Effects of Exposure of Lactating Female Rats to Polychlorinated Biphenyls (PCBs) on Testis Weight, Sperm Production and Sertoli Cell Numbers in the Adult Male Offspring

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ABSTRACT. To investigate the effects of intermittent and continuous exposure of lactating rats to Aroclor 1242 (a PCB congener), testis weight, daily sperm production (DSP) and Sertoli cell number per testis were examined in the adult male offspring. Thyroxine (T₄) was also measured because of the well-documented effects of polychlorinated biphenyls (PCBs) on this hormone. In experiment I, 3 groups of lactating female rats received daily subcutaneous injections of low (0.8 mg) or high (1.6 mg) doses of Aroclor 1242 in 0.1 ml corn oil from parturition to weaning of pups at 21 days. In experiment II, 3 groups of lactating rats received 2 subcutaneous injections per week of 0.8 or 1.6 mg Aroclor 1242, as in experiment I. In both experiments, control rats received vehicle alone. Serum T₄ was measured at 21 and 90 days of age, and testis weight, DSP and Sertoli cell numbers were examined at 90 days. In experiment I (continuous exposure), both the low (0.8 mg) and high (1.6 mg) doses suppressed T₄ concentrations at 21 days of age. Testis weight was increased by 14.8% (LD) and 16.5% (HD) compared with controls. DSP was increased by 20.4% in the low dose and 25% in the high dose animals compared with controls. The number of Sertoli cells per testis was increased by 32.6 and 39.4% in low and high dose animals, respectively. A similar study in which the lactating females were only dosed twice per week (experiment II) did not show any differences in these parameters. These results indicate that continuous exposure of lactating female rats to PCBs increases testis weight, sperm production and Sertoli cell numbers in the adult male offspring.

KEY WORDS: PCB, Sertoli cell number, sperm production, testis weight.


Polychlorinated biphenyls (PCBs) are a family of compounds with paired phenyl rings and various degrees of chlorination which were produced by the Monsanto Chemical Company of St. Louis, Missouri under the trade name of Aroclor. There are several different Aroclor mixtures, each of which contain a unique composition of PCB congeners [13]. Because of their widespread use, general stability, and the lack of control of their disposal, PCBs became a worldwide pollution problem between their introduction and their restriction by most governments during the 1970s [25]. Since 1966 PCBs have been detected in biological samples and it is believed that contaminated food constitute the major source of PCB exposure to the general population [24, 26]. PCBs have shown to be well absorbed from the gastro-intestinal tract of rats [1]. Moreover, PCBs have been reported to be transferred through the placenta to fetus and via milk to the neonate [2, 7, 16], with greater transfer occurring during lactation than gestation, at least in humans [16] and rats [30].

Previous studies have suggested that PCBs can have deleterious effects on various aspects of reproduction in humans [5], rats [27, 28], minks [3], guinea pigs [15], gray seals [11], and cockerels [23]. PCBs cause hypothyroidism in treated animals [7, 10, 20]; this hypothyroidism may be related to some of the disturbances in reproduction, growth, and development reported in animals and humans associated with PCB exposure.

Although PCB transfer could occur from mother to the suckling offspring, information concerning the effects of neonatal PCB exposure via milk on the reproductive function of the adult male offspring is sparse and the information is limited to the changes of the testicular and accessory gland weights [27, 29]. Therefore, the present study was conducted to investigate the effects of intermittent and continuous exposure of lactating female rats to Aroclor 1242, on testis weight, DSP and Sertoli cell numbers in the adult male offspring.

MATERIALS AND METHODS

Animals and treatments: Female Sprague Dawley rats were purchased from Bio-Safety Institute (Chonbuk National University, Chonju, Korea) at mid-pregnancy. They were maintained under conditions of controlled temperature (25°C) and lighting (14L:10D). All animals were fed with normal rat chow and water ad libitum until the birth of pups. After birth, pups were divided by sex, the male pups were retained, and litter size was adjusted to five to seven. In experiment I, lactating female rats were divided into 3 groups; mothers in each group received daily subcutaneous injections of 0.1 ml of corn oil, low (0.8 mg) and high (1.6 mg) doses of Aroclor 1242 (Ultra Scientific Co., North Kingstown, RI) in corn oil respectively, from parturition to weaning of pups at 21 days. In experiment II, 3 groups of lactating female rats received 2 injections per week of 0.1 ml of corn oil, low and high doses of Aroclor 1242 in corn oil respectively, as in experiment I. After weaning, all rats were fed with normal rat chow and water ad libitum. All pups were weighed to the nearest gram weekly throughout life.
Assay of thyroxine (T₄): Blood samples (8 rats per treatment) were taken from all groups by cardiac puncture under ketamine/xylazine anesthesia at 21 and 90 days to evaluate the severity of the hypothyroidism induced by the various treatments. Serum was separated by centrifugation and frozen at −20°C for determination of T₄. Total T₄ concentrations were determined using a commercially available radioimmunoassay kit (Coat-A-Count Total T₄, Diagnostic Products Corp., Los Angeles, CA). Each sample was assayed in triplicate. The lower limit of the T₄ assay was 0.6 µg/dl, with interassay and interassay coefficients of variation of 3.0 and 3.5%, respectively.

Determination of sperm production: At 90 days of age, control and treated rats (6 rats per treatment group) in each experiment were euthanized by inhalation of carbon dioxide gas. Daily sperm production (DSP) was determined by the procedure of Cooke et al. [8]. Briefly, after removal and weighing of testes, the tunica albuginea was removed. Testes were then homogenized for 2 min in 50 ml physiological saline containing 0.05% (vol/vol) Triton X-100 and 0.25 M thimerosal (Sigma Chemical Co., St. Louis, MO) using a semimicro blender. Step 17–19 spermatids (stage IV-VIII), which survive this homogenization, were counted using a hemocytometer to obtain total number of spermatids per testis. In the Sprague-Dawley rat, developing spermatids spend approximately 6.3 days in steps 17–19 of spermatogenesis [6]. Thus, the values for the number of spermatids per testis were divided by 6.3 to obtain total daily sperm production.

Fixation and processing of testicular tissue: One testis from each rat (4 rats per treatment group) was removed under ether anesthesia and weighed on a BP 210 S (Sartorius, Germany) balance to obtain the fresh testis weight. The specific gravity of the fresh testis was determined by the flotation technique [17–19] to determine fresh testis volume. The other testis of each 90 day old rat was fixed in situ by whole body perfusion using 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) as described previously [18]. During counting, the fields were selected randomly without overlap (area of the test frame = 4230 µm²). Thirty to forty areas per block and 10 blocks per rat were scored.

The number of Sertoli cells per testis was calculated by multiplying the numerical density by the fresh testis volume [17].

Statistics: ANOVA was performed followed by Duncan’s multiple-range Test. Data were expressed as mean ± standard error of the mean. Differences were considered to be significant when P<0.05.

RESULTS

Body weight: In both experiments, body weights in 21-day rats that exposed either 0.8 or 1.6 mg/day of Aroclor 1242 during lactation period were not significantly different than oil-treated controls (Exp I, 63–71 g; Exp II, 68–73 g). In experiments I and II, body weights in 90-day rats ranged from 426–452 g and 439–457 g, respectively. At 90 days of age, body weights of rats did not show any statistically significant differences among the Aroclor treated and control rats in both experiments.

Serum T₄ concentrations: In experiments I, both the low (0.8 mg) and high (1.6 mg) doses suppressed T₄ concentrations at 21 days of age. In experiment II, T₄ concentrations in the treated groups were not different than the control values at day 21 (Fig. 1). At 90 days of age, serum T₄ concentrations in all groups were normal (data not shown).

Testis weights: Testis weight from control and Aroclor-treated rats is shown in Fig. 2. In experiment I, testis weight of corn oil rats was determined as 1.88 ± 0.08 g. Testis weight was increased by 14.8% (low dose) and 16.5% (high dose) compared to controls. In experiment II, testis weight of corn oil rats was determined as 1.91 ± 0.12 g. Testis weight of the low and high dose rats was not significantly (P>0.05) different compared to corn oil-treated controls (Fig. 2).

Daily sperm production: Daily sperm production (DSP) in control and PCB-treated rats is shown in Fig. 3. In experiment I, DSP in control rats was 71.4 ± 6.3 million. Daily sperm production in PCB-treated rats was significantly (P<0.05) increased compared to controls. The magnitude of the increase was pronounced: low PCB rats, 20.4%; high PCB rats, 25%. In experiment II, DSP in control, low and high PCB rats was 73.6 ± 6.8, 71.3 ± 5.7 and 74.4 ± 7.6 million, respectively. DSP in PCB-treated rats (both the low and high doses) was not significantly (P>0.05) different com-
The number of Sertoli cells per testis: The number of Sertoli cells per testis in control and PCB-treated rats is shown in Fig. 4. In experiment I, control testes contained 35.1 ± 2.6 million Sertoli cells. The number of Sertoli cells per testis was increased by 32.6% in the low dose and 39.4% in the high dose animals compared to controls. In experiment II, the number of Sertoli cells per testis was 36.8 ± 2.1 million in controls. The number of Sertoli cells per testis in low and high PCB-treated rats was 37.6 ± 2.8 and 36.7 ± 3.7 million, respectively. The number of Sertoli cells per testis in PCB-treated rats (both the low and high doses) was not significantly (P>0.05) different compared to controls (Fig. 4).

DISCUSSION

The present study report the earliest findings ongoing investigations on the effect of continuous and intermittent PCB exposure of lactating rats on the reproductive function of the adult male offspring. With very high doses of PCBs, Sager [27] could not observe a change in body weight in neonatally PCB exposed rats at adulthood. Therefore, the unaltered body weights in the present study is not a surprise, because the doses used in the present study are 15 and 30 less than the high dose of Sager’s study [27].

Sager et al. [29] reported that early postnatal exposure to Aroclor 1254 do not affect the morphology or motility of sperm, however the ability of these sperm to fertilize eggs is severely impaired. Reduction in seminal vesicle and prostate...
weights in the adult male offspring of mothers expose to very high doses of Aroclor 1254 was previously report by Sager [27] which suggest a hypoandrogenic condition in such rats. However, it is difficult relate these findings with the effects of PCB via environmental exposure, because such high doses are not found in the environment. The high dose used in the present study is approximately 5 times lower than the lowest dose used previously to evaluate the effects of neonatal PCB exposure via mother’s milk on male reproductive function at adulthood [27, 29]. Therefore, the levels of PCB exposure used in the present study are more closer to what can be found in polluted sites have been used to test exposure of lactating mothers to PCBs on the testicular function of the adult male offspring.

PCB treatment is effective in increasing adult testis size and DSP if started at birth, but not if treatment begins at 12 days of age [10]. This neonatal window of responsiveness is similar to that described for PTU, an antithyroid drug [8], which also suggests that hypothyroidism is the causative factor responsible for the testis effects of PCBs. When either PCB or PTU treatment is started after the first week of life, Sertoli cell mitogenesis is nearly complete when systemic hypothyroidism is finally manifested, and this treatment therefore no longer affects Sertoli cell numbers of adult testis weight and DSP.

The mechanism by which PCB exposure beginning at birth increases adult testis size is unknown. Previous studies have found either no effect or a small increase in testis size and no increases in DSP following early PCB treatment. Gray et al. [12] reported that extended Aroclor 1254 treatment of rats starting at 31 days of age suppresses T4, but did not affect testis weight or sperm numbers. However, these treatments began after Sertoli cell proliferation was complete, and therefore could not produce the increased Sertoli cell numbers, testis weight, and DSP observed in the present study. Sager [27] reported that treatment of rats from birth to day 9 with 64 mg/kg of Aroclor 1254 every other day increased testis weight at 180 days of age but did not increase sperm production [29]. Cooke et al. [10] reported that neonatal injection of rats from birth to day 24 with Aroclor 1242 or 1254 (0.4–3.2 mg/day) every day increased testis weight and sperm production at 135 days of age. Exposure of fetal and neonatal guinea pigs to PCB in placenta and by the milk during days 18–60 of gestation with oral dose 2.2 mg of Clophen A50 every day until 3 weeks of age decreased testis weight at 90 days of age [15]. In the present study, both the low (0.8 mg) and high (1.6 mg) doses of experiment I increased in testis weight and sperm production, but experiment II did not affect. Therefore, possible explanations for the differences in testis weight and sperm production between earlier results and the present findings could be time and method of treatment, dosages used, kind of PCB congeners, and time of sacrifice.

Results of the present study showing that continuous PCB treatment in the early postnatal period increases sperm production in the adult. These findings are in agreement with rapidly emerging evidence that developing Sertoli cells, the primary regulators of sperm production [4], are responsive to thyroid hormones. For example, Sertoli cell development and insulin-like growth factor I production is inhibited in young hypothyroid rats [22], and T4 directly stimulates insulin-like growth factor I production by these cells in vitro [21].

Previous work on the effects of thyroid hormones on the developing testis and its constituent cells [9] supports the following proposed mechanism for PCB effects. Thyroid hormones directly suppress mitogenesis of neonatal Sertoli cells in vitro [9], presumably by acting through the thyroid hormone receptors expressed in these cells neonatally [14]. Therefore, in PCB-treated animals, decreased levels of thyroid hormones may directly result in increased Sertoli cell numbers. The increased Sertoli cell population may then be responsible for the increases in testis weight and daily sperm production which characterize this phenomenon. It is unclear why intermittent PCB exposure did not affect on testis weight, sperm production and Sertoli cell numbers. Additional studies are required to explain the differences between continuous and intermittent PCB effects.

In summary, data of the present study indicate that continuous exposure, but not intermittent exposure of male rats to Aroclor during the lactation period increases testis weight, sperm production and Sertoli cell numbers in the adult male offspring.

REFERENCES


