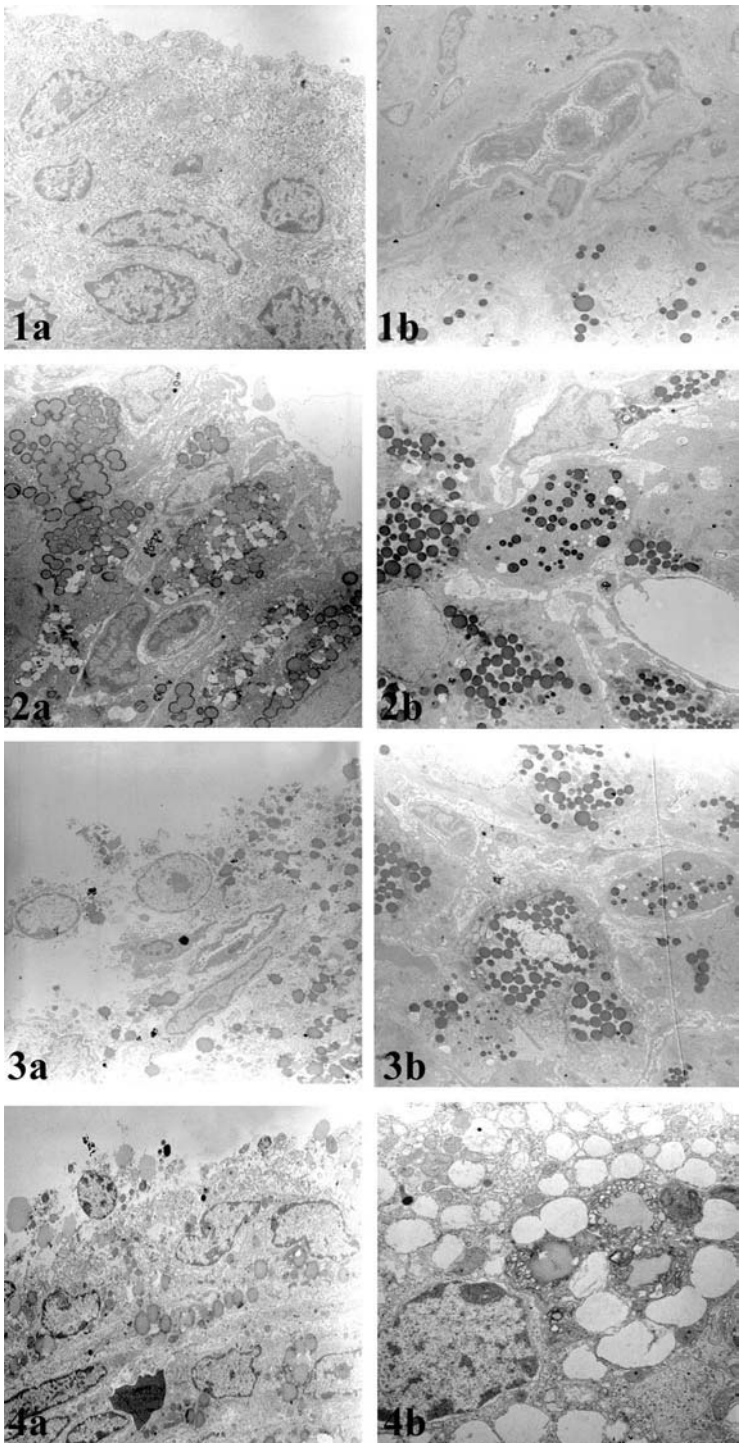
Methods and Materials

Animals and treatment: This report represents a portion of a comprehensive study. Wistar rats were mated once a day until a sperm positive vaginal smear was obtained which was designated as gestational day 0. On gestational day 6, gravid mothers were administered a single dose of either pharmacological grade peanut oil at 10ml/kg bw, 60 µg/kg bw PBDE-99 or 300µg/kg bw PBDE-99 per gavage. The doses administered to the dams were derived from an equation previously described by our group[10] and are respectively, 100 and 500-fold greater than concentrations found in human breast milk. An additional group of animals was treated with 0.5% of the goitrogen, 6n-

propyl-2-thiouracil (PTU), on gestation days 7-21 in the drinking water. The dose administered was approximately 940µg/kg bw/d. The female offspring were weaned on postnatal day 22 and necropsied in estrus (based on vaginal cytology) on approximately postnatal day 90. One ovary from one female offspring in each group was analyzed per electron microscopy. *Electron microscopy(EM):* Tangential sections were made in the middle of the ovary using a razor blade. Subsequently, the ovaries were cut crosswise for preparation of ultrathin sections. All samples were fixed in 1% glutaraldehyde plus 1% tannic acid in 0.1 M phosphate buffer (pH 7.4) and post-fixed in 1% OsO_4 in phosphate buffer. After rinsing and dehydration in ethanol, the samples were embedded in Epon, cut with an Ultracut E and the sections were stained with 2% uranyl acetate / lead citrate. The specimens were examined by transmission electron microscopy.

Results and Discussion

We found characteristic changes at the ultrastructural level in all ovary samples examined from PBDE-treated rats and the following describes the electron microscopic pictures presented below. **Figure 1a** shows the surface of the ovary from the control depicting a rather smooth luminal contour of the serosal epithelium. **Figure 1b** depicts the central portion of the ovary from the control. Stromal cells are present showing vesicular structures with homogeneously dense granular material. **Figure 2a** represents the surface of the ovary from the PTU exposed group which served as the reference control. There is an enormous accumulation of vesicular structures with homogeneously dense granular material which appear to fuse together forming large saccules. **Figure 2b** depicts the central portion of this ovary exhibiting an enormous accumulation of vesicular structures with homogeneously dense granular material in the cytoplasm of the stromal cells. **Figure 3a** demonstrates the surface of the ovary from the 60 µg/kg PBDE-99 exposed offspring. Destruction of the luminal surface of the serosal epithelial cells is apparent. These cells are undergoing necrosis and the organelles are in the process of dissolution. Substantial amounts of vesicular structures with homogeneously dense granular material within the cytoplasm can be seen. **Figure 3b** shows the central portion of the same ovary from the 60 µg/kg exposed offspring. Accumulation of vesicular structures with homogeneously dense granular material in the cytoplasm of the stromal cells are present. There is increased electron density of this granular material. The cells appear grossly intact. **Figures 4a** represents the surface of the ovary from the offspring exposed to 300 µg/kg PBDE-99. Destruction of the luminal surface of the serosal epithelial cells is present with the cells undergoing necrosis. Typical findings are degenerative changes, such as multiple vacuolization and large vesicles in the cytoplasm of ovarian cells that may have derived from swellings and dilatations of cell organelles. Of special interest are aggregates of small and large vesicles filled with homogeneously dense granular material in the cytoplasm and clumped chromatin within the condensed nucleus. **Figure 4b** shows the central portion of the same ovary. Typical findings include the peripheral accumulation of tubular structures surrounding central electron dense fine granular material. Such structures may be derived from swelling, dilatation and degeneration of cell organelles. The over abundance of vesicles appearing to be liposomes in the PTU and PBDE-99 ovaries are compatible with non-specific or uncontrolled synthesis of steroid products. The degenerative changes observed in the 300 µg/kg exposed offspring are suggestive of dissolution of the endoplasmic reticulum and tubular mitochondria which have lost their characteristic appearance. Exposure to low dose PBDE-99, therefore, may lead to altered mitochondrial regulation resulting in loss of controlled synthesis of cellular steroid products. Alterations in the mitochondria could lead to a cellular energy deficit and subsequent cell death. The possibilities regarding the mechanisms of action are numerous.


1a
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There may be a direct effect of the compound or metabolites on the ovary or an indirect action via disturbance of the thyroid hormone balance during development. The dams from the PTU group exhibited changes in thyroid hormone concentrations on PND 1 and the PBDE groups on both PNDs 1 and 22 (unpublished data-Kuriyama). It is known that thyroid hormone receptors exist on the mitochondria and recently, it is suggested that they may also serve as transcription factors, playing a role in regulating gene expression in the mitochondria[11]. Alterations in mitochondrial morphology have also been found in the cerebellum of offspring born to dams with chemically-induced hypothyroidism[12]. In addition, prepubertal hypothyroidism has been shown to alter the distribution of ovarian follicle types[13]. Depending on the concentration used in *in vitro* tests, PBDE-99 shows both agonist and antagonist activities for the AhR[6] . The physiological role of the AhR is unknown and it has been suggested that it plays a role in regulating the oocyte number by germ cell death during gametogenesis[14] or its involvement in the rate at which germ cells are surrounded by somatic cells and regulation of antral follicles has also be indicated[15]. We are in the process of examining whether the ultrastructural changes in the ovary following *in utero* administration of PBDE-99 observed per EM coincide with aberrations in follicle numbers, estrous cycle variations and/or decline in fertility parameters.

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