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ORGAN TOXICITY AND MECHANISMS

Cypermethrin exposure during puberty disrupts testosterone synthesis via downregulating StAR in mouse testes

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Abstract Cypermethrin is a widely used synthetic pyrethroid insecticide. Previous studies showed that cypermethrin significantly decreased the fertility and reduced the number of implantation sites and viable fetuses in females impregnated by males exposed to cypermethrin. As yet, little is known about the mechanism of cypermethrin-induced reproductive toxicity. In the present study, we investigated the effects of cypermethrin exposure during puberty on steroidogenesis in mice. Young male mice were administered with cypermethrin (25 mg/kg) by gavage daily from postnatal day (PND) 35 to PND70. Results showed that the level of serum and testicular testosterone (T) was markedly decreased in cypermethrin-treated mice. Additional experiment showed that cypermethrin exposure during puberty markedly downregulated mRNA level of steroidogenic acute regulatory protein (StAR) in testes. Correspondingly, protein level of testicular StAR was significantly decreased in cypermethrin-treated mice. Cypermethrin exposure during puberty did not affect the number of Leydig cells in testes. Although cypermethrin exposure during puberty did not affect the weight of testes and epididymides, the number of sperm in the cauda epididymides was significantly decreased in cypermethrin-treated mice. Taken together, these results indicate that cypermethrin exposure during puberty significantly disrupts T synthesis via downregulating the expression of testicular StAR. The decreased T synthesis might be associated with cypermethrin-induced impairment in spermatogenesis in mice.

Keywords Cypermethrin · Pyrethroid · Testes · Steroidogenesis · Testosterone · Steroidogenic acute regulatory protein (StAR)

Introduction

Cypermethrin is a synthetic pyrethroid insecticide, which has been commonly used to control noxious insects in agriculture, forestry, horticulture, public health, and so forth. Cypermethrin is a well-known neurotoxicant (Dési et al. 1986; McDaniel and Moser 1993; Wolansky and Harrill 2008). In vivo studies showed that cypermethrin exposure resulted in alterations of motor function and impairments of neurobehaviors in rodents (McDaniel and Moser 1993; Wolansky et al. 2006, 2008). Like other synthetic pyrethroid insecticides, cypermethrin is also a mutagen (Bhunya and Pati 1988; Surrallés et al. 1995). An earlier study showed that a single dose of cypermethrin significantly increased the frequency of sister chromatid exchange in mouse bone marrow cells (Chauhan et al. 1997). A recent study found that cypermethrin increased the incidence of sister chromatid exchange, chromosomal aberrations, and micronuclei in human peripheral lymphocytes (Kocaman and Topaktas 2009).

Recently, pyrethroids have been found to have potentially adverse effects on male reproduction. An earlier study showed that fenvalerate exposure to adult rats by inhalation obviously induced the decrease in testicular weight, epididymal sperm counts, and sperm motility (Mani et al. 2002). In addition, the expression of testicular 17β-hydroxysteroid dehydrogenase (17β-HSD), a testosterone (T) biosynthetic

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enzyme, was markedly downregulated in fenvalerate-exposed rats. Correspondingly, serum T was markedly decreased in rats exposed to one-fifth LC_{50} of fenvalerate by subchronic inhalation for 3 months (Mani et al. 2002). Nonetheless, relatively few studies have investigated the adverse effects of cypermethrin on male reproduction. According to an earlier report, cypermethrin significantly decreased the fertility and reduced the number of implantation sites and viable fetuses in females impregnated by males exposed to cypermethrin. In addition, the number of epididymal sperm as well as daily sperm production was significantly decreased in males exposed to cypermethrin (Elbetieha et al. 2001).

T, a primary male steroid hormone, which acts through the somatic cells to regulate germ cell differentiation, is a prerequisite for normal spermatogenesis (Holdcraft and Braun 2004). Steroidogenic acute regulatory (StAR) protein and T biosynthetic enzymes play a critical role in the synthesis of T in Leydig cells. Cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) initiates the first enzymatic step in T biosynthesis in the inner mitochondrial membrane of Leydig cells, whereas cholesterol is converted to pregnenolone. Pregnenolone is catalyzed by cytochrome P450 17α-hydroxysteroid dehydrogenase ($P450_{17\alpha}$) to produce 17-hydroxyprogesterone and androstenedione, with the latter then being converted to T by 17β-HSD (Payne and Youngblood 1995). According to a recent study, permethrin, another pyrethroid insecticide, significantly downregulated the expression of P450scc in mouse testes (Zhang et al. 2007). However, little is known about cypermethrin-induced testicular toxicity and endocrine disruption.

In this study, we investigated the effects of cypermethrin exposure during puberty on testicular T synthesis in mice. Our results indicate that cypermethrin exposure during puberty markedly disrupts T synthesis via downregulating the expression of StAR in mouse testes.

Materials and methods

Chemicals

Cypermethrin was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Sigma or as indicated in the specified methods.

Animals and treatments

Male CD-1 mice (5-week-old, 22–24 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25°C) and humidity (50 ± 5%) environment. Male mice were orally administered with cypermethrin (25 mg/kg) daily from postnatal day 35 (PND35) to PND 70. The corn oil-treated mice served as controls. All mice were slaughtered at PND70. Blood sera were collected for measurement of T. Cauda epididymides were removed for analysis of sperm quality. Testes were excised, dissected, weighted, and then divided in two parts: left one was kept at −80°C for subsequent measurement of T, RT-PCR and immunoblotting. The other part of the testes was immersed in modified Davidson's fluid (mDF) for 12–24 h for histology and apoptosis analysis (Latendresse et al. 2002). All procedures on animals followed the guidelines for humane treatment set by the association of laboratory animal sciences and the center for laboratory animal sciences at Anhui medical university.

Radioimmunoassay (RIA)

Serum was separated by centrifugation and stored at −80°C until assay for T. T in serum was measured using ^{125}I-based RIA kits from Beijing North Institute of Biological Technology (Beijing, China). For measuring testicular T in mice, testes were homogenized in 0.5 ml PBS (pH 7.4). T was extracted from homogenate using diethyl ether. After extraction, the organic phase was evaporated into dryness in a fume hood, the steroids were solubilized in an aliquot of PBS, and measured using ^{125}I-based RIA kits (Beijing, China) following the manufacturer's protocols for serum samples. Protein concentrations in testicular samples were determined by the Lowry method. The concentration of testicular T was expressed as ng/mg protein.

Semiquantitative RT-PCR

Total cellular RNA was extracted using TRI-Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions. RNase-free DNase (Promega,Madison, WI) was used to remove genomic DNA. The integrity and concentration of RNA was determined by measuring absorbance at 260 nm followed by electrophoresis on agarose gels. For the synthesis of cDNA, 1.0 μg of total RNA from each sample was reverse transcribed in a 40-μl reaction volume containing 0.5 μg oligo$(dT)_{15}$ (Promega, Madison, WI), 1 mM of each dNTP, 15 units AMV RT (Promega), and 1 U/μl recombinant RNasin RNase inhibitor (Promega) in 5 mM $MgCl_2$, 10 mM Tris · HCl, (pH 8.0), 50 mM KCl, and 0.1% Triton X-100. The mixture was heated to 70°C for 10 min, maintained at 42°C for 30 min, and then heated to 95°C for 5 min to terminate the reaction. Reaction mixtures (25 μl) for PCR were assembled using 2 μl cDNA template, 0.625

units Hot Start Polymerase (Promega), 200 μM of each dNTP, 1.5 mM $MgCl_2$, and 1.0 μM of each primer in 1 × Flexi buffer. The primers were synthesized by Sangon Biological Technology (Shanghai, China), according to sequence designs described by the literature (Akingbemi et al. 2003). The primer pairs, the number of cycles and annealing temperature are shown in Table 1. The amplified PCR products were subjected to electrophoresis at 75 V through 1.5% agarose gels (Sigma, St. Louis, MO) for 45 min. The pBR322 DNA digested with Alu I was used for molecular markers (MBI Fermentas). Agarose gels were stained with 0.5 mg/ml ethidium bromide (Sigma, St. Louis, MO) TBE buffer. The gel was scanned and analyzed by UVP gel-image analytic system. Band intensities were analyzed using the public domain NIH Scion Image Program.

Immunoblotting

Testicular samples were homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecylsulphate (SDS), and 1 mM phenylmethylsulfonyl fluoride (PMSF). Samples were then centrifuged at 15,000 × *g* for 15 min. Supernatants from each sample were added to a gel loading buffer (100 mM Tris, pH 6.8, 20% glycerol, 200 mM DTT, 4% SDS, 0.03% bromophenol blue) and boiled for 5 min. Proteins (50 μg/sample) in loading buffer were subjected to electrophoresis in 10% SDS–polyacrylamide gel for 3 h. The gel was transferred electrophoretically onto a polyvinylidene fluoride membrane (Immobilon-P; Millipore Corp., Bedford, Massachusetts, USA) and blocked in 5% nonfat powdered milk in Dulbecco's PBS (DPBS) overnight at 4°C. The membranes were then incubated for 2 h with rabbit polyclonal antibody against StAR, P450scc, 17*β*-HSD and $P450_{17\alpha}$ (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA, dilutions, 1:1,000) or *β*-actin (Beijing Biosynthesis Biotechnology, Beijing, China, dilution, 1:2,000) at room temperature. After washes in DPBS containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with goat anti-rabbit IgG antibody for 2 h. The membranes were then washed for four times in DPBS containing 0.05% Tween-20 for 10 min each, followed by signal development using an enhanced chemiluminescence (ECL) detection kit from Pierce (Pierce Biotechnology, Rockford, IL). For immunoblotting, developed films were scanned and band intensities were analyzed using the public domain NIH Scion Image Program.

Sperm analysis

The cauda epididymides were removed and immediately immersed into the F12 medium supplemented with 0.1% bovine serum albumin (BSA). Spermatozoa were released by mincing the epididymides in the collection medium. The sperm suspensions were incubated at 37°C. After incubation, sperm suspension was analyzed for the number of spermatozoa according to WHO (1999) laboratory manual 4th Edition.

Testicular histology and immunohistochemistry

Two cross sections from each testis were embedded in paraffin using standard procedures performed by Pathological Lab at Anhui Medical University. Paraffin-embedded tissues were serially sectioned. At least two nonserial sections were stained with hematoxylin and eosin (H&E) using standard procedures for morphological analyses. Leydig cells were identified by staining for 3*β*-hydroxysteroid dehydrogenase (3*β*-HSD). Sections of 5 μm were mounted onto coated slides, dewaxed, and rehydrated. Antigen retrieval was performed by pressure cooking slides for 5 min in 0.01 M citrate buffer (pH 6.0). Slides were incubated for 30 min in 0.3% (vol/vol) hydrogen peroxide in methanol to block endogenous peroxidase activity and then washed in Tris-buffered saline [TBS; 0.05 M Tris, 0.85% (wt/vol) NaCl (pH 7.4)]. Nonspecific binding sites were blocked with an appropriate normal serum diluted 1:5 in TBS containing 5% (wt/vol) BSA before the addition of goat

Table 1 Primers, the number of cycles and annealing temperature for RT-PCR

Name	Sequence	Denaturation (°C)	Annealing (°C)	Extension (°C)	Number of cycles (*n*)	Size (bp)
GAPDH	Forward: 5′-GAGGGGCCATCCACAGTCTTC-3′ Reverse: 5′-CATCACCATCTTCCAGGAGCG-3′	94	56	72	39	340
StAR	Forward: 5′-TGTCAAGGAGATCAAGGTCCTG-3′ Reverse: 5′-CGATAGGACCTGGTTGATGAT-3′	94	57	72	45	310
P450scc	Forward: 5′-AGGTGTAGCTCAGGACTTCA-3′ Reverse: 5′-AGGAGGCTATAAAGGACACC-3′	94	56	72	45	370
P450 17α	Forward: 5′-CCAGGACCCAAGTGTGTTCT-3′ Reverse: 5′-CCTGATACGAAGCACTTCTCG-3′	94	56	72	44	250
17*β*-HSD	Forward: 5′- ATTTTACCAGAGAAGACATCT-3′ Reverse: 5′- GGGGTCAGCACCTGAATAATG-3′	94	52	72	46	367

polyclonal antibody for 3β-HSD (Santa Cruz, USA) and overnight incubation at 4°C. After washing in TBS, slides were incubated for 30 min with the appropriate secondary antibody conjugated to biotin rabbit anti-goat (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA), diluted 1:500 in the blocking mixture. This was followed by 30-min incubation with horseradish peroxidase-labeled avidin–biotin complex (Dako). Immunostaining was developed by application of diaminobenzidine (liquid DAB$^+$; Dako), and slides were counterstained with hematoxylin, dehydrated, and mounted using Pertex mounting medium (Cell Path, Hemel Hempstead, UK). The number of 3β-HSD-positive cells was counted in twelve randomly selected fields from each slide at a magnification of $\times$400.

Terminal dUTP Nick-end labeling (TUNEL) staining

For the detection of apoptosis, paraffin-embedded sections were stained with the TUNEL technique using an in situ apoptosis detection kit (Promega) according to the manufacturer's protocols. The total numbers of seminiferous tubules, TUNEL-positive tubules, and TUNEL-positive cells per tubule were examined on six sections from six different litters.

Statistical analysis

For RT-PCR, StAR, P450scc, 17β-HSD and $P450_{17\alpha}$ mRNA were normalized to GAPDH mRNA level in the same samples. StAR, P450scc, 17β-HSD and $P450_{17\alpha}$ mRNA level of the control was assigned as 100%. For western blot studies, StAR, P450scc, 17β-HSD and $P450_{17\alpha}$ were normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. All quantified data were expressed as means $\pm$ SEM at each point. Student t test was used to determine differences between the cypermethrin-treated mice and controls. For serum or testicular T, data were analyzed by a student t test following logarithmic transformation of the data.

Results

Weights of reproductive organs and the number of sperm

No animals died during cypermethrin administration period. No significant difference on the body weight was observed between cypermethrin-treated mice and controls (data not shown). The effects of cypermethrin exposure during puberty on the weights of testes and epididymides were analyzed. Results showed that no significant difference on the weights of testes in adulthood was observed between cypermethrin-treated mice and controls (242.0 ± 4.9 vs. 243.5 ± 5.3 mg, $n = 12$, $P > 0.05$). In addition, there was no significant difference on the weights of epididymides at adulthood between cypermethrin-treated mice and controls (80.3 ± 1.8 vs. 78.4 ± 2.8 mg, $n = 12$, $P > 0.05$). The number of spermatozoa in the cauda epididymides was counted on PND70. Results showed that cypermethrin exposure during puberty significantly decreased the number of spermatozoa in the cauda epididymides [$(16.1 \pm 0.9) \times 10^6$ vs. $(13.0 \pm 1.3) \times 10^6$, $n = 12$, $P < 0.05$].

Testicular histology

The effects of cypermethrin exposure during puberty on testicular histology at adulthood are shown in Fig. 1. The morphology of testes in control mice at adulthood was normal (Fig. 1a, c). Cypermethrin exposure during puberty markedly increased the inside diameter of seminiferous tubules and disturbed the array of spermatogenic cells in testicular sections of mice at adulthood (Fig. 1b, d). The effects of cypermethrin exposure during puberty on apoptosis in testes were determined using TUNEL assay. As shown in Fig. 2 and Table 2, cypermethrin exposure during puberty significantly increased the percentages of TUNEL-positive tubules, TUNEL-positive cells per tubule, and apoptotic cell index in testes.

Serum and testicular T

The effects of cypermethrin exposure during puberty on serum and testicular T at adulthood are presented in Fig. 3. Results showed that cypermethrin exposure during puberty significantly reduced the level of serum T at adulthood (Fig. 3a). In addition, cypermethrin exposure during puberty significantly reduced the level of testicular T at adulthood (Fig. 3b).

Testicular mRNA of StAR and T synthetic enzymes

The effects of cypermethrin exposure during puberty on mRNA level of testicular StAR and T biosynthetic enzymes at adulthood are determined by RT-PCR. As shown in Fig. 4, cypermethrin exposure during puberty significantly decreased mRNA level of StAR and $P450_{17\alpha}$ in testes. No significant difference in mRNA level of testicular P450scc and 17β-HSD was observed between cypermethrin-treated mice and controls.

Protein expression of testicular StAR and T synthetic enzymes

The effects of cypermethrin exposure during puberty on protein expression of testicular StAR and T biosynthetic enzymes at adulthood are presented in Fig. 5. Results

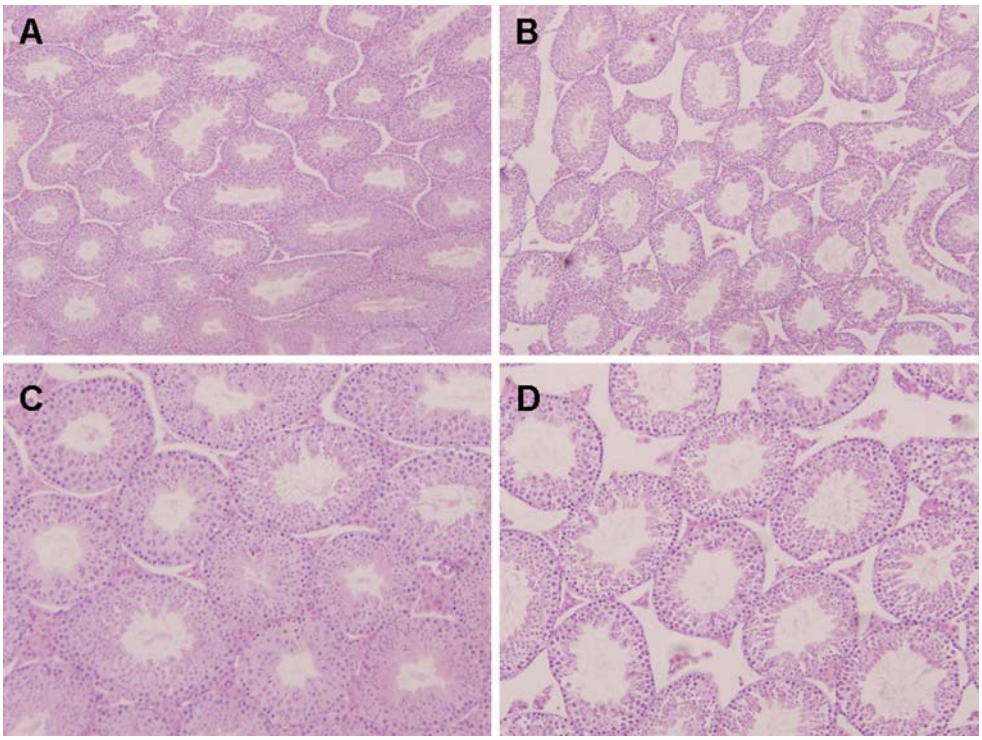

Fig. 1 Effects of cypermethrin exposure during puberty on testicular histology in mice. Male mice were administered with cypermethrin (25 mg/kg) by gavage daily from PND35 to PND70. Testes were collected at PND70. Testicular cross sections from control (**a** and **c**) and cypermethrin-treated (**b** and **d**) mice were stained with H & E at a magnification of ×100 (**a** and **b**), ×400 (**c** and **d**)

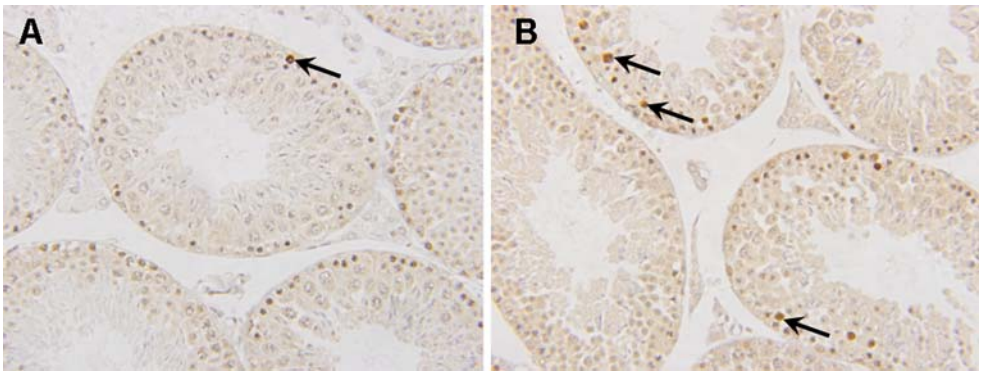

Fig. 2 Effects of cypermethrin exposure during puberty on apoptosis in testes of mice. Male mice were administered with cypermethrin (25 mg/kg) by gavage daily from PND35 to PND70. Testes were collected at PND70. Apoptosis was analyzed with TUNEL staining of testis sections from control (**a**) and cypermethrin-treated (**b**) mice. *Arrows* show TUNEL-positive germ cells

showed that cypermethrin exposure during puberty significantly reduced protein level of testicular StAR in adulthood. No significant difference on protein expression of testicular P450scc, $P450_{17\alpha}$ and 17β-HSD was observed between cypermethrin-treated mice and controls.

Leydig cells

Leydig cells in testes were identified by immunostaining for 3β-HSD. As shown in Fig. 6, no significant difference on the number of Leydig cells in testes was observed between cypermethrin-treated mice and controls.

Discussion

In the present study, we investigated the effects of cypermethrin exposure during puberty on T synthesis in testes of adult mice. We found that the level of serum T was significantly decreased in adult mice exposed to cypermethrin during

Table 2 Cypermethrin-induced germ cellular apoptosis in mouse testes at PND70

	Control	Cypermethrin
Percentage of positive tubule (%)	7.61 ± 0.52	15.16 ± 0.66**
Positive germ cells per tubule	0.13 ± 0.02	0.48 ± 0.07**
Apoptotic index	0.99 ± 0.18	7.29 ± 1.42**

Data were expressed as means ± SEM

** $P < 0.01$ as compared with controls

puberty. In addition, cypermethrin exposure during puberty significantly reduced the level of testicular T in adult mice. These results are in agreement with an earlier study, in which plasma T was markedly decreased in rabbits chronically exposed to cypermethrin for 12 weeks (Yousef et al. 2003).

StAR is essential in T synthesis in Leydig cells. StAR is responsible for the transport of cholesterol into mitochondria (Miller 2007). A recent study showed that the exposure of adult mice to permethrin, another pyrethroid insecticide, disrupted testicular T biosynthesis via inhibiting the mRNA and protein expression of StAR in testes (Zhang et al. 2007). In the present study, we investigated the effects of cypermethrin exposure during puberty on the expression of StAR in testes of adult mice. Our results showed that mRNA level of testicular StAR was significantly decreased in cypermethrin-treated mice. Correspondingly, cypermethrin exposure during puberty markedly reduced the protein expression of testicular StAR in adult mice. These results suggest that the decreased T synthesis might be associated with downregulation of StAR in testes of mice that were exposed to cypermethrin during puberty.

T synthetic enzymes, primarily P450scc, $P450_{17\alpha}$ and 17β-HSD, play a critical role in T synthesis in Leydig cells. P450scc initiates the first enzymatic step in T biosynthesis

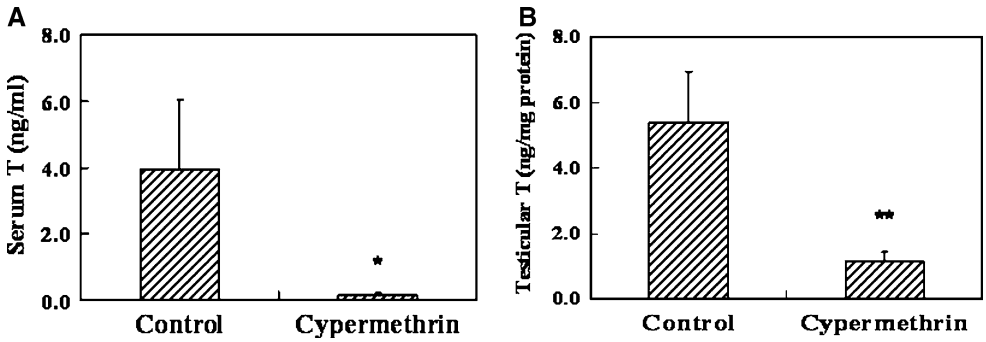

Fig. 3 Effects of cypermethrin during puberty on serum and testicular T in mice. Male mice were administered with cypermethrin (25 mg/kg) by gavage daily from PND35 to PND70. Sera and testes were collected at PND70. T in Sera (**a**) and testes (**b**) was measured by RIA. Data were expressed as means ± SEM of twelve samples from twelve different mice. *$P < 0.05$, **$P < 0.01$ when compared with controls

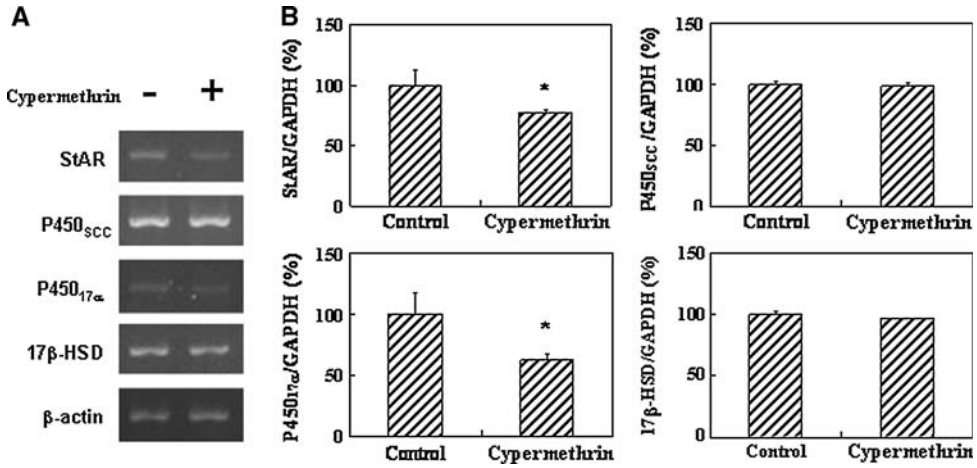

Fig. 4 Effects of cypermethrin exposure during puberty on mRNA levels of testicular StAR and T biosynthetic enzymes in mice. Male mice were administered with cypermethrin (25 mg/kg) by gavage daily from PND35 to PND70. Testes were collected at PND70. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA was measured using RT-PCR. Quantitative analysis for the level of StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA from six individual RNA samples at each point was performed. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA were normalized to GAPDH mRNA level in the same samples. StAR, P450scc, $P450_{17\alpha}$ and 17β-HSD mRNA level of the control was assigned as 100%. All data were expressed as means ± SEM of six samples from different mice. *$P < 0.05$ when compared with controls

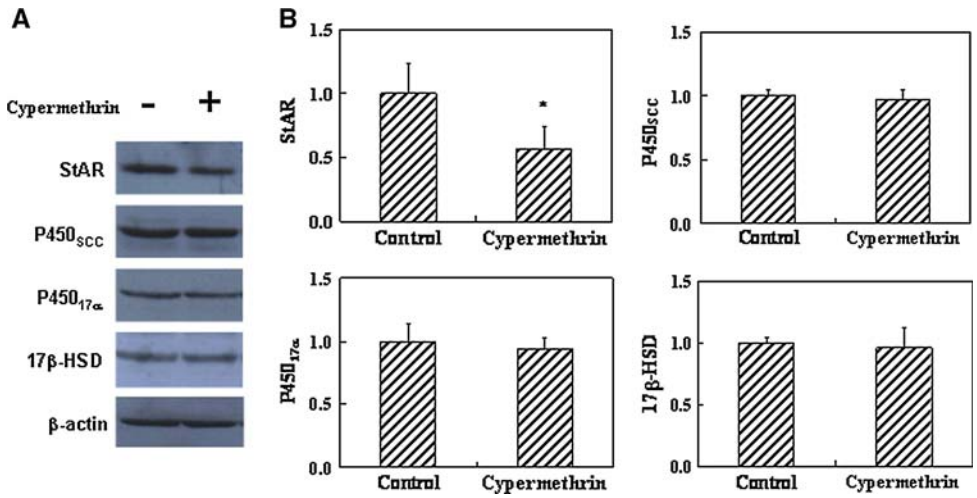

Fig. 5 Effects of cypermethrin exposure during puberty on protein expression of testicular StAR and T biosynthetic enzymes in mice. Male mice were administered with cypermethrin (25 mg/kg) by gavage daily from PND35 to PND70. Testes were collected at PND70. The protein expression of StAR, P450scc, 17β-HSD and P450$_{17\alpha}$ was measured using Western blot. Quantitative analysis of scanning densitometry was performed. StAR, P450scc, P450$_{17\alpha}$ and 17β-HSD were normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. All data were expressed as means ± SEM of six samples from six different mice. *$P < 0.05$ when compared with controls

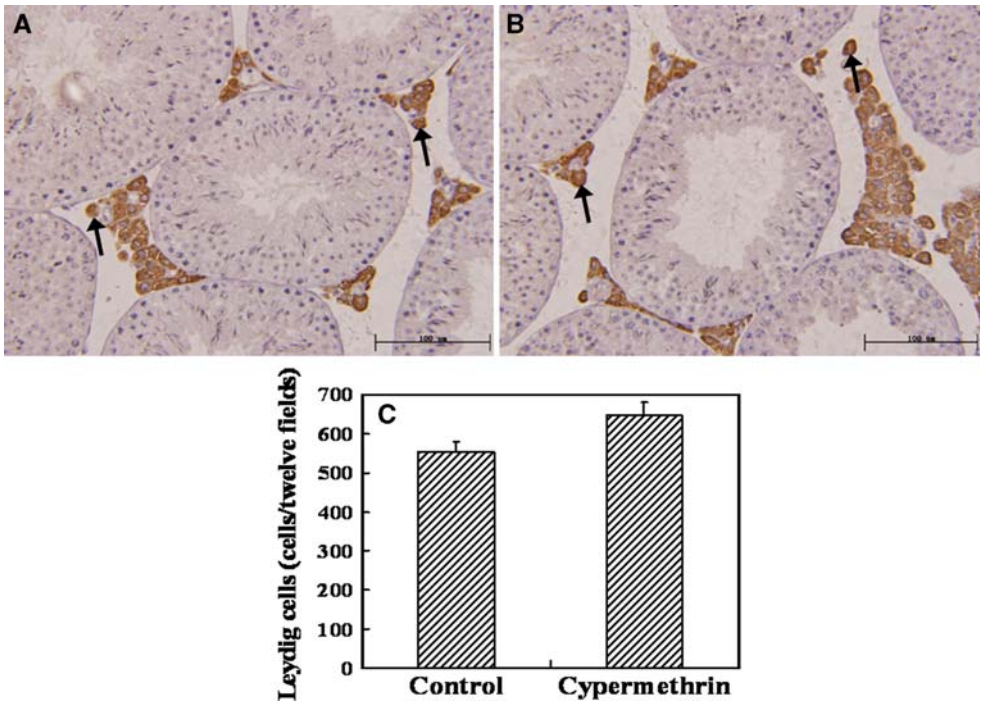

Fig. 6 Effects of cypermethrin exposure during puberty on the number of Leydig cells in testes of mice. Male mice were administered with cypermethrin (25 mg/kg) by gavage daily from PND35 to PND70. Testes were collected at PND70. Leydig cells in testes of control (**a**) and cypermethrin-treated (**b**) mice were immunolocalized in the interstitium by staining with a goat polyclonal antibody specific for 3β-HSD. The numbers of Leydig cells (**c**) in testes were counted in 12 randomly selected fields from each slide at 400× magnification. Data were expressed as means ± SEM of twelve samples from six different mice

in the inner mitochondrial membrane of Leydig cells, where cholesterol is converted to pregnenolone. Pregnenolone is catalyzed by P450$_{17\alpha}$ to produce 17-hydroxyprogesterone and androstenedione, with the latter then being converted to T by 17β-HSD (Payne and Youngblood 1995). The present study found that mRNA level of testicular P450$_{17\alpha}$ was significantly decreased in cypermethrin-treated mice, whereas cypermethrin exposure during puberty had little effect on protein expression of P450$_{17\alpha}$ in testes of adult mice. An earlier study showed that the expression of testicular 17β-HSD was obviously downregulated in fenvalerate-exposed rats by subchronic inhalation for 3 months (Mani et al. 2002). According to a recent study, the expression of testicular P450scc was significantly downregulated in adult mice exposed to permethrin (Zhang et al. 2007). However, we found that cypermethrin exposure during puberty had no effect on mRNA and protein expression of testicular P450scc and 17β-HSD in adult mice.

Aside from the T synthetic pathway, we searched for other possible factors involved in the decreased T production. Several studies showed that T production was correlated with the number of Leydig cells in testes (Suescun et al. 1997; Castro et al. 2002; Gould et al. 2007; Saraiva et al. 2008). In the present study, we analyzed the number of leydig cells in testes of adult mice. Results showed that cypermethrin exposure during puberty had no effect on the number of Leydig cells in testes of adult mice. These results suggest that cypermethrin-induced decrease in testicular T synthesis cannot be attributed to the decrement in the number of Leydig cells. Recently, several studies showed that abnormal Leydig cell aggregation in the fetal testes of rats exposed to di-(n-butyl) phthalate (DBP) or di-(2-ethylhexyl) phthalate (DEBP) was associated with the decreased level of testicular T (Mahood et al. 2005, 2007; Lin et al. 2008). Additional work is required to determine whether cypermethrin exposure during puberty induces abnormal Leydig cell aggregation in testes.

Several studies showed that exposure of adult male animals to cypermethrin resulted in a decline in semen quality (Elbetieha et al. 2001; Ahmad et al. 2009). In the present study, we also investigated the effects of cypermethrin exposure during puberty on sperm quality in adult mice. Our results showed that although cypermethrin exposure during puberty did not affect the weight of testes and epididymides, the number of sperm in the cauda epididymides was significantly decreased in cypermethrin-exposed mice. T acts through the somatic cells to regulate germ cell differentiation and is a prerequisite for testicular development and normal spermatogenesis (Holdcraft and Braun 2004). Recent studies found that a Sertoli cell-selective knockout of the androgen receptor resulted in spermatogenic arrest in meiosis and infertility with defective spermatogenesis and hypotestosteronemia (Chang et al. 2004; De Gendt et al. 2004). As stated above, cypermethrin exposure during puberty markedly disrupted T synthesis in testes. These results suggest that cypermethrin-induced impairment in spermatogenesis might be associated with the decreased T synthesis in testes. However, recent studies demonstrated that some various endocrine disruptors induced germ cell apoptosis in testes (Ichimura et al. 2003; Borch et al. 2005; Sobarzo et al. 2006; McClusky et al. 2007; Saradha et al. 2009). A recent study showed that mono-(2-ethylhexyl) phthalate (MEHP) significantly reduced the number of germ cells by increasing apoptosis without change in T production in human fetal testes (Lambrot et al. 2009). The present study revealed that the number of apoptotic cells per tubule in testes was significantly increased in cypermethrin-exposed mice. Therefore, we cannot exclude the possibility that cypermethrin exposure during puberty impairs spermatogenesis, at least partially, via a proapoptotic mechanism in testes.

In summary, the present study indicates that cypermethrin exposure during puberty disrupts T synthesis via down-regulating the expression of StAR in testes. The decreased T synthesis might be associated with cypermethrin-induced impairment in spermatogenesis in mice.

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