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Lactational exposure of polychlorinated biphenyls impair Leydig cellular steroidogenesis in F_1 progeny rats

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ABSTRACT

The present study was aimed to determine the effects of lactational exposure of PCBs (Aroclor 1254) on Leydig cellular steroidogenesis in F_1 progeny rats. Lactating dams were orally treated by gavage with different doses of PCBs (1, 2 and 5 mg/kg b.wt./day). Male progenies were sacrificed on PND60. Our results demonstrated that exposure to PCBs decreased the body weight, testis weight and anogenital distance (AGD) index in the F_1 progeny rats. Importantly, PCBs exposure reduced the serum levels of LH, testosterone and estradiol. Interestingly, PCBs caused a decrease in the Leydig cell population along with decreased activities of steroidogenic enzymes 3β- and 17β-HSD. Additionally, we observed a significant decrease in LHR, SR-B1, StAR protein, Cyp11a1, 3β-HSD, Cyp17a1, 17β-HSD, 5α-reductase, Cyp19a1 and AR gene expression in the Leydig cells of progeny rats. In conclusion, our study demonstrates that lactational exposure of PCBs alters Leydig cellular steroidogenesis in the F_1 progeny rats.

1. Introduction

Polychlorinated biphenyls (PCBs) are well recognized endocrine disrupting chemical (EDC)[1], which mimics hormones, thus modulate hormone-dependent gene expression including inhibition of steroidogenic enzymes that leads to defect inmale reproductive function [2,3]. PCBs are halogenated, lipophilic, aromatic hydrocarbon mixtures of 209 congeners, which are generally used as insulators in electrical equipment, plasticizers in polyvinyl carbonate (PVC) products, de-inking solvents for recycling of carbonless newspaper, and waterproofing agents. PCBs are slow to biodegrade in the environment and once released into the environment, they tend to partition to the more organic components of the environment. As a result, they have been detected as contaminants in almost every component of the global ecosystem including the air, water, sediments, fish, wildlife, and human adipose tissue, milk and serum [1–5].

The testes are the most sensitive target organ for PCBs, which can disrupt the Leydig cellular steroidogenesis and sperm count [6–8]. The toxic effect of PCBs are exerted through the arylhydrocarbon receptor (AhR) pathway and also through cross-talk of the AhR and AhR nuclear transport protein (ARNT) transcription factors with other nuclear receptors that disrupts cellular functions in male offspring [9,10].

Leydig cells in the testis are the primary source of testosterone in rodents, which is essential for development of male reproductive system and the maintenance of reproductive function [11]. Leydig cells are recognized into two waves as, foetal Leydig cells up to PND10 [12] and adult Leydig cells approximately from day 56 to 70 postpartum, which continues for the reminder of life [13,14]. Luteinizing hormone (LH) is the most important hormone for control of Leydig cell functions and steroid production [15]. LH acts on Leydig cell through LH receptors leading to activation of cAMP pathway [16]. Subsequent activation of cAMP cascade triggers a serious of reactions including de-estrification of cholesterol and transport of cholesterol into mitochondria through steroidogenesis acute regulatory protein (StAR) [17–19]. Leydig cells utilize scavenger receptor class B-type 1 (SR-B1) mediated cholesterol esters for testosterone production [20]. Transported cholesterol is metabolized into pregnenolone by the cytochrome P_{450} side chain cleavage enzyme (Cyp11a1) [21–24] and pregnenolone is subsequently metabolized in the smooth endoplasmic reticulum by a series of enzymes namely cytochrome P_{450} (Cyp17a1) [25], 3β-HSD and 17β-HSD to form

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testosterone [24,26]. The testosterone is further metabolized in the Leydig cells by enzyme 5α-reductase, irreversibly reduced to delta 4,5 bond of c-19 and c-21 steroids to 5α-stereoisomers dihydrotestosterone (DHT) [27,28]. Consequently, cytochrome P450 aromatase (Cyp19a1) transforms androgens into estrogens in Leydig cells [29], which is also vital for male reproductive physiology and fertility [30].

Early developmental exposures to PCBs are particularly devastating, and can have different outcomes from adult exposure. However, there are many studies demonstrating that premature postnatal day exposure of male offspring to PCBs leads to hypoandrogenic condition which is harmful to normal adult reproductive function [31,32]. Our previous study also showed that lactational exposure to PCBs downregulated the 5α-reductase, aromatase (Cyp19a1) and androgen receptor (AR) genes expression in Leydig cells of PND21 (prepuberal) rats with reduced anogenital distance (AGD), testis weight and Leydig cell count [33]. Both *in vitro* and *in vivo* studies from our laboratory have also confirmed that impairment in male reproductive function due to PCBs exposure [8,34,35].

The effect of acute exposure to PCBs resulted in decreased LH secretion in adult rats [36] perturbed with significantly reduced cholesterol transporter steroidogenic acute regulatory protein (StAR) expression [35,37]. Decreased steroidogenesis and serum testosterone levels were due to dowenregulated transcriptional and translational levels of P_{450} side chain cleavage and P_{450} 17α enzymes in the PCBs injected rats [38]. The potential acute toxic effect of PCBs were confirmed by reduced activity of 3β-HSD, which interferes with the conversion of pregnenolone to progesterone and reduced activity of 17β-HSD, which converts androstenedione to testosterone [39]. Furthermore, PCBs impaired steroidogenesis by inhibiting steroidogenic enzymes extreme up to terminal metabolism of testosterone through SRD5A1 and Cyp19a1 to DHT and estradiol [40–42]. Earlier studies from our laboratory by Murugesan et al. [8] revealed reduced concentrations of testosterone in PCB exposed adult rats. In addition, PCBs also reduced the semen quality, Sertoli cell function and fertility [6]. Our recent findings suggest that lactational exposure of PCBs downregulated the critical genes of Leydig cells in F_1 male progeny and altered the testicular architecture [33,43]. Sekaran and Arunakaran [44] demonstrated that *in utero* exposure to another EDC namely phthalate (DEHP) downregulated the critical genes in Leydig cells of F_1 male progeny on PND60. Therefore, we hypothesized that lactational exposure of PCBs may disrupt Leydig cell steroidogenesis in F_1 progeny male rats. The objective of the study was to determine the effects of PCBs on the expression of genes involved in Leydig cell steroidogenic machinery on PND60 F_1 progeny male rats. We also studied the effects of PCBs on serum hormone levels, Leydig cell count, enzyme activities of 3β-HSD and 17β-HSD. Further, the mRNA and protein expressions of LHR, scavenger receptor class B-type 1 (SR-B1), StAR protein, cytochrome P_{450} side chain cleavage enzyme (Cyp11a1), 3β- and 17β-HSD, cytochrome P_{450} (Cyp17a1), 5α-reductase, aromatase (Cyp19a1) and androgen receptor (AR) were determined in the Leydig cells of F_1 male progeny rats.

2. Materials & methods

2.1. Materials

PCBs sandy loam 1 (DG913), total RNA isolation reagent (TRIR) and β-actin monoclonal antibody were purchased from Sigma-Aldrich Pvt. Ltd. (St. Louis, MO, USA). RT-PCR kit RevertAid reverse transcriptase was purchased from Thermo Fisher Scientific (Waltham, MA USA) and quantitative PCR (qPCR) reagents was purchased from KAPA Biosystems (Boston, USA). LH, testosterone and estradiol levels were determined using ELISA kit purchased from CUSABIO (USA). The polyclonal LHR, SR-B1, StAR protein, Cyp11a1, 3β-HSD, Cyp17a1, 17β-HSD, 5α-reductase, Cyp19a1 and AR primary antibodies and horseradish peroxidase (HRP)-conjugated anti-mouse and goat-anti-rabbit secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). PVDF membrane was purchased from Millipore (USA). All other analytical grade (AR) chemicals were purchased from Sisco Research Laboratories (Mumbai), India.

Table 1
List of Primer Sequences Used in the Study.

S. no	Target gene	Primer sequence (5′ → 3′)	Ampliconsize (bp)	Annealing temp (°C)/ no of cycle	Genebank accession number
1	Luteinizing hormone receptor (LHR)	5′ ACGCTCATCGCCCTGTCTTCCT 3′ 5′ ATTCCTGAAGGCGCAGGAGTGG 3′	114	58 °C/40	>NM_012978.1
2	Scavenger receptor class B, member 1(SR-B1)	5′TGCGGGAGTTTGGAGTTGTT 3′ 5′AGCACTCAGGCCAACCAAAT 3′	160	58 °C/40	XM_017598269.1
3	Steroidogenic acute regulatory protein (STAR)	5′ GCAGGCATGGCCACACAC 3′ 5′ TGAGCAGCCAGCTGAGTT 3′	133	58 °C/40	NM_031558.3
4	Cytochrome P450 side-chain cleavage enzyme (Cyp11a1)	5′ CCAAGACTTTGGTGCAGG 3′ 5′ AACACCCCAGCCAAAGCC 3′	146	58 °C/40	NM_017286.2
5	3-beta-hydroxysteroid dehydrogenase(3β-HSD)	5′ GTTGTCATCCACACCGCT 3′ 5′ CTTCGACGCAGGCCTCCA 3′	112	58 °C/40	NM_001007719.3
6	Cytochrome P450 (Cyp17a1)	5′ CCATACCTCAACACCCACAG 3′ 5′ CCCATTGCTGCCAACTAGAA 3′	100	58 °C/40	XM_006231435.3
7	17-beta-hydroxysteroid dehydrogenase (17βHSD)	5′ATGCTCCCCAACCTGCTCCCAA3′ 5′AGGCCTCTCCTTGATTCCA 3′	214	58 °C/40	NM_054007.1
8	5-alpha-reductase(Srd5a1)	5′ACCAGAGCGAAGCAGCACCA 3′ 5′ TGGGAGGCAACAGCGCTAACA 3′	116	58 °C/40	NM_017070.3
9	Aromatase (Cyp19a1)	5′AGGAGCCTTTACCTGCTCTTGGT 3′ 5′GCCCTTGAGTGGGTAGAGTGACG3′	122	58 °C/40	NM_017085.2
10	Androgen receptor(AR)	5′ GGTAATATCCGAAGGCAGCA 3′ 5′ TCCCCTGGACTCAGATGTTC 3′	183	58 °C/40	NM_012502.1
11	β-actin	5′GGGAAATCGTGCGTGACATT 3′ 5′CGGATGTCAACGTCACACTT 3′	253	58 °C/40	NM_031144.3