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Effects of pubertal fenvalerate exposure on testosterone and estradiol synthesis and the expression of androgen and estrogen receptors in the developing brain

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ABSTRACT

Fenvalerate is a potential endocrine disruptor. Several studies have demonstrated that fenvalerate disrupts testosterone (T) synthesis in testes. T and estradiol (E_2) are de novo synthesized in the developing brain. Thus, the aim of the present study was to investigate the effects of pubertal fenvalerate exposure on the synthesis of T and E_2 and the expression of androgen receptor (AR) and estrogen receptors (ERs) in cerebral cortex. CD-1 mice were orally administered daily with either vehicle or fenvalerate (7.5 or 30 mg/kg) from postnatal day (PND) 28 to PND56. The level of T and E_2 in cerebral cortex was significantly decreased in males exposed to fenvalerate. In agreement with the decrease in T and E_2 syntheses, the expression of 17β-HSD, a key enzyme for T synthesis, was significantly reduced in cerebral cortex of fenvalerate-exposed males. Conversely, in females, the expression of 17β-HSD in cerebral cortex was mildly up-regulated by fenvalerate and the level of T and E_2 was mildly increased. Pubertal fenvalerate exposure had no effect on the expression of StAR, $P450_{17\alpha}$ and P450scc, the key enzymes for T synthesis, and P450 aromatase, the key enzyme for E_2 synthesis, in cerebral cortex of males and females. Interestingly, the expression of AR in cerebral cortex was up-regulated in male and female mice exposed to fenvalerate, whereas pubertal fenvalerate exposure did not affect the level of ERα and ERβ in cerebral cortex. Taken together, these results suggest that pubertal fenvalerate exposure disrupts T and E_2 synthesis and the expression of AR in cerebral cortex. These changes of steroid status in the developing brain might be deleterious for neurobehavioral development.

1. Introduction

Hundreds of substances have been identified as so-called "endocrine disrupting chemicals (EDCs)" because exposure to them results in disruption of normal endocrine function with possible adverse health outcomes. EDCs may interfere with the synthesis, release, transport, metabolism and binding or elimination of natural hormones in gonads (Caserta et al., 2008) and in the developing brain (Andrade et al., 2006). Fenvalerate, a type-II pyrethroid widely used in agricultural and domestic environments to control noxious insects, has been well demonstrated to disrupt endocrine function in humans and animals (Garey and Wolff, 1998; Go et al., 1999; Meeker et al., 2009). According to an epidemiological investigation, a statistically significant relationship was observed between the concentrations of pyrethroid insecticide metabolite and the levels of circulating testosterone (T) in non-occupationally exposed Chinese men (Han et al., 2008). Another epidemiological investigation showed that the decreased level of T was in relation to urinary metabolites of pyrethroid insecticides among the US general population (Meeker et al., 2009). Several in vitro studies found that fenvalerate inhibited the release of steroid hormones in primary cultured rat preantral ovarian follicles and mouse Leydig tumor cells (Fei et al., 2010; Qu et al., 2008). Several earlier studies demonstrated that perinatal fenvalerate exposure not only reduced plasma level of T or estradiol (E_2) but also delayed sexual maturation in the male and female offspring (Moniz et al., 1999, 2005). Recently, we found that maternal fenvalerate exposure disrupted the synthesis of T in testes of male offspring (Zhang et al., 2010a). In addition, lactational and pubertal fenvalerate exposure reduced the level of serum and testicular T in male mice (Zhang et al., 2009, 2010b).

It has been well established that T and E_2 are de novo synthesized in the developing brain. Indeed, the key enzymes for T synthesis, such as steroidogenic acute regulatory protein (StAR),

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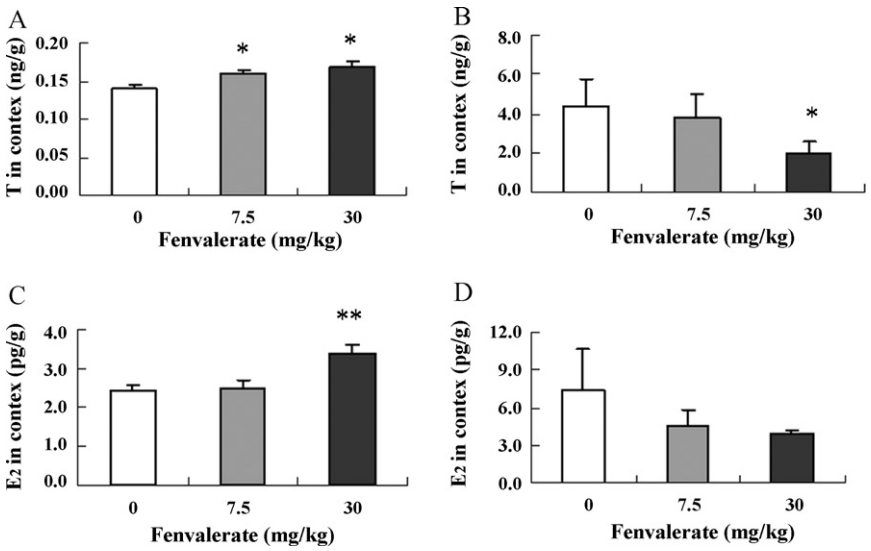

Fig. 1. The effects of pubertal fenvalerate exposure on T and E_2 in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. The level of T and E_2 in cerebral cortex was measured by RIA. (A) T in cerebral cortex of female mice. (B) T in cerebral cortex of male mice. (C) E_2 in cerebral cortex of female mice. (D) E_2 in cerebral cortex of male mice. Data were presented as mean ± SEM of twelve samples. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the controls.

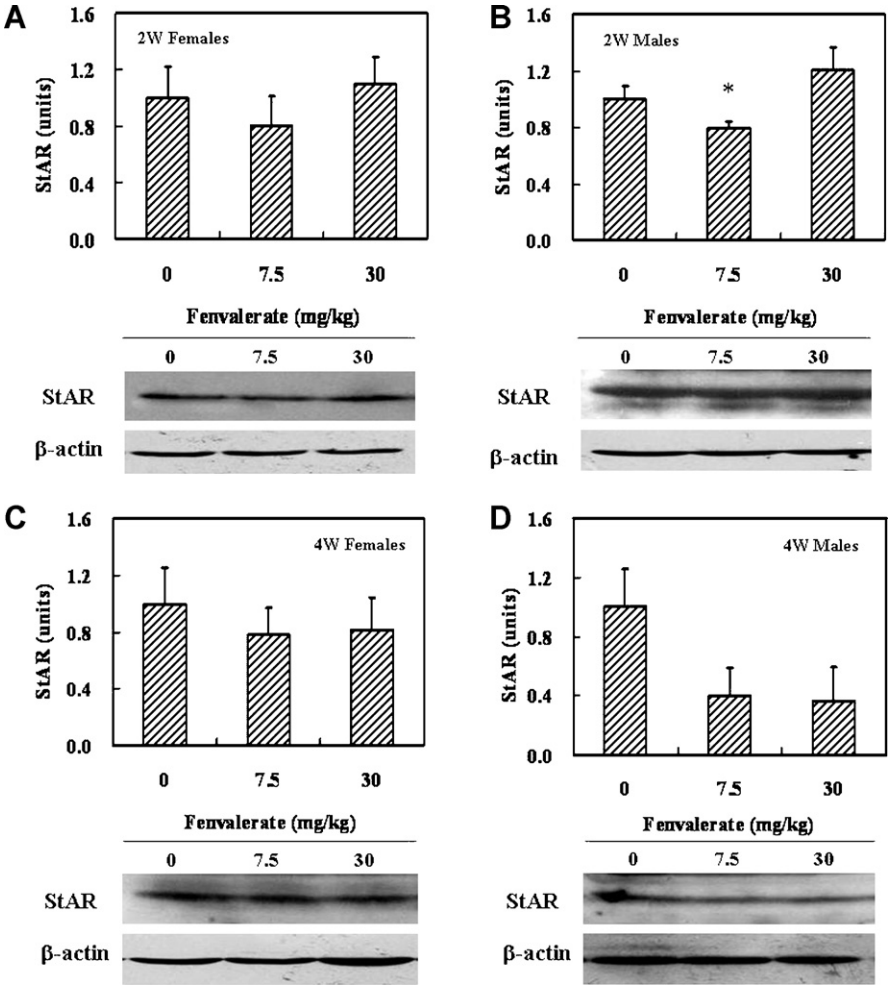

Fig. 2. The effects of pubertal fenvalerate exposure on the expression of StAR in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. Cerebral cortex was collected at PND42 or PND56. StAR in cerebral cortex was measured using immunoblotting. Blots are representative of four independent experiments. Quantitative analysis of scanning densitometry was performed. StAR was normalized to β-actin level in the same samples. The densitometry unit of the control was assigned as 1. (A) For female mice administered with fenvalerate for two weeks. (B) For male mice administered with fenvalerate for two weeks. (C) For female mice administered with fenvalerate for four weeks. (D) For male mice administered with fenvalerate for four weeks. Data were presented as mean ± SEM. $^{*}P < 0.05$ compared with the controls.

P450 side-chain cleavage (P450scc), P450 17α-hydroxysteroid dehydrogenase ($P450_{17\alpha}$) and 17β-hydroxysteroid dehydrogenase (17β-HSD), are expressed in cerebral cortex and hippocampus (Furukawa et al., 1998; Zwain and Yen, 1999; Kimoto et al., 2001; Hojo et al., 2004). In addition, protein expression of cytochrome P450 19 (CYP19, P450 aromatase), the key enzyme for E_2 synthesis, is detected in hypothalamus, cerebral cortex and hippocampus (Beyer et al., 1994; MacLusky et al., 1994; Kato et al., 1997). An earlier study showed that treatment of juvenile salmon with nonylphenol caused a significant induction of StAR mRNA in brain (Arukwe, 2005). A similar result was found in a recent study, in which the expression of StAR and P450scc was significantly increased in brain of juvenile salmon exposed to ethynylestradiol, a synthetic pharmaceutical endocrine disruptor (Lyssimachou and Arukwe, 2007). According to a recent study, maternal exposure to di-(2-ethylhexyl)-phthalate from pregnancy to lactation altered aromatase activity in brain of offspring in a dose- and gender-dependent manner (Andrade et al., 2006). However, the potential effects of fenvalerate on endocrine disruption in the developing brain remain to be determined.

The aim of the present study was to investigate the effects of pubertal fenvalerate exposure on the synthesis of T and E_2 and the expression of AR and ERs in cerebral cortex. We found that pubertal fenvalerate exposure not only disrupts T and E_2 synthesis but also inhibits the expression of AR in cerebral cortex.

2. Materials and methods

2.1. Chemicals

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

2.2. Animals and treatment

ICR mice (3-week-old) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. They were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50% ± 5%) environment for one week before use. Food and water were provided *ad libitum*. Mice were administered with different doses (7.5 or 30 mg/kg) of fenvalerate (Sigma Chemical Co., St. Louis, MO) by gavage daily from postnatal day (PND) 28 to PND56. According to our previous studies, the dose of 30 mg/kg, about 1/8 LD_{50} of the fenvalerate, was administered in the high dose group in the present study. Our previous studies showed that no signs of toxicity were observed in mice exposed to 30 mg/kg or a higher dose of fenvalerate (Zhang et al., 2009, 2010a,b). The corn oil treated mice served as controls. Twelve mice (six males and six females) each group were sacrificed at PND42. The remaining mice (twelve males and twelve females each group) were sacrificed at PND56. The whole cerebral cortex was dissected. Half of cerebral cortex was used to measure T and E_2 in cerebral cortex. The other cerebral cortex was collected for immunoblotting. Animals were treated humanely and with regard for alleviation of suffering according to proto-

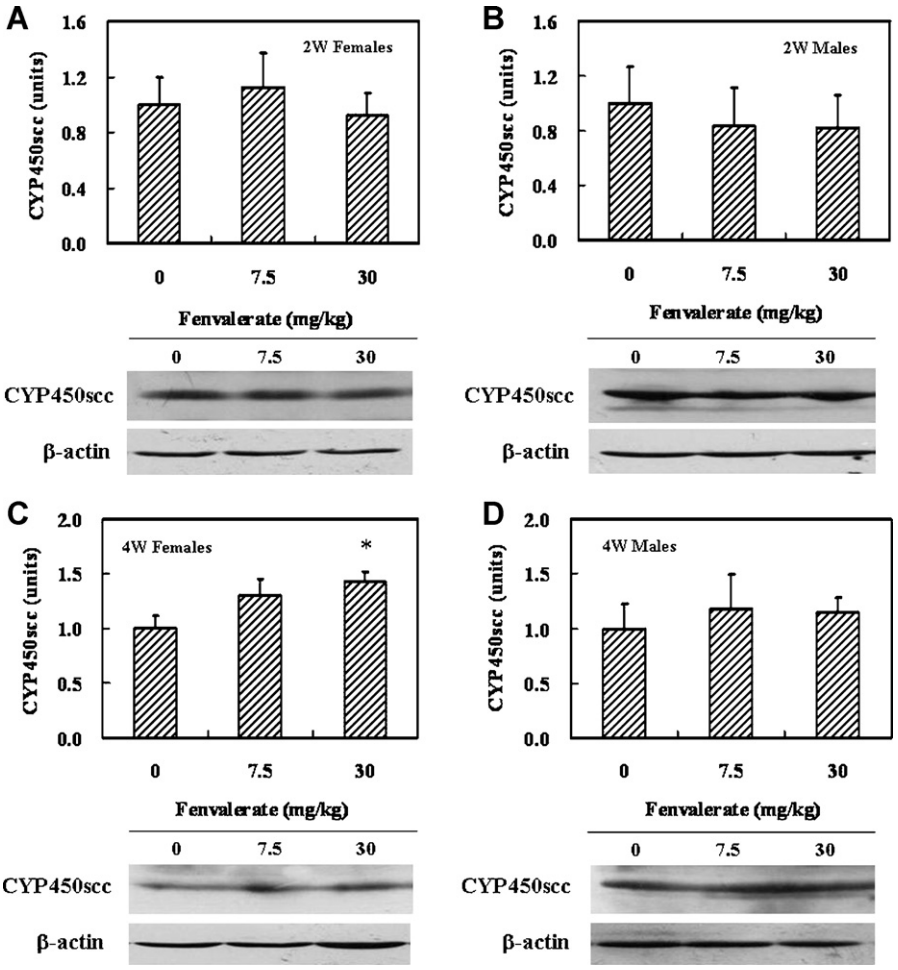

Fig. 3. The effects of pubertal fenvalerate exposure on the expression of P450scc in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. Cerebral cortex was collected at PND42 or PND56. P450scc in cerebral cortex was measured using immunoblotting. Blots are representative of four independent experiments. Quantitative analysis of scanning densitometry was performed. P450scc was normalized to β-actin level in the same samples. The densitometry unit of the control was assigned as 1. (A) For female mice administered with fenvalerate for two weeks. (B) For male mice administered with fenvalerate for two weeks. (C) For female mice administered with fenvalerate for four weeks. (D) For male mice administered with fenvalerate for four weeks. Data were presented as mean ± SEM. $^*P < 0.05$ compared with the controls.

cols approved by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Radioimmunoassay (RIA)

For measuring cerebral cortex T and E_2 in mice, cerebral cortex was homogenized in 0.5 ml PBS (pH 7.4). T and E_2 were 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. The concentration of cerebral cortex T and E_2 was expressed as ng/g and pg/g protein.

2.4. Immunoblotting

Cerebral cortex was dissected and stored at −80 °C. Tissues 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 dodecylsulfate (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 (Santa Cruz Biotechnology, USA), P450scc (Santa Cruz Biotechnology, USA), $P450_{17\alpha}$ (Santa Cruz Biotechnology, USA), 17β-HSD (Santa Cruz Biotechnology, USA) AR (Santa Cruz Biotechnology, USA), ERα (Santa Cruz Biotechnology, USA), ERβ (Santa Cruz Biotechnology, USA) or β-actin (Beijing Biosynthesis Biotechnology, Beijing, China), and with goat polyclonal antibody against CYP19 (Santa Cruz Biotechnology, USA) 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 or donkey anti-goat 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).

2.5. Statistical analysis

For immunoblotting, developed films were scanned and band intensities were analyzed using the public domain NIH Scion Image Program. StAR, P450scc, $P450_{17\alpha}$, 17β-HSD, CYP19, AR, ERα and ERβ were normalized to β-actin level in the same samples. The densitometry unit of the control was assigned as 1. All quantified data were expressed as mean ± SEM. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences among different groups. The significance level was set at $P < 0.05$. All statistical analyses were performed using the statistical package for social sciences (SPSS, version 12.0).

3. Results

3.1. Effects of pubertal fenvalerate exposure on the level of T and E_2 in cerebral cortex

The effects of pubertal fenvalerate exposure on the level of T in cerebral cortex were analyzed. As shown in Fig. 1A, the level of T in cerebral cortex was significantly increased in female mice exposed to fenvalerate ($F_{(2, 33)} = 7.548$, $P = 0.001$). Conversely, the level of T in cerebral cortex was significantly decreased in male mice exposed to

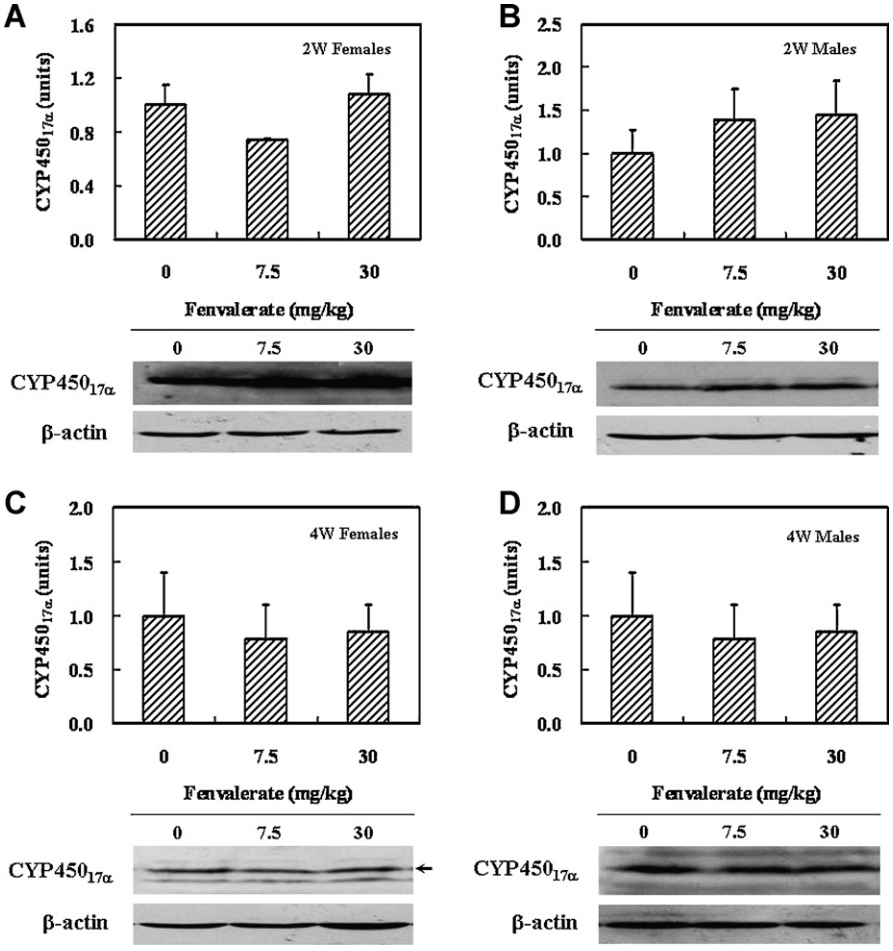

Fig. 4. The effects of pubertal fenvalerate exposure on the expression of $P450_{17\alpha}$ in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. Cerebral cortex was collected at PND42 or PND56. $P450_{17\alpha}$ in cerebral cortex was measured using immunoblotting. Blots are representative of four independent experiments. Quantitative analysis of scanning densitometry was performed. $P450_{17\alpha}$ was normalized to β-actin level in the same samples. The densitometry unit of the control was assigned as 1. (A) For female mice administered with fenvalerate for two weeks. (B) For male mice administered with fenvalerate for two weeks. (C) For female mice administered with fenvalerate for four weeks. (D) For male mice administered with fenvalerate for four weeks. Data were presented as mean ± SEM.

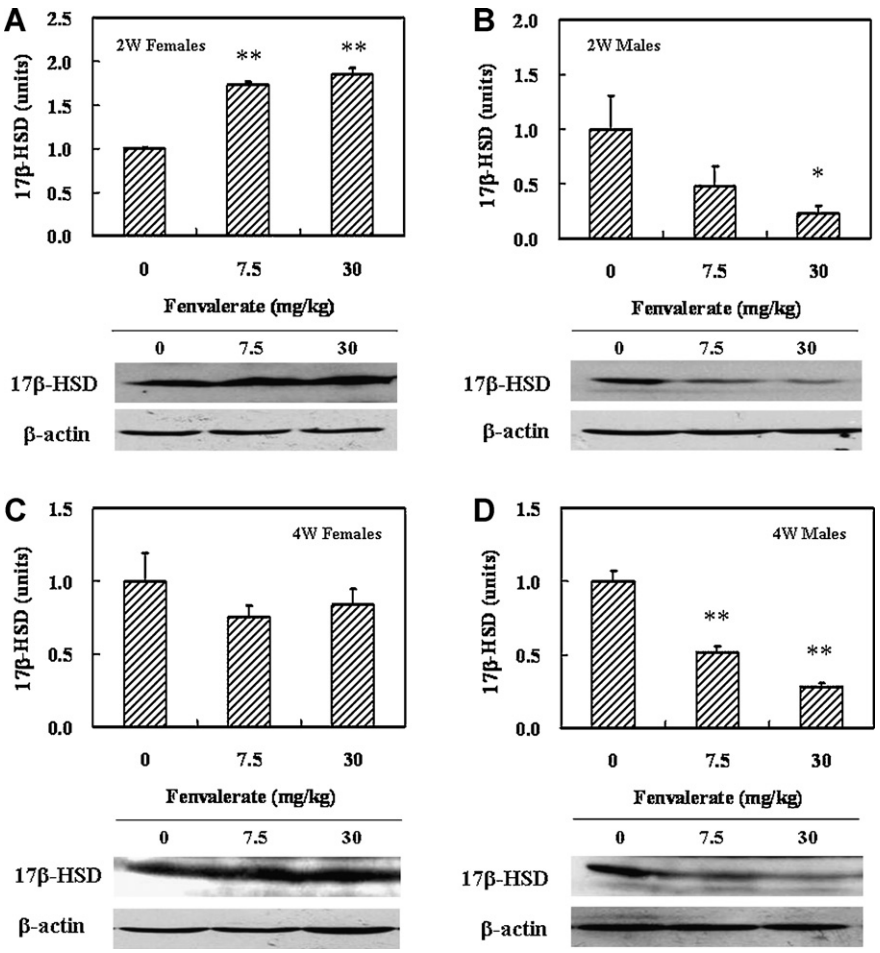

Fig. 5. The effects of pubertal fenvalerate exposure on the expression of 17β-HSD in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. Cerebral cortex was collected at PND42 or PND56. 17β-HSD in cerebral cortex was measured using immunoblotting. Blots are representative of four independent experiments. Quantitative analysis of scanning densitometry was performed. 17β-HSD was normalized to β-actin level in the same samples. The densitometry unit of the control was assigned as 1. (A) For female mice administered with fenvalerate for two weeks. (B) For male mice administered with fenvalerate for two weeks. (C) For female mice administered with fenvalerate for four weeks. (D) For male mice administered with fenvalerate for four weeks. Data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the controls.

30 mg/kg of fenvalerate (Fig. 1B). The effects of pubertal fenvalerate exposure on the level of E_2 in cerebral cortex are presented in Fig. 1C and D. The level of E_2 in cerebral cortex was significantly increased in female mice treated with 30 mg/kg of fenvalerate during puberty (Fig. 1C). By contrast, a trend of the decreased E_2 in cerebral cortex was observed in male mice exposed to fenvalerate during puberty ($F_{(2, 33)} = 0.884$, $P = 0.422$; Fig. 1D).

3.2. Effects of pubertal fenvalerate exposure on the expression of StAR and T synthetic enzymes in cerebral cortex

The effects of pubertal fenvalerate exposure on protein level of StAR and T synthetic enzymes in cerebral cortex were analyzed. As shown in Fig. 2A and C, no significant difference on the expression of StAR in cerebral cortex was observed in female mice exposed to fenvalerate for two weeks or four weeks ($F_{(2, 15)} = 0.638$, $P = 0.542$; $F_{(2, 15)} = 0.336$, $P = 0.720$). The effects of pubertal fenvalerate exposure on protein level of StAR in cerebral cortex of male mice are presented in Fig. 2B and D. The expression of StAR in cerebral cortex was mildly decreased in mice exposed to 7.5 mg/kg fenvalerate for two weeks (Fig. 2B). No statistically significant difference on the level of StAR in cerebral cortex was observed among different groups although there was a trend of reduction on the expression of StAR in cerebral cortex of male mice exposed to fenvalerate for four weeks ($F_{(2, 15)} = 3.279$, $P = 0.066$; Fig. 2D). The effects of pubertal fenvalerate exposure on protein level of P450scc in cerebral cortex are presented in Fig. 3. Results showed that pubertal exposure to fenvalerate for two weeks had little effect on the expression of P450scc in cerebral cortex (female: $F_{(2, 15)} = 0.294$, $P = 0.749$; male: $F_{(2, 15)} = 0.181$, $P = 0.836$), whereas the level of P450scc in cerebral cortex was mildly increased in female mice exposed to 30 mg/kg of fenvalerate for four weeks. In addition, pubertal fenvalerate exposure did not affect the expression of $P450_{17\alpha}$ in cerebral cortex (for two weeks: female, $F_{(2, 15)} = 2.606$, $P = 0.107$; male: $F_{(2, 15)} = 0.636$, $P = 0.543$. for four weeks: female, $F_{(2, 15)} = 0.138$, $P = 0.872$; male: $F_{(2, 15)} = 0.328$, $P = 0.725$, Fig. 4). The effects of pubertal fenvalerate exposure on the expression of 17β-HSD in cerebral cortex are presented in Fig. 5. Results showed that the level of 17β-HSD in cerebral cortex was significantly increased in female mice exposed to fenvalerate for two weeks ($F_{(2, 15)} = 98.064$, $P = 0.000$; Fig. 5A). Conversely, pubertal fenvalerate exposure significantly inhibited the expression of 17β-HSD in cerebral cortex of male mice in a dose-dependent manner (for two weeks: $F_{(2, 15)} = 4.323$, $P = 0.033$. for four weeks: $F_{(2, 15)} = 62.418$, $P = 0.000$, Fig. 5B and D).

3.3. Effects of pubertal fenvalerate exposure on the expression of CYP19 in cerebral cortex

The effects of pubertal fenvalerate exposure on the expression of CYP19 in cerebral cortex were analyzed. As shown in Fig. 6A and C,

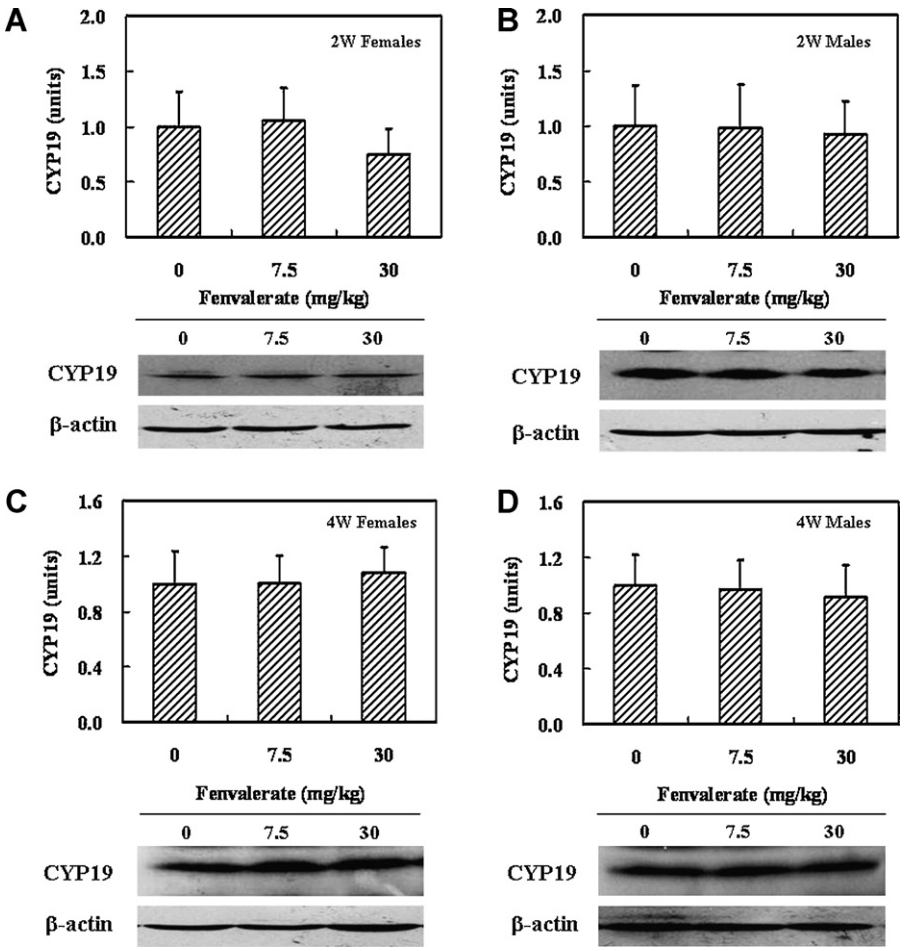

Fig. 6. Effects of pubertal fenvalerate exposure on the expression of CYP19 in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. Cerebral cortex was collected at PND42 or PND56. CYP19 in cerebral cortex was measured using immunoblotting. Blots are representative of four independent experiments. Quantitative analysis of scanning densitometry was performed. CYP19 was normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. (A) For female mice administered with fenvalerate for two weeks. (B) For male mice administered with fenvalerate for two weeks. (C) For female mice administered with fenvalerate for four weeks. (D) For male mice administered with fenvalerate for four weeks. Data were presented as mean ± SEM.

no significant difference on the level of aromatase in cerebral cortex was observed between fenvalerate-exposed female mice and controls (for two weeks: $F_{(2, 15)} = 0.401$, $P = 0.677$; for four weeks: $F_{(2, 15)} = 0.066$, $P = 0.936$). In addition, pubertal fenvalerate exposure had no effect on the expression of CYP19 in cerebral cortex of male mice (for two weeks: $F_{(2, 15)} = 0.015$, $P = 0.985$; for four weeks: $F_{(2, 15)} = 0.050$, $P = 0.951$, Fig. 6B and D).

3.4. Effects of pubertal fenvalerate exposure on the expression of androgen and estrogen receptors in cerebral cortex

The effects of pubertal fenvalerate exposure on the expression of AR in cerebral cortex were analyzed. As shown in Fig. 7B, there was a trend of elevation on the level of AR in cerebral cortex of male mice exposed to fenvalerate for two weeks ($F_{(2, 15)} = 0.826$, $P = 0.457$), whereas the expression of AR in cerebral cortex was significantly upregulated in male mice exposed to fenvalerate for four weeks ($F_{(2, 15)} = 56.023$, $P = 0.000$, Fig. 7D). Surprisingly, the level of AR in cerebral cortex was markedly increased in female mice exposed to fenvalerate (for two weeks: $F_{(2, 15)} = 8.405$, $P = 0.004$; for four weeks: $F_{(2, 15)} = 6.575$, $P = 0.009$, Fig. 7A and C). The effects of pubertal fenvalerate exposure on the expression of ERs in cerebral cortex are presented in Fig. 8. Results showed that no statistically significant difference on the expression of ERα and ERβ in cerebral cortex of female mice was observed among different groups although there was a trend of elevation on the expression of ERα in cerebral cortex of female mice exposed to fenvalerate for two weeks (for two weeks: $F_{(2, 15)} = 1.057$, $P = 0.372$; for four weeks: $F_{(2, 15)} = 0.103$, $P = 0.903$, Fig. 8A and C). By contrast, no significant difference on the expression of ERα in cerebral cortex of male mice was observed among different groups (for two weeks: $F_{(2, 15)} = 1.661$, $P = 0.223$; for four weeks: $F_{(2, 15)} = 0.658$, $P = 0.532$, Fig. 8B and D), whereas the level of ERβ in cerebral cortex was significantly increased in male mice exposed to 30 mg/kg fenvalerate for four weeks (Fig. 8D). In addition, a trend of elevation on the expression of ERβ in cerebral cortex was observed in male mice exposed to fenvalerate for two weeks ($F_{(2, 15)} = 0.513$, $P = 0.609$; Fig. 8B).

4. Discussion

Fenvalerate is a potential endocrine disruptor. According to our recent study, the level of serum and testicular T at weaning was significantly decreased in male pups whose mothers were exposed to fenvalerate during lactation (Zhang et al., 2009). In addition, pubertal fenvalerate exposure reduced the level of serum and testicular T in mice (Zhang et al., 2010a,b). In the present study, we found for the first time that the level of T in cerebral cortex was significantly decreased in male mice exposed to fenvalerate during puberty. Conversely, the level of T in cerebral cortex was mildly increased in fenvalerate-exposed female mice. StAR, P450scc, $P450_{17\alpha}$, 17β-HSD are the key enzymes for T synthesis. Several in vitro studies found that fenvalerate inhibited the expression of StAR and P450scc

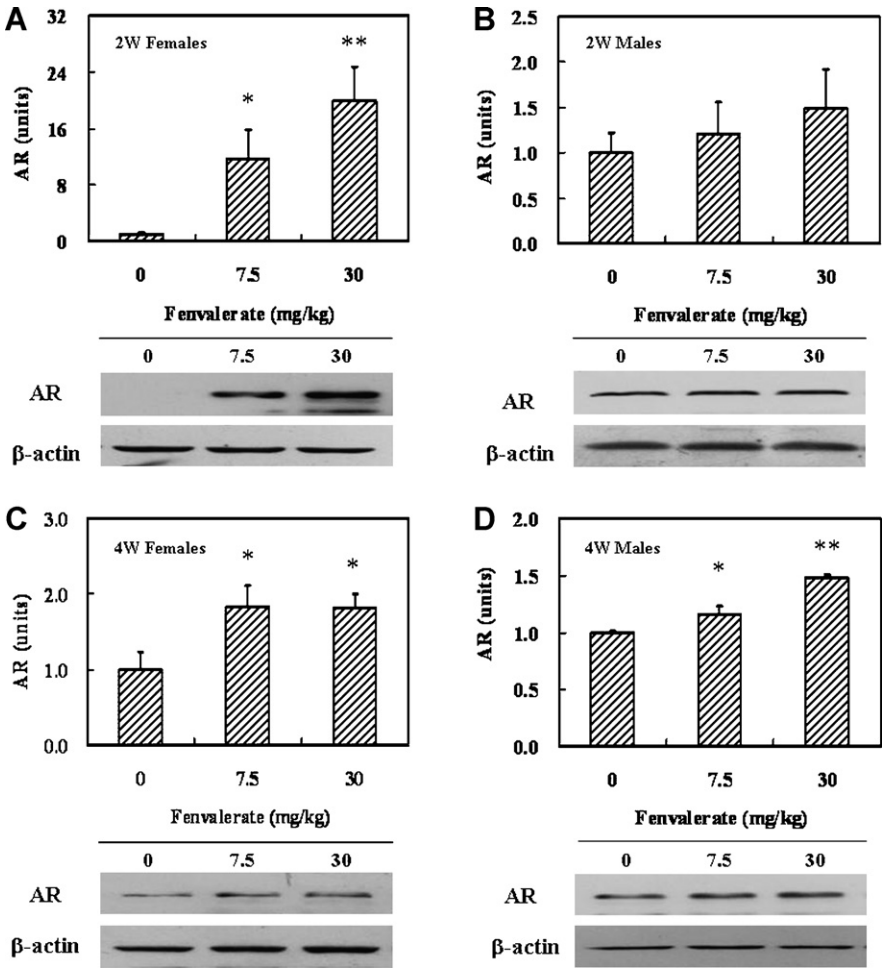

Fig. 7. Effects of pubertal fenvalerate exposure on the expression of AR in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. Cerebral cortex was collected at PND42 or PND56. AR in cerebral cortex was measured using immunoblotting. Blots are representative of four independent experiments. Quantitative analysis of scanning densitometry was performed. AR was normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. (A) For female mice administered with fenvalerate for two weeks. (B) For male mice administered with fenvalerate for two weeks. (C) For female mice administered with fenvalerate for four weeks. (D) For male mice administered with fenvalerate for four weeks. Data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the controls.

in primary cultured rat preantral ovarian follicles and mouse Leydig tumor cells (Fei et al., 2010; Qu et al., 2008). Our recent studies showed that lactational fenvalerate exposure reduced the level of P450scc mRNA and protein in testes (Zhang et al., 2009). In addition, pubertal fenvalerate exposure down-regulated the expression of P450scc and $P450_{17\alpha}$ in testes (Zhang et al., 2010a,b). Increasing reports have demonstrated that all the key enzymes for T synthesis are expressed in cerebral cortex (Hojo et al., 2004). In the present study, we showed that the expression of 17β-HSD, one of the key enzymes for T synthesis, was significantly down-regulated in cerebral cortex of male mice exposed to fenvalerate. Conversely, the expression of 17β-HSD was mildly up-regulated in cerebral cortex of female mice exposed to fenvalerate, in agreement with the elevation of T in cerebral cortex. These results suggest that the down-regulation or the up-regulation of 17β-HSD contributes, at least partially, to fenvalerate-induced reduction or elevation of T level in cerebral cortex.

In the present study, we investigated the effects of pubertal fenvalerate exposure on E_2 synthesis in cerebral cortex. We found that the level of E_2 in cerebral cortex was significantly increased in fenvalerate-exposed female mice. Conversely, a trend of the decreased E_2 in cerebral cortex was observed in male mice exposed to fenvalerate during puberty. P450 aromatase is the key enzyme for E_2 synthesis. Several studies have demonstrated that P450 aromatase is expressed in hypothalamus, cerebral cortex and hippocampus (Beyer et al., 1994; MacLusky et al., 1994; Kato et al., 1997). To investigate whether the effects of pubertal fenvalerate exposure on E_2 synthesis in cerebral cortex are associated with the altered expression of P450 aromatase, the level of P450 aromatase in cerebral cortex was measured in mice exposed to fenvalerate. Surprisingly, pubertal fenvalerate exposure had little effect on the expression of P450 aromatase in cerebral cortex in male and female mice. These results suggest that the increased or the decreased of E_2 synthesis in cerebral cortex is independent of the alteration of P450 aromatase in cerebral cortex. The elevation or the reduction of E_2 synthesis in cerebral cortex might be associated with the increase or the decrease in the level of T in cerebral cortex.

Numerous reports have demonstrated that in both the developing and adult brains, AR and ERs are present which might mediate the action of steroids on sexual differentiation (Attardi and Ohno, 1976; Vito and Fox, 1981; Vito et al., 1983; Pomerantz et al., 1985). Interestingly, the expression of AR and ERs in cerebral cortex is regulated by T and E_2 in a age- and sex-specific manner (Thakur et al., 2000; Kumar and Thakur, 2004; Sharma and Thakur, 2006). Several in vitro studies showed that fenvalerate had weak estrogenic activity (Lemaire et al., 2006; Xu et al., 2006). Thus, it is especially interesting whether pubertal fenvalerate exposure regulates the expression of AR and ERs in cerebral cortex. In the present study,

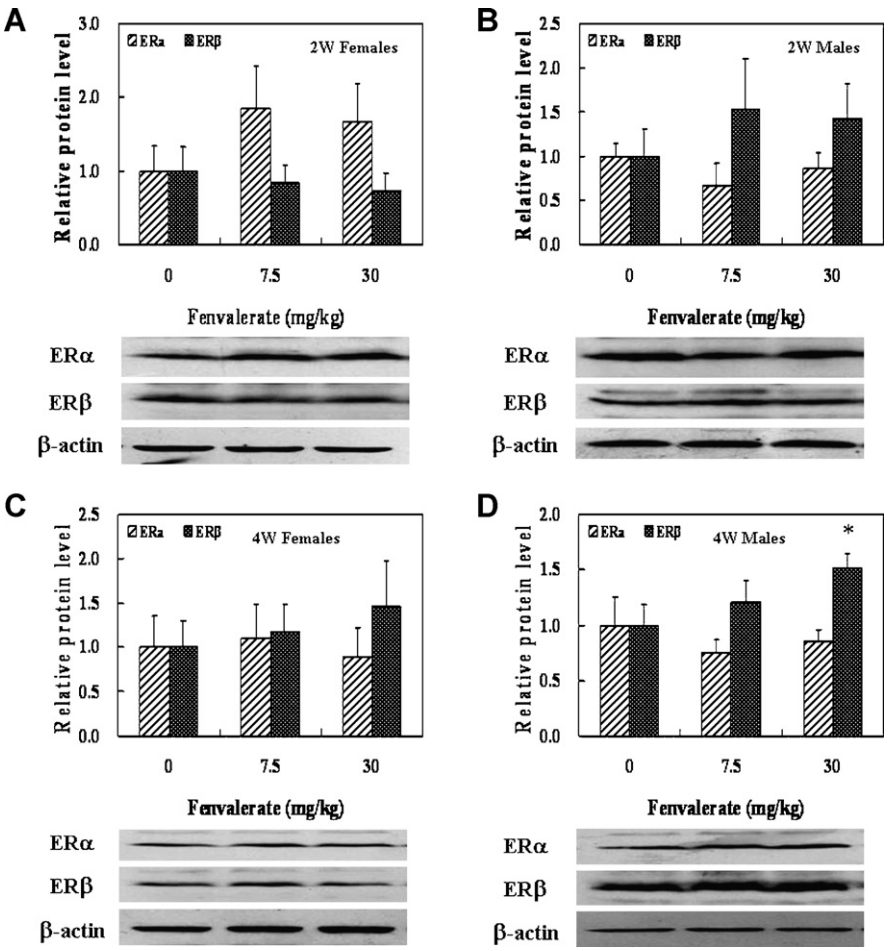

Fig. 8. Effects of pubertal fenvalerate exposure on the expression of ERs in cerebral cortex. Mice were administered with fenvalerate (30 mg/kg or 7.5 mg/kg) by gavage daily from PND28 to PND56. Cerebral cortex was collected at PND42 or PND56. ERα and ERβ in cerebral cortex was measured using immunoblotting. Blots are representative of four independent experiments. Quantitative analysis of scanning densitometry was performed. ERα and ERβ were normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. (A) For female mice administered with fenvalerate for two weeks. (B) For male mice administered with fenvalerate for two weeks. (C) For female mice administered with fenvalerate for four weeks. (D) For male mice administered with fenvalerate for four weeks. Data were presented as mean ± SEM. *$P < 0.05$ compared with the controls.

the effects of pubertal fenvalerate exposure on the expression of AR and ERs in cerebral cortex were investigated. Results showed that protein level of ERα and ERβ in cerebral cortex was scarcely altered in male and female mice exposed to fenvalerate during puberty. Conversely, the expression of AR in cerebral cortex was markedly upregulated in fenvalerate-exposed mice. Surprisingly, the effects of pubertal fenvalerate exposure on the expression of AR in cerebral cortex are more obvious in female mice than in male mice. Indeed, EDC-induced endocrine disruption in the developing brain has been reported to be gender-dependent in different animal models. According to a recent report, bisphenol A resulted in endocrine alteration and sexually dimorphic changes in rodent brain (Rubin et al., 2006). In addition, polychlorinated biphenyls produced differential effects of endocrine disruption upon the developing brain in male and female animals (Colciago et al., 2006). In the present study, our results suggest that pubertal fenvalerate exposure disrupts T and E_2 synthesis and differentially regulates the expression of androgen and estrogen receptors in cerebral cortex in a gender-dependent manner.

In human and rodent models, T or E_2 in the developing brain plays a critical role in sexual differentiation of brain during developmental period (Paus et al., 2010; Taziaux et al., 2007). Several studies showed that T could influence behavioral development in males not only through AR but also through ERs (Zuloaga et al., 2008; Frye et al., 2008; Raskin et al., 2009). Another study found that aromatization of T into E_2 is important for the development of aggressive behavior (Wu et al., 2009). On the other hand, the level of T or E_2 in the developing brain might influence spatial learning and memory performance. According to an earlier report, a positive correlation was observed between T and spatial learning and memory performance in adult male white-footed mice (Pyter et al., 2006). Interestingly, T or its metabolites profoundly improve spatial learning and memory performance in mice (Osborne et al., 2009). The present study found that T and E_2 synthesis in cerebral cortex was disrupted in fenvalerate-exposed mice. In addition, the expression of androgen and estrogen receptors in cerebral cortex was also altered in mice exposed to fenvalerate during puberty. These changes of steroid hormones and their receptors in the developing brain might be deleterious for sexual differentiation and the development of spatial learning and memory performance. Thus, additional work is required to determine whether pubertal fenvalerate exposure disrupts neurobehavioral development. On the other hand, the dose levels used in the present study are much higher than the human exposure levels. Therefore, further study is necessary to investigate the effects of low dose fenvalerate exposure on the synthesis of T and E_2 and the expression of androgen receptor (AR) and estrogen receptors (ERs) in cerebral cortex.

In summary, the present study found that pubertal fenvalerate exposure inhibits T and E_2 synthesis in cerebral cortex of male mice. Conversely, the level of T and E_2 in cerebral cortex was increased

in female mice exposed to fenvalerate during puberty. In addition, pubertal fenvalerate exposure differentially regulates the expression of T synthetic enzymes and androgen and estrogen receptors in cerebral cortex in a gender-dependent manner. These changes of steroid status and its receptor in the developing brain might be deleterious for sexual differentiation in the developing brain and spatial learning and memory performance.

Conflict of interest statement

None declared.

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