Toxicology and Applied Pharmacology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration

Fucui Ma, Daicheng Liu *

Key Laboratory of Animal Resistance, College of Life Science, Shandong Normal University, 88 East Wenhua Road, Jinan 250014, PR China

ARTICLE INFO

Article history:
Received 17 February 2014
Revised 12 November 2014
Accepted 17 November 2014
Available online xxxx

Keywords:
17β-trenbolone
Neurodegeneration
Alzheimer's disease
Presenilin-1
Aβ42

ABSTRACT

Both genetic and environmental factors contribute to neurodegenerative disorders. In a large number of neurodegenerative diseases (for example, Alzheimer's disease (AD)), patients do not carry the mutant genes. Other risk factors, for example the environmental factors, should be evaluated. 17β-trenbolone is a kind of environmental hormone as well as an anabolic–androgenic steroid. 17β-trenbolone is used as a growth promoter for livestock in the USA. Also, a large portion of recreational exercisers inject 17β-trenbolone in large doses and for very long time to increase muscle and strength. 17β-trenbolone is stable in the environment after being excreted. In the present study, 17β-trenbolone was administered to adult and pregnant rats and the primary hippocampal neurons. 17β-trenbolone's distribution and its effects on serum hormone levels and Aβ42 accumulation *in vivo* and its effects on AD related parameters *in vitro* were assessed. 17β-trenbolone accumulated in adult rat brain, especially in the hippocampus, and in the fetus brain. It altered Aβ42 accumulation. 17β-trenbolone induced apoptosis of primary hippocampal neurons *in vitro* and resisted neuroprotective function of testosterone. Presenilin-1 protein expression was down-regulated while β-amyloid peptide 42 (Aβ42) production and caspase-3 activities were increased. Both androgen and estrogen receptors mediated the processes. 17β-trenbolone played critical roles in neurodegeneration. Exercisers who inject large doses of trenbolone and common people who are exposed to 17β-trenbolone by various ways are all influenced chronically and continually. Identification of such environmental risk factors will help us take early prevention measure to slow down the onset of neurodegenerative disorders.

Introduction

Alzheimer's disease (AD) is a progressive, irreversible, and so far incurable dementia. The genetic factors contributing to AD have been studied extensively. Familial Alzheimer's disease (FAD) is primarily caused by dominantly inherited mutations in the genes that encode presenilin (PS-1 and PS-2) and amyloid precursor protein (APP) (Hardy and Gwinn-Hardy, 1998). Other factors such as other genetic factors, aging, and environmental factors may lead to a chronic imbalance between β-amyloid peptide (Aβ) production and Aβ clearance in the brain (Mattson, 2000). Considering neuropathology changes may occur many years earlier than the clinical dementia, we assumed that some environmental factors which are continuously influencing the human body may play roles in the neurodegeneration of AD.

The main hallmarks of AD in the brain are extracellular β-amyloid peptide (Aβ) plaques (senile plaques) and intracellular neurofibrillary tangles (NFTs). The senile plaques consist mainly of Aβ40 and Aβ42. The initial Aβ deposition begins with Aβ42 because it is more prone to aggregate than Aβ40 (Suzuki et al., 1994). The Aβ hypothesis is one of the most prevailing hypotheses that have been proposed to explain the pathogenesis of AD. Aβ is a peptide released by proteolysis of APP. APP is a type I transmembrane protein and is ubiquitously expressed in both neuronal and nonneuronal tissues. Three secretases are involved in proteolysis of APP, α-secretase, β-secretase, and γ-secretase. Cleavage of APP by β- and γ-secretases will produce Aβ. On the contrast, Aβ production will be avoided if APP is cleaved first by α-secretase instead by β-secretase. APP is first cleaved in the extracellular domain by β-secretase, and the remnant is cleaved at least twice within the membrane by γ-secretase to produce the Aβ peptide and the intracellular domain. The produced Aβ variants contain 38–43 residues. The major Aβ variant is 40 residues in length (Wolfe and Selkoe, 2010; Wolfe, 2013). Although Aβ42 represents only 10% of total Aβ, it is the major form found in the plaques of AD. The "amyloid hypothesis"

Abbreviations: Δψm, mitochondrial membrane potential; AAS, anabolic–androgenic steroid; AD, Alzheimer's disease; APP, amyloid precursor protein; AR, androgen receptor; Aβ, β-amyloid peptide; BBB, blood brain barrier; CSF, cerebrospinal fluid; DHT, dihydrotestosterone; E_2, estradiol; ER, estrogen receptor; Flu, flutamide; Fulv, fulvestrant; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol carbocyanine iodide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-dipenyl tetrazolium bromide; Na-DOC, sodium deoxycholate; PBST, phosphate-buffered saline containing 0.5% Tween-20; PFA, paraformaldehyde; PI, propidium iodide; PR, progestin receptor; PROG, progesterone; PS, presenilin; T, testosterone; TB, 17β-trenbolone; Tri, trilostane.

* Corresponding author. Fax: +86 531 86185360.
E-mail addresses: mafucui@hotmail.com (F. Ma), daichengliu@gmail.com, liudch@sdnu.edu.cn (D. Liu).

http://dx.doi.org/10.1016/j.taap.2014.11.007
0041-008X/

identifies Aβ deposition as the primary cause of AD (Hardy, 1997; Tanzi and Bertram, 2005; Suzuki et al., 1994). The Aβ overproduction in the brain is thought to be the primary pathogenic process which causes various physiological events, such as oxidative damage, synaptic loss, formation of tau pathology, microglial and astrocytic activation, and progressive cognitive decline (Wojda and Kuznicki, 2013).

PS is a 50–55 kDa protein which contains nine transmembrane domains (Laudon et al., 2005). PS and three other proteins were discovered as essential for γ-secretase activity (Wolfe, 2013). PS-1 mRNA was expressed predominantly in the neuronal cells of the CNS, but only at low level in glial cells (Suzuki et al., 1996). Mutations in PS-1 can lead to alteration of APP processing and an increase and aggregation of Aβ42 (Haass and Strooper, 1999; Doan et al., 1996; Akbari et al., 2004). PS activity is important in learning, memory, and neuronal survival. PS-1 may control neurite outgrowth in neurons (Dowjat et al., 1999). PS is essential for synaptic contact and in regulation neurotransmitter release during synaptic transmission. Inactivation of presynaptic PS will decrease long-term potentiation (LTP) and alter short-term plasticity and synaptic facilitation (Georgakopoulos et al., 1999; Ho and Shen, 2011). PS-1 is also involved in regulation of apoptosis (Fluhrer et al., 2004).

Environmental hormones are also called endocrine disrupting compounds which are released from domestic, agricultural, and industrial sources and can interfere with the endocrine system of human beings and animal kingdom (Zeng et al., 2011). Since endogenous hormones, such as testosterone (T), dihydrotestosterone (DHT), and estradiol (E_2), exhibit protective actions in AD, the environmental hormones which may mimic or antagonize the role of endogenous hormones become our suspect for AD onset. Trenbolone acetate (TBA, 17β-hydroxyestra-4,9,11-trien-3-one 17-acetate) is a synthetic anabolic steroid that has been used extensively since the 1970s as a growth promoter for livestock in the USA. TBA is administered to livestock by subcutaneous slow-release implant (Yarrow et al., 2010). After being released, TBA is rapidly hydrolyzed to 17β-trenbolone (17β-hydroxyestra-4,9,11-trien-3-one) in blood stream of the animals. 17β-trenbolone is a potent agonist of mammalian androgen receptor (AR) with a binding affinity to the human AR comparable to DHT (Bauer et al., 2000). Abundant studies have focused on the reproductive toxicity of 17β-trenbolone (Hemmer et al., 2001; Wilson et al., 2002; Ankley et al., 2003; Sone et al., 2005; Yarrow et al., 2010).

Besides, as an anabolic–androgenic steroid, trenbolone is used by a large portion of recreational exercisers to increase muscle size and strength (Perry et al., 2005; Parkinson and Evans, 2006; Ip et al., 2011).

Humans are at high risk of being exposed to 17β-trenbolone. There are four possible ways through which humans are exposed to 17β-trenbolone. The first one is 17β-trenbolone residue in meat. Although 17β-trenbolone is banned in livestock by some organizations, its usage is still allowed in the USA and some merchants in other areas also use 17β-trenbolone in pursuit of profit. The second way 17β-trenbolone goes to human body is through the food chains. 17β-trenbolone can be excreted by the animals and humans and it has long half-life and stable properties in the environment (Schiffer et al., 2001). It's worrying that 17β-trenbolone may be absorbed by aquatic animals (Yarrow et al., 2010) as well as plants (Schiffer et al., 2001; Blackwell et al., 2012) and can be incorporated into food chains. Thirdly, 17β-trenbolone is regarded as a promising candidate in clinical application. 17β-trenbolone can reduce incidence of androgenic and/or estrogenic side effects associated with androgen administration (Yarrow et al., 2010). The last way by which 17β-trenbolone goes into human body is direct injection. Since 17β-trenbolone can promote muscle growth and reduce fat (Yarrow et al., 2010), it is used in athletics and fit center, which is actually forbidden. The surveys indicated that trenbolone is widely used as an anabolic–androgenic steroid (AAS) mostly by recreational exercisers, in very large doses for very long times (Perry et al., 2005; Parkinson and Evans, 2006; Ip et al., 2011).

To our knowledge, no article related to the effects of environmental hormone on AD onset has been reported in the current literature. Both the *in vivo* and *in vitro* effects of 17β-trenbolone on AD-related parameters were assessed. The experiment systems we used did not carry mutant genes that are correlated with AD.

Materials and methods

Animals and cell culture. Wistar rats were purchased from the Center of Experimental Animal of Shandong University (Shandong, China). The studies were conducted according to the regulations of the Center of Experimental Animal of Shandong University. Male and female rats were of 250 ± 10 g. Pregnant rats were shipped on the day after mating and housed individually in clean plastic cages (20 cm × 25 cm × 47 cm). The day after mating was designated as gestation day 1 (GD 1). Photoperiod was 14 h light and 10 h dark, lights on at 06:00 and off at 20:00. Rats were allowed free access to rodent chow and water. Temperature was 20–22 °C and relative humidity was 45–55%.

Hippocampal neurons from newborn rats (postnatal day 0) were cultured according to previously established procedures (Nunez, 2008). The culture medium was Neurobasal A (phenol red free, Invitrogen, USA) containing 2 mM L-Glutamine (Sigma, USA) and 2% B27 Supplement (Invitrogen, USA). All experiments were performed on 9- to 12-day-old cultures.

Drugs and treatments. 17β-trenbolone was purchased from Dr. Ehrenstorfer GmbH (Germany). T, DHT, flutamide, fulvestrant, and trilostane were purchased from Sigma (USA). In animal experiments, 17β-trenbolone was dissolved in laboratory-grade corn oil (Sigma, USA) with final concentration of 5 mg/ml, 1 mg/ml, and 0.2 mg/ml, respectively. Rats were divided into several groups with each group having six rats. Rats were injected with corn oil (control) or 17β-trenbolone solution (0.1 ml/100 g body weight) once intramuscularly on the right hind limbs. Pregnant rats were injected on GD 16. Male rats in groups Am, Bm, Cm, Dm, Em, and Fm were injected with 5 mg/ml 17β-trenbolone solution and the treating time were 0.5 h, 2 h, 6 h, 12 h, 24 h, and 48 h, respectively. Male rats in groups Gm and Hm were injected with 17β-trenbolone solution of 1 and 0.2 mg/ml, respectively. The treating time was 48 h. Male rats in group Om was control. Accordingly, female rats in groups Af, Bf, Cf, Df, Ef, and Ff were injected with 5 mg/ml 17β-trenbolone solution and the treating times were 0.5 h, 2 h, 6 h, 12 h, 24 h, and 48 h, respectively. Female rats in groups Gf and Hf were injected with 17β-trenbolone solution of 1 and 0.2 mg/ml, respectively. The treating time was 48 h. Female rats in group Om was control. Pregnant rats in group P were treated with 5 mg/ml 17β-trenbolone solution for 48 h. Group Op was control.

The largest dose used in the animal experiment was 5 mg/kg body weight. According to the dose conversion formula below (Chen, 1993) and the data in the previous paper presented, the equivalent injection dose for human should be 0.85 mg/kg body weight which is much lower than the dose injected by exercisers.

$$d_{human} = d_{rat} \times \frac{R_{human}}{R_{rat}} \times \frac{\sqrt[3]{W_{rat}}}{\sqrt[3]{W_{human}}} \quad (187)$$

d, dose; R, body size coefficient; W, body weight.

$d_{rat} = 5$ mg/kg, $R_{human} = 0.11$, $R_{rat} = 0.09$, $W_{rat} = 0.25$ kg, $W_{human} = 94.4$ kg (Perry et al., 2005).

The survey published in 2005 reported that 21.3% of the 207 respondents injected trenbolone at the dose of 117.1 mg every other day (*i.e.*, 1.24 mg/kg body weight) with an average cycle of 6.8 weeks (Perry et al., 2005). In 2006 it was reported that the trenbolone injection dose by exercisers was 700 mg/week with a cycle of 11–20 weeks (Parkinson and Evans, 2006). A most recent research indicated that

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007

20.8% of the respondents used trenbolone, and trenbolone is one of the three most commonly used AAS (Ip et al., 2011). In general, trenbolone is used in much larger dose and much longer times in the human body.

The medium supplement B27 contains antioxidants which allow for long term neuron survival and it was used before drug treatment. To avoid interference of the antioxidants with the results, B27 supplement was substituted with B27 Supplement Minus AO (Invitrogen, USA) which is free of antioxidants and with which neurons will survive for a few days. In cell experiments, drugs were dissolved in dimethyl sulfoxide (DMSO, Sigma, USA) and then diluted with culture medium. Final 17β-trenbolone concentrations were 100 nM, 10 nM, and 1 nM. Final concentrations of T, DHT, flutamide, fulvestrant, and trilostane were 10 nM, 10 nM, 10 μM, 1 μM, and 10 μM, respectively. Final DMSO concentration in the medium was no more than 0.1%. To investigate the toxicity of 17β-trenbolone, it was added into the medium and cells were incubated for 48 h. In order to clarify whether AR was involved in the mechanism, flutamide (an AR antagonist) or DHT (an AR agonist) was added, followed 1 h later by 100 nM 17β-trenbolone (groups Flu + TB and DHT + TB) for 48 h. Fulvestrant was added, followed 1 h later by 100 nM 17β-trenbolone for 48 h, to investigate the involvement of estrogen receptor (ER, group Fulv + TB). There was also group Tri + DHT + TB in which trilostane (a 3β-hydroxysteroid dehydrogenase inhibitor) was added, followed 1 h later by DHT and 100 nM 17β-trenbolone for 48 h. Neurons were also treated with T 24 h before or after 100 nM 17β-trenbolone or they were used together (groups T then TB, TB then T + TB, TB then T, and T + TB). Control neurons were treated by replacing the medium with medium containing 0.1% DMSO (vehicle).

Animal sample collection. After drug treatment, rats were anesthetized by exposure to diethyl ether between the hours of 08:00 and 11:00. Cerebrospinal fluid (CSF) samples were taken using the cisternal puncture technique. Blood collected was divided into two parts which were put into tubes with or without $EDTAK_2$ anticoagulant, respectively. The blood was centrifuged at 5000 *g*, at 4 °C for 10 min, and then plasma or serum was collected. Serum is best for hormone determination and plasma is best for other parameter determination. Rats were euthanized by decapitation and the brains of the adult rats and fetuses were obtained. Hippocampus of adult rats was isolated from the brain on ice. The hippocampus and the brain without hippocampus were stored separately. Biceps femoris muscles on the left leg were collected. We did not choose the right side because 17β-trenbolone was injected on the right side and results from samples on the left side would be more reliable. All samples were stored at −80 °C until analysis.

Drug concentration determination. This experiment aimed at investigating whether 17β-trenbolone could penetrate through the blood brain barrier (BBB) and placental barrier and the distribution of 17β-trenbolone in the body. 17β-trenbolone concentrations in the samples were determined by Trenbolone ELISA Kit (Cat. #DE-100170, Alpha Diagnostic Intl. Inc., USA) according to the Instruction Manual (No. M-DE-100170). Each test was performed in triplicate and the mean value was obtained.

Serum hormone determination. To assess effect of 17β-trenbolone on endocrine system, concentrations of serum hormones, such as T, E_2, and progesterone (PROG) were determined. The procedure was carried out on the Access Immunoassay Systems (Beckman Coulter Access 2, USA) according to the manual instruction of the three hormone testing kits, *i.e.*, Access Testosterone (33560, Beckman, USA), Access Estradiol (33540, Beckman, USA), and Access Progesterone (33550, Beckman, USA). Each test was performed in triplicate and the mean value was obtained.

Cell viability assay. Cell viability was assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-dipenyl tetrazolium bromide (MTT) method. After drug treatment, 0.5 mg/ml MTT was added into the culture plate and incubated for 2 h in the cell incubator. Then 100 μl of DMSO was added after removal of supernatants. The formazan crystals formed inside the viable cells would be solubilized. The absorbance at 540 nm was read with microplate reader. Each test was performed in triplicate and the mean value was obtained.

Cell morphology and nuclear staining assay. Nuclear of the cell was stained by Hoechst 33258 (Sigma, USA). Culture medium was discarded and cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 10 min. Cells were then rinsed with PBS for several times, followed by staining with 5 μg/ml Hoechst 33258 in PBS for 10 min. The stained cells were washed with PBS for several times.

Plasma membrane translocation of phosphatidylserine. Cells were incubated with FITC-conjugated Annexin-V (Sigma, USA) for 10 min at room temperature, followed by PI (propidium iodide, Sigma, USA) staining.

Mitochondrial membrane potential ($\Delta\psi_m$) measurement. $\Delta\psi_m$ was estimated using 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol carbocyanine iodide (JC-1). Normal mitochondria can concentrate JC-1 from aggregates, whereas de-energized mitochondria cannot. Fluorescence of JC-1 monomer is green, while fluorescence of JC-1 aggregate is red. Cells were incubated with JC-1 (5 μM) for 20 min at 37 °C and washed.

Cells were photographed by Olympus I × 71 Inverted Fluorescent Microscope equipped with DP2-BSW camera software. Photographs were processed using Adobe Photoshop CS2 (Adobe Systems, San Jose, CA) to obtain the optimal display of the image.

Caspase-3 activity. Caspase-3 activity was measured using spectrophotometer caspase-3 assay kit (Beyotime, China). Ac-DEVD-*p*NA can be catalyzed by caspase-3 to *p*NA which has strong absorbance at 405 nm. Units of caspase-3 (nmol *p*NA) were determined from a standard curve, and the values were normalized to protein content. Each test was performed in triplicate and the mean value was obtained.

Aβ42 assay. Aβ42 was measured using Human/Rat β Amyloid (42) ELISA Kit (Wako, Japan). The samples were hippocampus, brain (without hippocampus), CSF, plasma, and cell culture medium. Each test was performed in triplicate and the mean value was obtained.

Protein precipitation and Western blot analysis. Cells lysates were concentrated using trichloroacetic acid-sodium deoxycholate/acetone (TCA-DOC/acetone) method according to previous method (Cheng et al., 2009). The protein samples were separated on a SDS-polyacrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Germany). The membrane was blocked and then incubated with primary antibody (Rb pAb to Presenilin 1, dilution 1:300, Abcam, England) overnight at 4 °C. The membrane was washed with PBST (phosphate-buffered saline containing 0.5% Tween-20) and incubated with secondary HRP-conjugated antibody (Anti-rabbit IgG, HRP-linked antibody, dilution 1:3000, Cell Signaling Technology, Inc., USA) for 1 h at room temperature. Antibody binding was detected using Enhanced Chemiluminescence Substrates for Western Blotting (Perkin Elmer, USA). After developing, the membrane was stripped, followed by reprobing to detect β-actin to confirm equal loading. Primary antibody was β-actin (13E5) Rabbit mAb (dilution 1:1000, Cell Signaling Technology, Inc., USA). QuantiScan Software for Windows (BIOSOFT, USA) was used to quantify the band intensity. Photographs were processed using Adobe Photoshop CS2 (Adobe Systems, San Jose, CA) to obtain the optimal display of the image. Each test was performed in triplicate and the mean value was obtained.

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007

Statistical analysis. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Inc., USA). Results were expressed as mean ± SEM. Student's *t* test and ANOVA were carried out. A level of $p < 0.05$ was required to obtain statistical significance.

Results

17β-trenbolone distribution in rats

17β-trenbolone was observed to exist in brain, hippocampus, CSF, plasma, and muscle of male and female rats, and in fetus brain (Fig. 1). The ratio of 17β-trenbolone concentration in brain tissues to that in plasma of male rats reached the highest at 12 h. The ratio at 24 h was the highest for hippocampus and 12 h for the brain (without hippocampus) in female rats (Fig. 2). The results indicated that 17β-trenbolone could cross both the BBB and placental barrier and could exert its effect on CNS of both adult and fetal rats. 17β-trenbolone accumulated predominantly in brain, especially in the hippocampus. 17β-trenbolone concentration in hippocampus was higher than in the rest of the brain. 17β-trenbolone levels in tissues and fluids exhibit a dose-dependent property. There were some differences between male rats and female rats. We could see from the figures that 17β-trenbolone concentrations in male rats were commonly higher than in female rats.

Effect of 17β-trenbolone treatment on serum hormone levels of the rats

Administration of 17β-trenbolone caused serum hormone fluctuation in both male and female rats (Fig. 3). In male rats, 48 h after 17β-trenbolone treatment, serum T levels of the three dose groups were slightly lower than that of the control group ($p > 0.05$) while in female rats serum T levels of all the groups were higher than that of the control

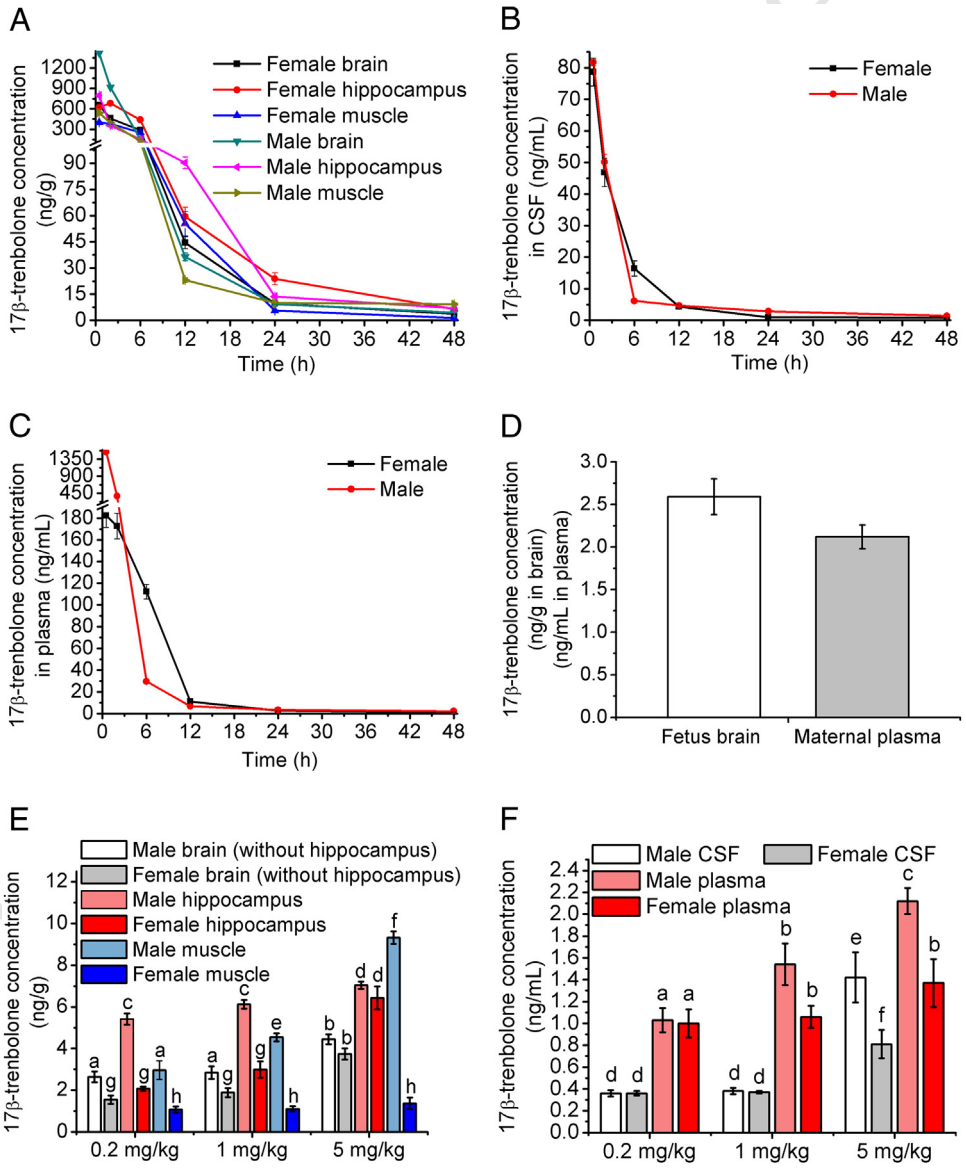

Fig. 1. 17β-trenbolone concentration in rats. (A–D) 17β-trenbolone concentration in rat brain (without hippocampus), hippocampus, muscle (A), CSF (B), plasma (C), and fetus brain and maternal plasma (D). (E) Comparison of 17β-trenbolone concentration in male and female rat brain (without hippocampus), hippocampus, and muscle with different 17β-trenbolone injection doses. a–h, different letters indicate significant difference ($p < 0.05$, $n = 6$). (F) Comparison of 17β-trenbolone concentration in male and female rat CSF and plasma with different 17β-trenbolone injection doses. a–f, different letters indicate significant difference ($p < 0.05$, $n = 6$).

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007

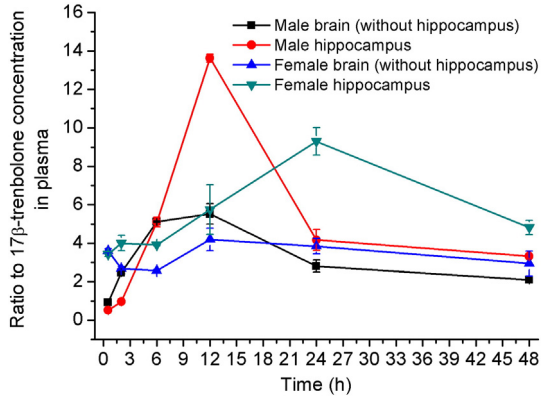

Fig. 2. Ratio of 17β-trenbolone concentration in brain tissues to that in plasma. 17β-trenbolone dose was 5 mg/kg body weight. n = 6.

group and the E_2 level was elevated. Serum E_2 level was not altered while PROG level was very significantly higher after 17β-trenbolone treatment of the pregnant rats.

Effect of 17β-trenbolone treatment on Aβ42 level in vivo

Aβ42 levels in hippocampus, brain (without hippocampus), CSF, and plasma were measured. Results were shown in Fig. 4. Since plasma Aβ42 is derived from peripheral tissues so it may not reflect the situations in brain well, the Aβ42 levels in plasma in our present studies were examined as a parameter evaluating the toxicity of 17β-trenbolone. In male rats, 48 h after 17β-trenbolone injection, Aβ42 levels were increased in a dose-dependent manner in the brain (without hippocampus), hippocampus, and plasma. Aβ42 level was only significantly decreased in the female brain (without hippocampus). Aβ42 level in the control embryo rat brain was very low. 17β-trenbolone treatment of the mother rats could significantly increase Aβ42 level in embryo rat brain.

17β-trenbolone induced cell viability reduction and apoptosis in primary hippocampal neurons

As is shown in Fig. 5, cell viability was reduced and 17β-trenbolone induced neuron morphological changes, and possible chromatin condensation and nuclear fragmentation. It also induced translocation of phosphatidylserine. Change of the fluorescence color from red to green indicated the decrease of $\Delta\psi m$ (Fig. 5C). These results indicated apoptosis in the neurons.

Increase of caspase-3 activity in vitro was mediated by both AR and ER

Caspase-3 activities increased after treatment with 17β-trenbolone at concentrations of 1-100 nM (Fig. 6). Addition of T or DHT alone into the cultures would not alter the caspase-3 activity. The inhibitors (flutamide, fulvestrant, and trilostane) pre-treated groups have lower caspase-3 activities compared with the 100 nM 17β-trenbolone treated group, but still higher than control group. When DHT and 17β-trenbolone were added together to treat the neurons, caspase-3 activity increased very significantly. Treatment with trilostane, the inhibitor of 3β-hydroxysteroid dehydrogenase, suggested that DHT might be partly metabolized by 3β-hydroxysteroid dehydrogenase to 5α-androstan-3β, 17β-diol (3β-diol) (Pak et al., 2005; Lund et al., 2006; Handa et al., 2008) in primary hippocampal neurons. The result suggested that AR and ER were involved in the regulation of caspase-3 activity. T was proved to be able to protect primary cultured rat hippocampal neurons. Addition of T could decrease the caspase-3 activity compared with the 17β-trenbolone-only treated group.

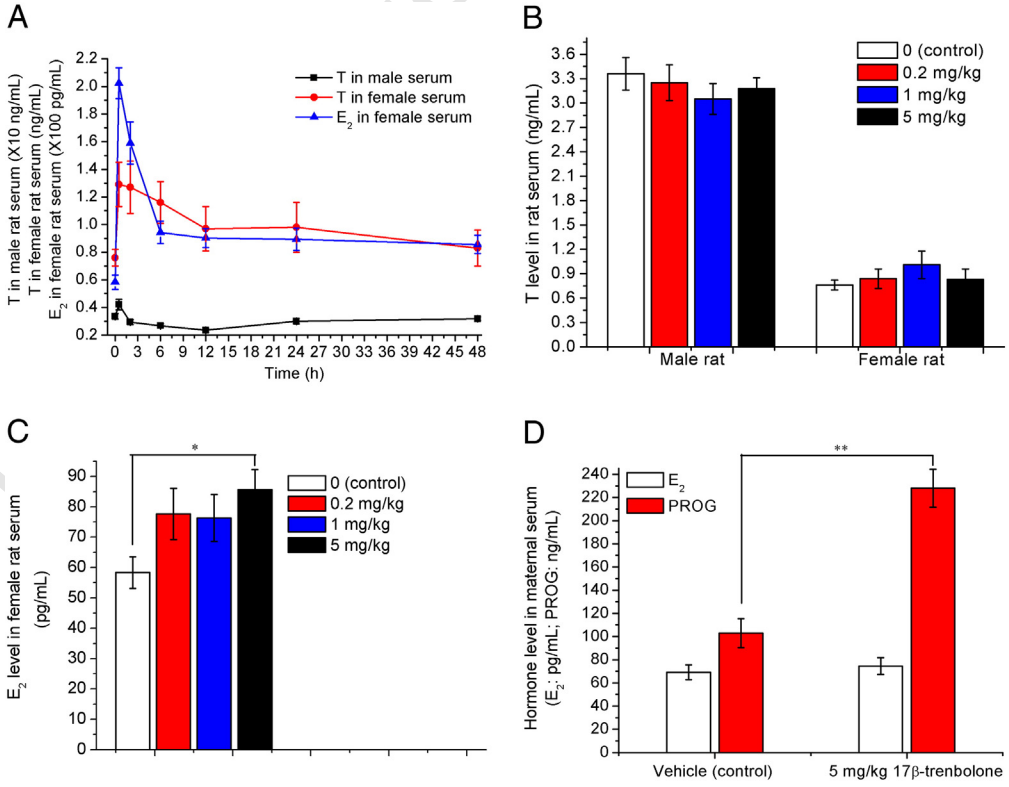

Fig. 3. Rat serum hormone levels. (A) T and E_2 levels in rat serum changed over time. (B) Effect of 17β-trenbolone injection dose on T level in rat serum. (C) Effect of 17β-trenbolone injection dose on E_2 level in female rat serum. (D) Effect of 17β-trenbolone treatment on E_2 and PROG levels in serum of pregnant rats. *p < 0.05, **p < 0.01 (n = 6).

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007

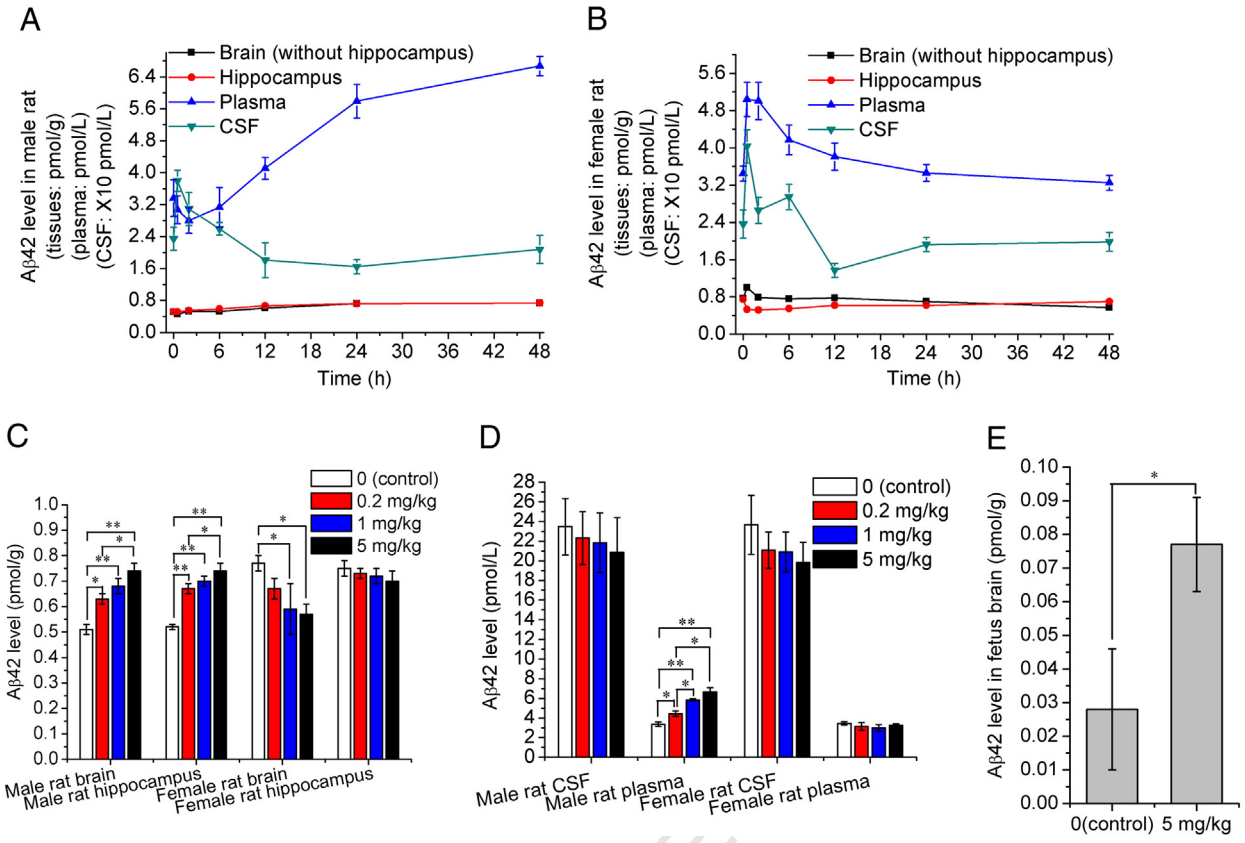

Fig. 4. Effect of 17β-trenbolone treatment on Aβ42 level in rats. (A and B) Aβ42 level in male and female rat brain (without hippocampus), hippocampus, plasma, and CSF changed over time. (C) Effect of 17β-trenbolone injection dose on Aβ42 level in rat brain (without hippocampus) and hippocampus. (D) Effect of 17β-trenbolone injection dose on Aβ42 level in rat CSF and plasma. (E) Effect of 17β-trenbolone treatment on Aβ42 level in fetus brain. $^{*}p < 0.05$, $^{**}p < 0.01$ ($n = 6$).

Reduction of PS-1 protein expression level in vitro was mediated by both AR and ER

17β-trenbolone caused down-regulation of PS-1 protein levels in the neurons (Fig. 7) and PS-1 protein expression was inhibited on a same level by treatment with 17β-trenbolone from 1 nM to 100 nM. Down-regulation of PS-1 protein expression was inhibited by antagonists (flutamide, fulvestrant, and trilostane) and promoted by agonist (DHT), indicating both involvement of AR and ER, and DHT was partly metabolized to 3β-diol. Addition of T could up-regulate PS-1 protein expression level. The result shows that T and 17β-trenbolone competed for exerting their own effects on primary hippocampal neurons.

Increase of Aβ42 secretion by primary hippocampal neurons was mediated by both AR and ER

Change of Aβ42 peptide level in the conditioned medium was negatively related to PS-1 protein expression level and positively related to caspase-3 activity (Fig. 6). The result shows that 17β-trenbolone treatment promoted Aβ42 production and both AR and ER were involved in the process. T and 17β-trenbolone resisted each other by playing their neuroprotection and neurotoxic actions, respectively.

Interactions of T and 17β-trenbolone

The activities of PS-1 include increasing caspase-3 activity and Aβ42 secretion and down-regulating PS-1 protein expression. The different combinations of T and 17β-trenbolone treated groups showed there interactions. T could protect the neurons by resisting activities of PS-1. When T was added prior to 17β-trenbolone (group T then TB), the PS-1 activities were reduced, especially the activity of promoting Aβ42 production. When 17β-trenbolone was added before T (group TB then T), the PS-1 activities were also reduced. But when 17β-trenbolone was added first and there was 17β-trenbolone all the time (group TB then T + TB), the protecting role of T was limited.

Discussion

Our experiment indicated that 17β-trenbolone could cross the BBB. 17β-trenbolone is a small hydrophobic molecular sharing similar structure with T and DHT, so it's not difficult to understand why 17β-trenbolone can cross the BBB. Hippocampus is known to be a target for the modulator actions of androgens and estrogens (Hatanaka et al., 2009). Maybe that's why brain tissue, especially the hippocampus, had stronger affinity with 17β-trenbolone than muscle. 17β-trenbolone was injected by athletes and bodybuilders once every two days. Our results showed that 17β-trenbolone concentrations in rat brain and especially hippocampus were still high even 48 h after 17β-trenbolone injection at the lowest administration dose (0.2 mg/kg body weight). If 17β-trenbolone is injected again, its concentrations in the brain and hippocampus will be increased. That means 17β-trenbolone will accumulate in the brain and hippocampus, which will lead to serious consequences. Intense attention should be paid to the fact that 17β-trenbolone was also detected in fetal rats' brain. 17β-trenbolone could cross the placental barrier and exert effects on the fetus brain during development. Aβ42 concentration in fetus brain was increased by maternal exposure to 17β-trenbolone. The damage to neurons and then to the CNS may occur since as early as fetus. The consequences may be irreversible. The negative effects on developmental and growth

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007

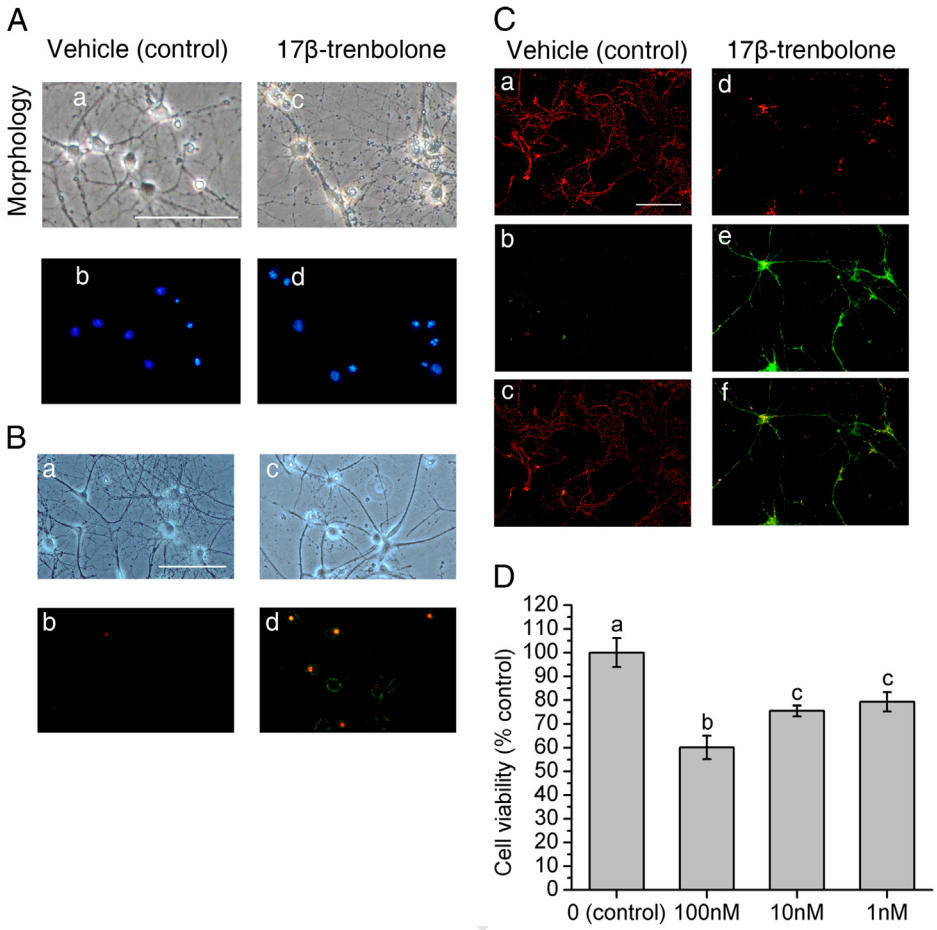

Fig. 5. 17β-trenbolone induced cell apoptosis and decrease of cell viability. (A) Light microscopy of neuron morphology (a and c) and H33258 (Hoechst 33258) staining of nucleus (b and d). Cells were treated with either vehicle (control, a and b) or 100 nM 17β-trenbolone (c and d). Scale bar = 100 μm. (B) Annexin V/PI double staining assay. Cells were treated with either vehicle (control, a and b) or 100 nM 17β-trenbolone (c and d). The micrograph shows the morphology of the neurons under light microscopy (a and c) and the overlay of the Annexin V-FITC and PI images (b and d). Scale bar = 100 μm. (C) Alteration of $\Delta\psi_m$ in the neurons. Cells were treated with either vehicle (control, a, b, and c) or 100 nM 17β-trenbolone (d, e, and f). Red fluorescence is emitted by the JC-1 aggregates (a and d) and green fluorescence by JC-1 monomer (b and e). Images c and f are overy of images a and b, and d and e, respectively. Scale bar = 100 μm. (D) Cell viability assay of primary cultured rat hippocampal neurons. The percentage of viable cells was calculated as a ratio of A540 of treated cells to control cells. a–c, different letters indicate significant difference (n = 9). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of offspring will be far-reaching. Subsequent observations on the neurotoxicity in the offspring should be carried out in future studies.

The "amyloid hypothesis" identifies Aβ overproduction and deposition as the primary cause of AD (Tanzi and Bertram, 2005). Aβ42 levels were altered in both *in vivo* and *in vitro* experiments by 17β-trenbolone administration. Increase of Aβ42 concentration in the brain and hippocampus will increase the Aβ42 burden, lead to its aggregation and deposition, and cause damages to neurons. Decreased Aβ42 levels in CSF were regarded as predictor of AD (Blennow, 2005). In our experiment, the CSF Aβ42 concentration did not change significantly. However, the fact that neurons did produce more Aβ42 cannot be neglected. This can only be explained as the overproduced Aβ42 did not diffuse into CSF and the deposition of Aβ42 might have already occurred in the brain. Moreover, the rats were treated with 17β-trenbolone for only 2 days. Long term exposure to 17β-trenbolone will have a chronic influence on Aβ42 level in the CSF. 17β-trenbolone caused increase of E_2 which can reduce Aβ accumulation (Pike et al., 2009), maybe that's why there were sex differences in Aβ42 accumulation.

17β-trenbolone induced apoptosis of the primary hippocampal neurons. Neuronal cell apoptosis is the cardinal feature of both acute and chronic neurodegenerative diseases, including AD (Mattson, 2000). 17β-trenbolone caused Aβ42 overproduction in the conditioned medium and increase of caspase-3 activity in the neuron lysates. PS-1 levels in the neurons changed reversely. Mutations in PS-1 can lead to alteration of APP processing and increase and aggregation of Aβ42 (Haass and De Strooper, 1999). We can interpret PS-1 mutation as another way of losing normal PS-1. They share certain identical consequences. Loss of PS-1 in the neurons leads to weaken of its normal functions and increases the vulnerability of neurons to apoptosis. As to how the PS-1 protein expression was down-regulated, we hypothesized that the endoproteolysis of PS-1 was promoted (Wolfe, 2013) and/or PS-1 was cleavage by activated caspase-3 (Fluhrer et al., 2004).

17β-trenbolone activities of decreasing PS-1 protein expression level and increasing Aβ42 level and caspase-3 activity in primary hippocampal neurons were all both AR and ER dependent. 17β-trenbolone has an affinity to AR similar to DHT and three times the affinity of T (Bauer et al., 2000; Yarrow et al., 2010). The competition of 17β-trenbolone with T and DHT for the AR may have altered AR activation and associated nuclear translocation and transcription. 17β-trenbolone could induce androgen-dependent translocation of the AR into the cell nucleus (Wilson et al., 2002) and cause up-regulation of ARα and ARβ mRNAs expression (Sone et al., 2005). The ER was also reported to be present in hippocampus (Meyer and Korz, 2013). The activation of ER by 17β-trenbolone may be different from E_2. 17β-trenbolone is a substrate for neither 5α-reductase nor aromatase

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007

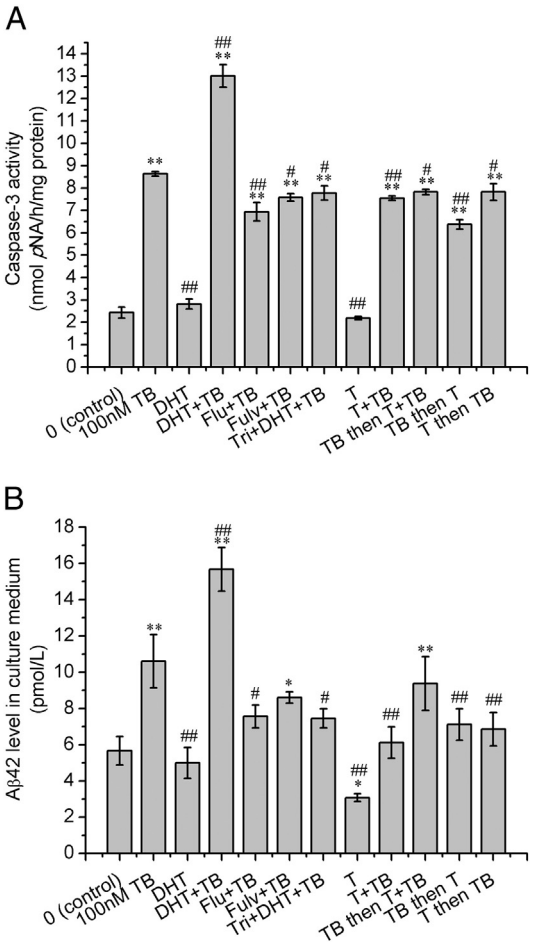

Fig. 6. Effects of 17β-trenbolone treatment on caspase-3 activity and Aβ42 production by primary hippocampal neurons. (A) Caspase-3 activity. $^{**}p < 0.01$ *vs.* control group, #p < 0.05, ##p < 0.01 *vs.* 100 nM 17β-trenbolone group (n = 6). (B) Aβ42 levels in culture medium of primary rat hippocampal neurons. $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* control group, #p < 0.05, ##p < 0.01 *vs.* 100 nM 17β-trenbolone group (n = 6). TB = 17β-trenbolone, Flu = flutamide, Fulv = fulvestrant, Tri = trilostane.

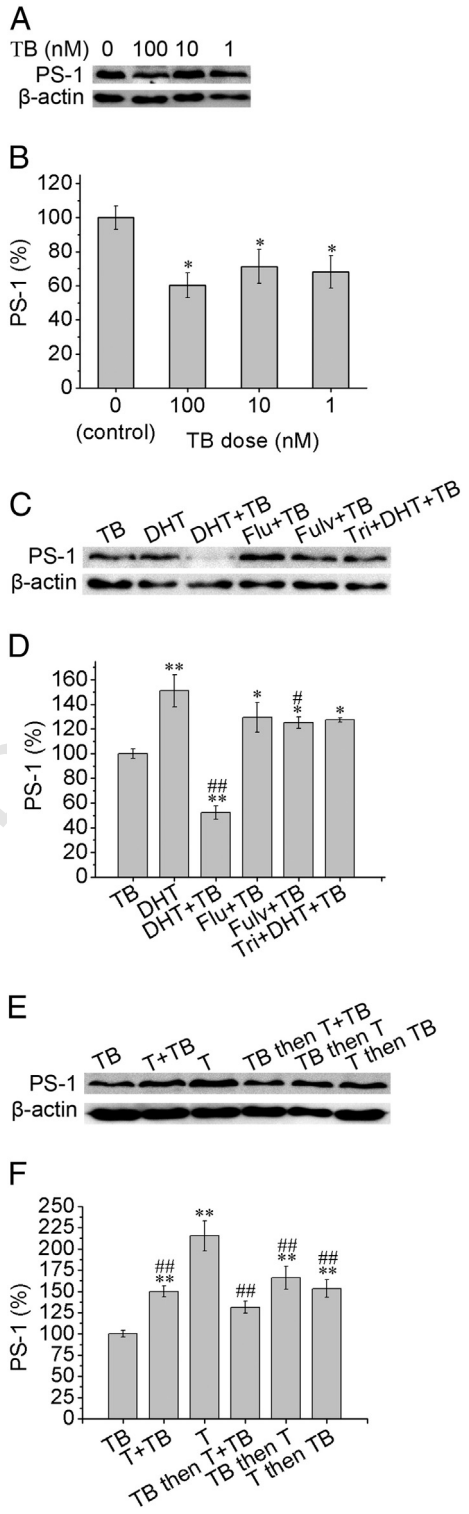

Fig. 7. Effects of 17β-trenbolone treatment on PS-1 protein expression level in primary hippocampal neurons. (A, C, and E) Representative Western blot shows analysis of PS-1 (upper panel) protein levels. β-actin (lower panel) was used as a negative control. (B, D, and F) Relative levels of PS-1 protein were quantified by densitometry scanning of Western blots. $^{*}p < 0.05$ *vs.* control group (B), $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* 100 nM 17β-trenbolone group, #p < 0.05, ##p < 0.01 *vs.* 10 nM DHT group (D), $^{**}p < 0.01$ *vs.* 100 nM 17β-trenbolone group, ##p < 0.01 *vs.* 10 nM T group (F). n = 3. TB = 17β-trenbolone, Flu = flutamide, Fulv = fulvestrant, Tri = trilostane.

(Yarrow et al., 2010). So 17β-trenbolone will not be converted into estrogens. 17β-trenbolone activities through ER are direct rather than indirect by converting to estrogens. 17β-trenbolone was reported to have low-affinity with ER and could activate ER (Ankley et al., 2003). Since DHT is an AR agonist and it can also be metabolized to 3β-diol which activates ER, the 17β-trenbolone activities were promoted to a very large extent.

T could attenuate Aβ toxicity in cultured hippocampal neurons with a non-genomic mechanism (Pike, 2001). Our results indicated that T could exert its neuron protection effects by resisting the cytotoxic effects of 17β-trenbolone (Fig. 6, Fig. 7). However, when T and 17β-trenbolone were used together to treat the cells, the toxic effects of 17β-trenbolone still appeared and protection function of T was reduced whenever 17β-trenbolone was added.

In conclusion, current results indicated that 17β-trenbolone played roles in neurodegeneration. We found that 17β-trenbolone could influence the brain, especially the hippocampus, and promote Aβ42 production in developmental brains if the mother has been exposed to 17β-trenbolone. As an AAS, it is used widely in large doses for long times by exercisers. As an environmental androgen, common people may also be exposed to 17β-trenbolone through various ways. Since damages of neurons may occur much earlier than the clinical symptoms of neurodegenerative disorders, exposure to 17β-trenbolone should be regarded as a high risk environmental factor in AD onset. Thus, the use of 17β-trenbolone should be monitored.

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007

Declaration of interest

The authors declare that they have no financial or personal conflicts of interests that could influence the work presented in this manuscript.

Funding

The work was supported by the Graduate Innovation Foundation of Shandong Normal University [grant number BCX1306].

Authors' contributions

FM designed and performed the experiments in this study and was the primary author in writing this manuscript. DL assisted in the design of the experiments and in the preparation of this manuscript. All authors read and approved the final manuscript.

Uncited references

Q5

Boelsterli, 2007
Kovacs et al., 1996
Nguyen et al., 2007
Selkoe and Wolfe, 2007

Acknowledgments

We thank Prof. Ling Gai from Key Laboratory for Improving Birth Outcome Technique of Shandong Province, Shandong Provincial Family Planning Institute of Science and Technology, for assistance in hormone determination.

References

Akbari, Y., Hitt, B.D., Murphy, M.P., Dagher, N.N., Tseng, B.P., Green, K.N., Golde, T.E., LaFerla, F.M., 2004. Presenilin regulates capacitative calcium entry dependently and independently of gamma-secretase activity. Biochem. Biophys. Res. Commun. 322, 1145–1152.

Ankley, G.T., Jensen, K.M., Makynen, E.A., Kahl, M.D., Korte, J.J., Hornung, M.W., Henry, T.R., Denny, J.S., Leino, R.L., Wilson, V.S., 2003. Effects of the androgenic growth promoter 17-β-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. Environ. Toxicol. Chem. 22, 1350–1360.

Bauer, E., Daxenberger, A., Petri, T., Sauerwein, H., Meyer, H., 2000. Characterisation of the affinity of different anabolics and synthetic hormones to the human androgen receptor, human sex hormone binding globulin and to the bovine progestin receptor. AMPIS 108, 838–846.

Blackwell, B.R., Karnjanapiboonwong, A., Anderson, T.A., Smith, P.N., 2012. Uptake of 17beta-trenbolone and subsequent metabolite trendione by the pinto bean plant (*Phaseolus vulgaris*). Ecotoxicol. Environ. Saf. 85, 110–114.

Blennow, K., 2005. CSF biomarkers for Alzheimer's disease: use in early diagnosis and evaluation of drug treatment. Expert. Rev. Mol. Diagn. 5, 661–672.

Boelsterli, U.A., 2007. Mechanistic Toxicology: the Molecular Basis of How Chemicals Disrupt Biological Targets. Informa Health Care, London.

Chen, Q., 1993. Traditional Chinese Medicine Pharmacology Research Methodology. People's Medical Publishing House, Beijing.

Cheng, S.M., Li, J.C., Lee, D.C., Liu, L., Chen, Z., Lau, A.S., 2009. HIV-1 transactivator protein induction of suppressor of cytokine signaling-2 contributes to dysregulation of IFNγ signaling. Blood 113, 5192–5201.

Doan, A., Thinakaran, G., Borchelt, D.R., Slunt, H.H., Ratovitsky, T., Podlisny, M., Selkoe, D.J., Seeger, M., Gandy, S.E., Price, D.L., 1996. Protein topology of presenilin 1. Neuron 17, 1023–1030.

Dowjat, W.K., Wisniewski, T., Efthimiopoulos, S., Wisniewski, H.M., 1999. Inhibition of neurite outgrowth by familial Alzheimer's disease-linked presenilin-1 mutations. Neurosci. Lett. 267, 141–144.

Fluhrer, R., Friedlein, A., Haass, C., Walter, J., 2004. Phosphorylation of presenilin 1 at the caspase recognition site regulates its proteolytic processing and the progression of apoptosis. J. Biol. Chem. 279, 1585–1593.

Georgakopoulos, A., Marambaud, P., Efthimiopoulos, S., Shioi, J., Cui, W., Li, H.-C., Schütte, M., Gordon, R., Holstein, G.R., Martinelli, G., 1999. Presenilin-1 forms complexes with the cadherin/catenin cell–cell adhesion system and is recruited to intercellular and synaptic contacts. Mol. Cell 4, 893–902.

Haass, C., De Strooper, B., 1999. The presenilins in Alzheimer's disease—proteolysis holds the key. Science 286, 916–919.

Handa, R.J., Pak, T.R., Kudwa, A.E., Lund, T.D., Hinds, L., 2008. An alternate pathway for androgen regulation of brain function: activation of estrogen receptor beta by the metabolite of dihydrotestosterone, 5α-androstane-3β, 17β-diol. Horm. Behav. 53, 741–752.

Hardy, J., 1997. Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci. 20, 154–159.

Hardy, J., Gwinn-Hardy, K., 1998. Genetic classification of primary neurodegenerative disease. Science 282, 1075–1079.

Hatanaka, Y., Mukai, H., Mitsuhashi, K., Hojo, Y., Murakami, G., Komatsuzaki, Y., Sato, R., Kawato, S., 2009. Androgen rapidly increases dendritic thorns of CA3 neurons in male rat hippocampus. Biochem. Biophys. Res. Commun. 381, 728–732.

Hemmer, M.J., Hemmer, B.L., Bowman, C.J., Kroll, K.J., Folmar, L.C., Marcovich, D., Hoglund, M.D., Denslow, N.D., 2001. Effects of p-nonylphenol, methoxychlor, and endosulfan on vitellogenin induction and expression in sheepshead minnow (*Cyprinodon variegatus*). Environ. Toxicol. Chem. 20, 336–343.

Ho, A., Shen, J., 2011. Presenilins in synaptic function and disease. Trends Mol. Med. 17, 617–624.

Ip, E.J., Barnett, M.J., Tenerowicz, M.J., Perry, P.J., 2011. The anabolic 500 survey: characteristics of male users versus nonusers of anabolic-androgenic steroids for strength training. Pharmacotherapy 31, 757–766.

Kovacs, D.M., Fausett, H.J., Page, K.J., Kim, T.-W., Moir, R.D., Merriam, D.E., Hollister, R.D., Hallmark, O.G., Mancini, R., Felsenstein, K.M., 1996. Alzheimer-associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. Nat. Med. 2, 224–229.

Laudon, H., Hansson, E.M., Melén, K., Bergman, A., Farmery, M.R., Winblad, B., Lendahl, U., von Heijne, G., Näslund, J., 2005. A nine-transmembrane domain topology for presenilin 1. J. Biol. Chem. 280, 35352–35360.

Lund, T.D., Hinds, L.R., Handa, R.J., 2006. The androgen 5α-dihydrotestosterone and its metabolite 5α-androstan-3β, 17β-diol inhibit the hypothalamo–pituitary–adrenal response to stress by acting through estrogen receptor β-expressing neurons in the hypothalamus. J. Neurosci. 26, 1448–1456.

Mattson, M.P., 2000. Apoptosis in neurodegenerative disorders. Nat. Rev. Mol. Cell Biol. 1, 120–130.

Meyer, K., Korz, V., 2013. Age dependent differences in the regulation of hippocampal steroid hormones and receptor genes: relations to motivation and cognition in male rats. Horm. Behav. 63, 376–384.

Nguyen, T.-V.V., Yao, M., Pike, C.J., 2007. Flutamide and cyproterone acetate exert agonist effects: induction of androgen receptor-dependent neuroprotection. Endocrinology 148, 2936–2943.

Nunez, J., 2008. Primary culture of hippocampal neurons from P0 newborn rats. JoVE (10. 3791/895).

Q6

Pak, T.R., Chung, W.C., Lund, T.D., Hinds, L.R., Clay, C.M., Handa, R.J., 2005. The androgen metabolite, 5α-androstane-3β, 17β-diol, is a potent modulator of estrogen receptor-β1-mediated gene transcription in neuronal cells. Endocrinology 146, 147–155.

Parkinson, A.B., Evans, N.A., 2006. Anabolic androgenic steroids: a survey of 500 users. Med. Sci. Sports Exerc. 38, 644.

Perry, P.J., Lund, B.C., Deninger, M.J., Kutscher, E.C., Schneider, J., 2005. Anabolic steroid use in weightlifters and bodybuilders: an internet survey of drug utilization. Clin. J. Sport Med. 15, 326–330.

Pike, C.J., 2001. Testosterone attenuates β-amyloid toxicity in cultured hippocampal neurons. Brain Res. 919, 160–165.

Pike, C.J., Carroll, J.C., Rosario, E.R., Barron, A.M., 2009. Protective actions of sex steroid hormones in Alzheimer's disease. Front. Neuroendocrinol. 30, 239–258.

Schiffer, B., Daxenberger, A., Meyer, K., Meyer, H., 2001. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: environmental studies. Environ. Health Perspect. 109, 1145.

Selkoe, D.J., Wolfe, M.S., 2007. Presenilin: running with scissors in the membrane. Cell 131, 215–221.

Sone, K., Hinago, M., Itamoto, M., Katsu, Y., Watanabe, H., Urushitani, H., Tooi, O., Guillette Jr., L.J., Iguchi, T., 2005. Effects of an androgenic growth promoter 17beta-trenbolone on masculinization of mosquitofish (*Gambusia affinis affinis*). Gen. Comp. Endocrinol. 143, 151–160.

Suzuki, N., Cheung, T.T., Cai, X.D., Odaka, A., Otvos Jr., L., Eckman, C., Golde, T.E., Younkin, S.G., 1994. An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. Science 264, 1336–1340.

Suzuki, T., Nishiyama, K., Murayama, S., Yamamoto, A., Sato, S., Kanazawa, I., Sakaki, Y., 1996. Regional and cellular *Presenilin 1* gene expression in human and rat tissues. Biochem. Biophys. Res. Commun. 219, 708–713.

Tanzi, R.E., Bertram, L., 2005. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 120, 545–555.

Wilson, V.S., Lambright, C., Ostby, J., Gray, L., 2002. In vitro and in vivo effects of 17β-trenbolone: a feedlot effluent contaminant. Toxicol. Sci. 70, 202–211.

Wojda, U., Kuznicki, J., 2013. Alzheimer's disease modeling: ups, downs, and perspectives for human induced pluripotent stem cells. J. Alzheimers Dis. 34, 563–588.

Wolfe, M.S., 2013. Chapter 65 — presenilin-1. In: Rawlings, N.D., Salvesen, G. (Eds.), Handbook of Proteolytic Enzymes. Academic Press, London, pp. 273–278.

Wolfe, M.S., Selkoe, D.J., 2010. Giving Alzheimer's the old one-two. Cell 142, 194–196.

Yarrow, J.F., McCoy, S.C., Borst, S.E., 2010. Tissue selectivity and potential clinical applications of trenbolone (17beta-hydroxyestra-4,9,11-trien-3-one): a potent anabolic steroid with reduced androgenic and estrogenic activity. Steroids 75, 377–389.

Zeng, Y., Li, B., Ma, W., Zhou, K., Fan, H., Wang, H., 2011. Discussion on current pollution status and legislation of environmental hormone in China. Procedia Environ. Sci. 11, 1267–1277.

Please cite this article as: Ma, F., Liu, D., 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration, Toxicol. Appl. Pharmacol. (2014), http://dx.doi.org/10.1016/j.taap.2014.11.007