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EFFECT OF POLYBROMINATED DIPHENYLETHER AND PCB ON THE DEVELOPMENT OF THE BRAIN-GONADAL AXIS AND GENE EXPRESSION IN RATS

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Introduction

A marked increase of PBDE levels in human milk during the last decade has first been noted in Sweden[1]. PBDE levels are also rising rapidly in North America[2]. This increase is in remarkable contrast to the steady decrease of PCB levels. Since human milk is an indicator of both, potential exposure of nursing babies but also body burden of the pregnant woman, it seems necessary to investigate in more depth the developmental toxicity of PBDE.

We investigated possible effects on sexual development in rats with a dual focus on brain regions involved in central control of reproductive processes, and male and female reproductive organs. mRNA levels of sex hormone target genes were determined in brain regions and reproductive organs in order to obtain information on delayed effects at the molecular level. PBDE99 (2,2',4,4'5-pentabromoBDE) was selected because it had been found to exhibit developmental neurotoxicity in mice and was one of the congeners present in human samples[3]. The effects of PBDE99 were compared with those of a commercial PCB mixture, Aroclor 1254, which had previously been studied with regard to sexual brain differentiation[4].

Methods and Materials

The study was conducted on Long Evans rats (Møllegard, Denmark) housed under controlled conditions (lights on 02.00-16.00, 22 ± 1 °C). Time-pregnant females were given one daily subcutaneous injection of chemical dissolved in olive oil or of vehicle from gestational day (GD)10 to GD18 (9 injections) (GD1 = 24 hr after onset of mating period). Experimental groups included: **PBDE99** (2,2',4,4',5-pentabromoBDE, Promochem GmbH, Wesel, Germany, purity > 99%), 1.0 mg/kg/day or 10 mg/kg/day; the commercial **PCB mixture, Aroclor 1254** (Promochem, Wesel), 10 mg/kg/day or 30 mg/kg/day, and vehicle controls (olive oil) accompanying each treatment group. Offspring were analyzed for litter size, body weight gain, perinatal mortality, developmental landmarks including sex-related parameters: sex ratio, anogenital distance, onset of puberty (preputial separation in males, vaginal opening in females), estrous cycle. Litter size was adjusted to 8-10 pups on postnatal day (PN) 2 (PN 1 = day of birth). At 120 days of age, offspring were sacrificed by decapitation (females in diestrus). Uterus, ventral prostate, and dorsal + lateral prostate (designed as „dorsal prostate") were weighed (wet weight) and stored in liquid nitrogen, the brain at -80°C. Two brain pieces, medial preoptic area and ventromedial hypothalamus with ventromedial hypothalamic nucleus, were dissected from 100 µm frontal cryostat sections.

Total RNA was extracted by RNeasy mini Kit 250 (Quiagen) plus a DNAse step, for reverse transcription PCR an Applied Biosystems Kit was used. mRNAs encoding for estrogen receptor (ER) alpha, ER beta, progesterone receptor (PR), androgen receptor (AR), insulin-like growth factor-I

(IGF-I), preproenkephalin (PPE), and cyclophilin were determined by Real Time PCR using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland), and the TaqMan universal master mix (Applied Biosystems). Forward and reverse primers and TaqMan probe were ordered at Microsynth (Balgach, Switzerland). mRNA levels were normalized to cyclophilin as reference gene. Experimental groups were compared with ANOVA followed by pairwise comparisons with Bonferroni correction

Results and Discussion

Prenatal treatment with both, PBDE99 (10 mg/kg/day) and Aroclor 1254 (30 mg/kg/day) delayed the onset of **puberty** in female offspring (Fig. 1, $p < 0.01$). Male puberty was slightly advanced by Aroclor (10 mg/kg/day, $p < 0.05$), and a similar tendency was noted with 1 mg/kg/day PBDE99. The effect of PBDE99 occurred in the absence of signs of general toxicity, whereas Aroclor 1254 reduced postnatal survival rate (30 mg/kg/day) and body weight in adult female offspring (both doses). PBDE99 and the PCB mixture both affected the development of **reproductive organs** as indicated by changes in adult organ weights. Absolute and relative weight of ventral and dorsal prostate (Fig. 2) of adult offspring was increased by PBDE99, but decreased by Aroclor 1254. Adult (absolute and relative) epididymis weight (Fig. 2) was decreased by PBDE99 and increased by Aroclor 1254. Uterine weight of adult offspring was decreased by the PCB mixture ($p < 0.01$) but remained unchanged after prenatal PBDE99.

mRNA levels of estrogen-regulated genes exhibited delayed changes in sexually dimorphic brain areas and reproductive organs of adult offspring. Prenatal treatment with PBDE 99 caused changes in AR, ER alpha, ER beta, and IGF-I mRNA levels in prostate, with differences between prostate lobes (Fig. 3a), and of PR and ER beta mRNA in uterus. Delayed effects of PBDE99 on brain mRNA levels were sex- and region-specific (Fig. 3b). The sex difference in PR mRNA of VMN, with higher leves in female controls ($p < 0.001$), was abolished after prenatal PBDE99 by a reduction of PR mRNA in females and an increase in males. PR mRNA in MPO was unaffected. ER alpha mRNA increased in male and female MPO and in female VMN, a small rise was also seen in male VMN. Analyses of Aroclor 1254 were limited to the lower dose because of the signs of general toxicity in offspring after 30 mg/kg/day. Developmental exposure to the PCB mixture also resulted in delayed changes in mRNA levels, with effect patterns different from PBDE99 (Fig. 3).

PBDE, specifically PBDE99, thus can interact with the development of the mammalian hypo-thalamo-pituitary-gonadal axis, both at brain and reproductive organ levels. This action would not have been predicted from in vitro assays. PBDE99 exhibits low, if any, estrogenic activity in MCF-7 cells[5]. We did not detect in vitro androgenic or antiandrogenic activity on MDA-kb2 cells which express AR and are transfected with a luciferase reporter plasmid (data not shown). The treatment period (GD10-GD18) covered sexual differentiation processes from late embryonic stages. Since PBDE accumulate, we assume that exposure continued beyond birth and overlapped also with the second part of sexual brain differentiation in the first postnatal week. Developmental exposure to PBDE99 affected the sexual dimorphism of PR mRNA expression in VMN of adult offspring. PR expression in VMN is of critical importance for female sexual behavior in rats[6].

Conclusions

Our data indicate that PBDE99 exhibits features of endocrine disrupters in vivo, at the organ level and molecular level. It induces effect patterns that differ in part from PCB.

Fig. 1. Effect of prenatal treatment with PBDE99 (P1 = 1.0 mg/kg/d, P10 = 10 mg/kg/d) or Aroclor 1254 (Aro10 = 10 mg/kg/d, Aro30 = 30 mg/kg/d) on vaginal opening and preputial separation in Long Evans rats. Differences from vehicle control: * p <0.05, ** p <0.01.

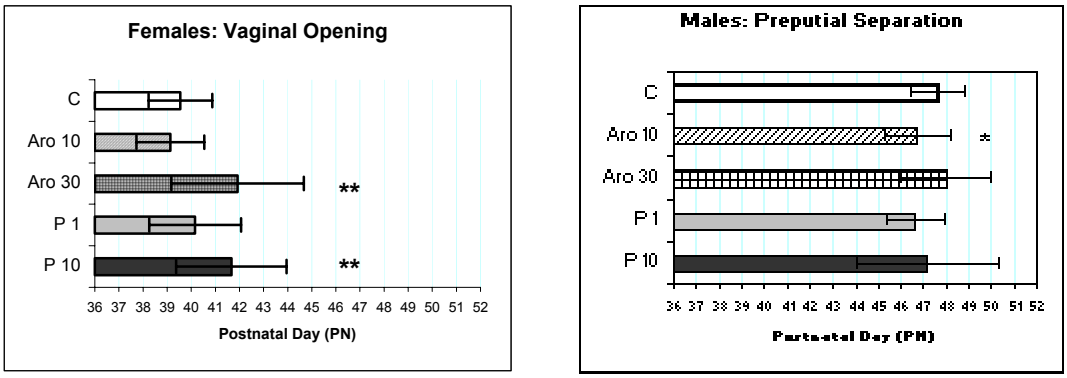


Fig. 2. Effect of prenatal treatment with PBDE99 or Aroclor 1254 on reproductive organ weights of adult male and female rat offspring.

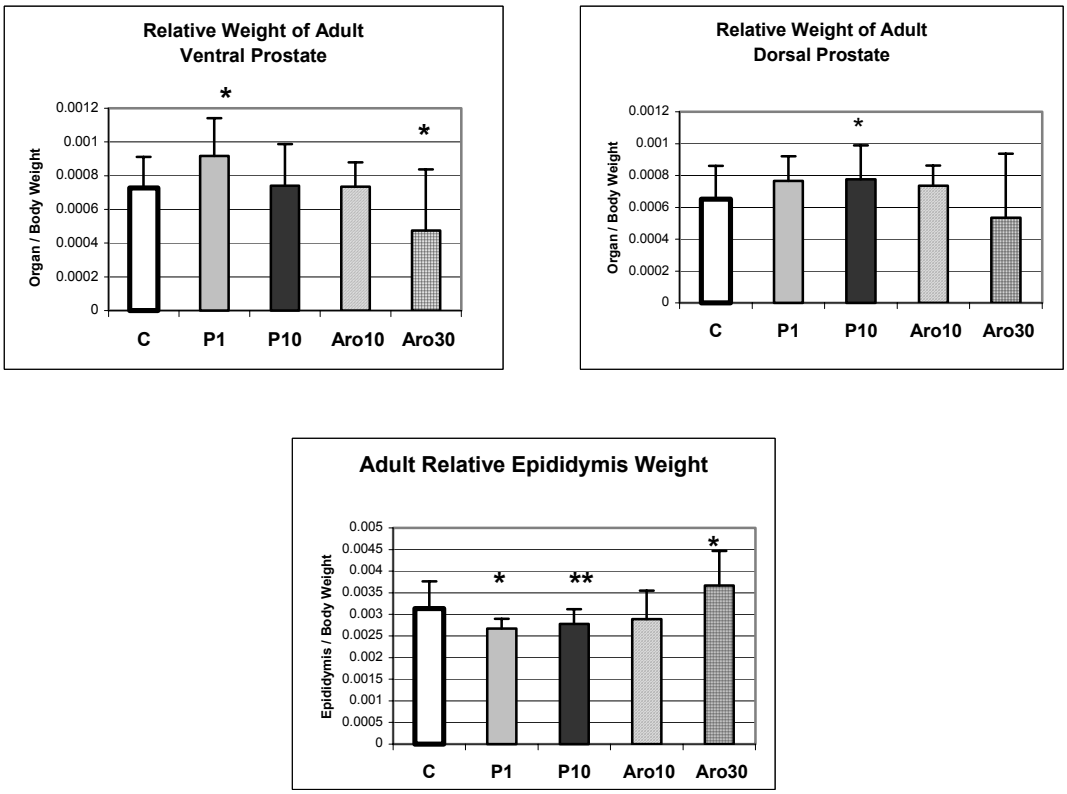
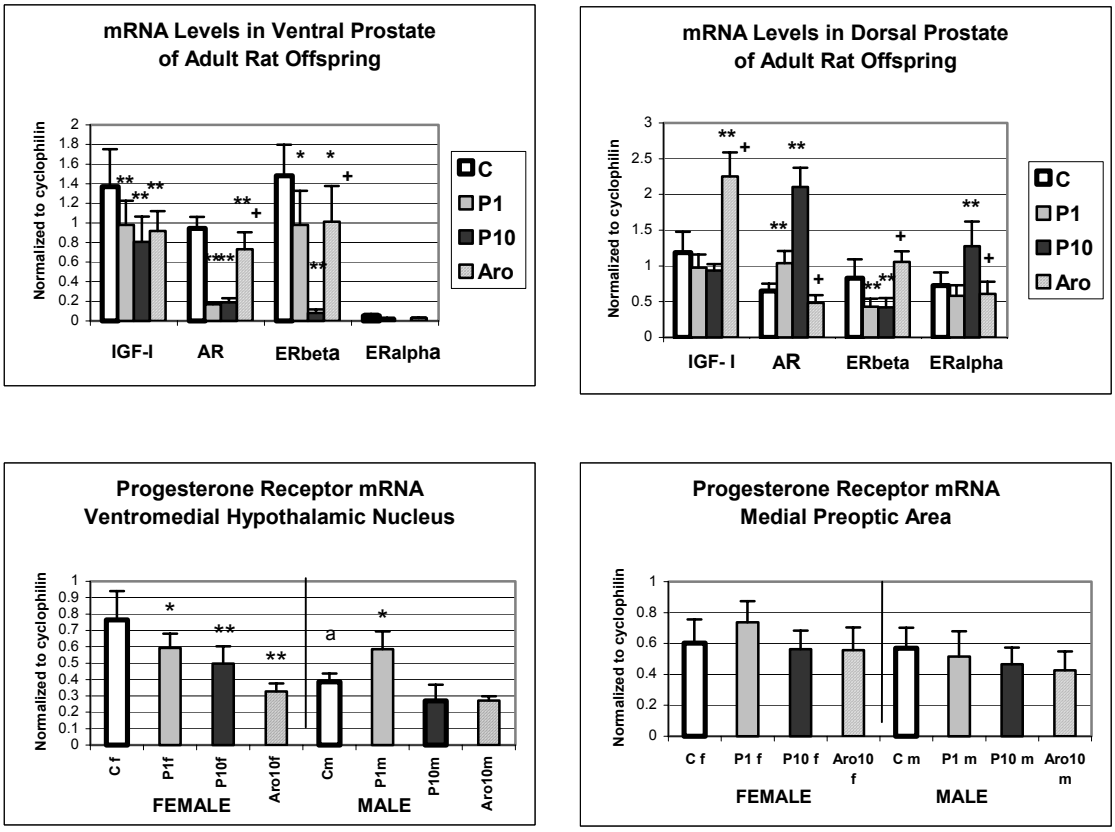


Fig. 3. Effect of prenatal treatment with PBDE99 or Aroclor 1254 (10 mg/kg/d) on sex hormone target gene mRNA levels in prostate (top), and brain regions (bottom) of adult male and female rat offspring. Mean ± SD, n = 7–10 animals. * / **: different from vehicle control for p <0.05/0.01, +: difference between Aro10 and P10 for p<0.01, a: sex difference in controls for p <0.001.



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