Effects of Subchronic Finasteride Treatment and Withdrawal on Neuroactive Steroid Levels and their Receptors in the Male Rat Brain

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EFFECTS OF SUBCHRONIC FINASTERIDE TREATMENT AND WITHDRAWAL ON NEUROACTIVE STEROID LEVELS AND THEIR RECEPTORS IN THE MALE RAT BRAIN

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Abstract

The enzymatic conversion of progesterone and testosterone by the enzyme 5alpha-reductase exerts a crucial role in the control of nervous function. The effects in the brain of finasteride, an inhibitor of this enzyme used for the treatment of human benign prostatic hyperplasia and androgenic alopecia, have been poorly explored. Therefore, the effects of a subchronic treatment of finasteride at low doses (3mg/kg/day) and the consequences of its withdrawal on neuroactive steroid levels in plasma, cerebrospinal fluid and some brain regions as well as on the expression of classical and non-classical steroid receptors have been evaluated in male rats. After subchronic treatment (i.e., 20 days) the following effects were detected: i) depending on the compartment considered, alteration in the levels of neuroactive steroids, not only in 5alpha-reduced metabolites but also in its precursors and in neuroactive steroids from other steroidogenic pathways and ii) upregulation of androgen receptor in the cerebral cortex and beta3 subunit of GABA-A receptor in the cerebellum. One month after the last treatment (i.e., withdrawal period), some of these effects persisted (i.e., upregulation of androgen receptor in the cerebral cortex, increase of dihydroprogesterone in cerebellum, decrease of dihydrotestosterone in plasma). Moreover, other changes in neuroactive steroid levels, steroid receptors (i.e., upregulation of estrogen receptor alpha and downregulation of estrogen receptor beta in cerebral cortex) and GABA-A receptor subunits (i.e., decrease of alpha 4 and beta 3 mRNA levels in cerebral cortex) were detected. These findings suggest that finasteride treatment may have broad consequences for brain function.

Introduction

Neuroactive steroids, such as progesterone (PROG) and testosterone (T), are converted by the enzyme 5alpha-reductase (5α-R) into dihydroprogesterone (DHP) and dihydrotestosterone (DHT), respectively. These metabolites are further converted in other steroids, such as tetrahydroprogesterone (THP) and isopregnanolone in the case of DHP and 5α-androstane-3α,17β-diol (3α-diol) or 5α-androstane-3β,17β-diol (3β-diol) in the case of DHT. These enzymatic conversions occur not only in the peripheral glands but also in the nervous system [1].

Three 5α-R isozymes, defined as type 1, 2 and 3, have been identified in the brain [2, 3], even if it is important to highlight that 5α-R type 3 is still under intensive investigation. At variance to type 1 and 2, type 3 is thought to play a role in protein glycosylation via dolichol phosphate pathway [3, 4]. It is still unclear whether specific isozymes are responsible for the conversion of T or PROG [2, 3]. The 5α-R inhibitor finasteride inhibits both type 1 and type 2 isozymes, although it has higher affinity for the isozyme type 2 [2, 3].

The enzymatic conversion mediated by 5α-R exerts a crucial role in the control of nervous function. Indeed, the metabolites of PROG and T are involved in the regulation of neuroendocrine events, behavior, affection, learning and memory, synaptic and glial plasticity and adult hippocampal neurogenesis as well as in the response of brain tissue to injury and neurodegeneration (i.e., regulating neuronal survival, axonal regeneration and gliosis) [1, 5-7]. Finasteride has been used as a tool to evaluate whether the effects of PROG or T in the nervous tissue could be ascribed to their conversion into 5α-reduced metabolites [2, 8-18]. However, despite of the wide therapeutic use of this inhibitor (e.g., human benign prostatic hyperplasia and androgenic alopecia), the effects of finasteride *per se* in the nervous system have been poorly explored. The effects of short exposures (i.e., few days) to high doses (i.e., 50mg/kg) of finasteride have been assessed in experimental models [2, 8-18]. However, the effects of subchronic treatments with finasteride at low doses, more similar to those used in clinical practice, and the consequences of its withdrawal have yet to be clarified.

Sexual dysfunction and anxious/depressive symptomatology have been reported in a subpopulation of male patients with androgenic alopecia that received finasteride [19-23]. These side-effects were also reported in a subset of patients after discontinuation of the therapy [23-28]. Interestingly, these patients also showed altered neuroactive steroid levels in plasma and cerebrospinal fluid (CSF) in comparison to healthy individuals [29, 30]. Our hypothesis was that the treatment with low doses of finasteride may also cause alterations in neuroactive steroid levels in the central nervous system. Therefore, the aim of this study was to determine if a subchronic treatment with low doses of finasteride affects the levels of neuroactive steroids in the brain of male rats. In particular, since finasteride inhibits 5α-R activity, we expected that the treatment with this drug would affect the levels of 5α-R substrates and products. We also hypothesized that by the alteration of the levels of neuroactive steroids, finasteride would also affect the expression of steroid receptors in the brain.

On this basis, we have here evaluated in male rats the effects of a subchronic treatment with low doses of finasteride (i.e., 3 mg/kg/day for 20 days) and the consequences of its withdrawal (i.e., evaluated 1 month after the last treatment) on neuroactive steroid levels in plasma, CSF, cerebral cortex, cerebellum and hippocampus. Neuroactive steroids assessed (Fig. 1) were: pregnenolone (PREG), PROG, DHP, THP, isopregnanolone, dehydroepiandrosterone (DHEA), T, DHT, 3α-diol, 3β-diol, 17α-estradiol (17α-E) and 17β-estradiol (17β-E). On the basis of the changes observed in the levels of neuroactive steroids, we analyzed possible changes of their receptors. To this aim, the expression of steroid receptors was evaluated in those brain structures in which neuroactive steroid levels were affected by finasteride. Thus, depending on the specific binding capability of the neuroactive steroids affected by finasteride, the expression of androgen (AR), estrogen (ER), progesterone (PR) receptor or GABA-A receptor subunits (i.e., α2, α4, β3, δ and γ2) were assessed.

Material and Methods

Animals

Sprague-Dawley male rats (150-175 g at the arrival, Charles River, Italy) were housed in the animal care facility of the Neuroscience Institute Cavalieri-Ottolenghi (NICO) at the University of Torino. Animals were maintained in standard rat cages (cage size: 59.5 cm x 38.0 cm x 20.0 cm, Eurostandard type IV) with food and tap water available *ad libitum* and under controlled temperature (21 ± 4°C), humidity (40-60%), room ventilation (12.5 air changes per hour) and light cycle (12 h light / dark cycle; on 8 AM / off 8 PM).

Rats were acclimated to the new environment for ten days before being randomly assigned to one of the experimental groups described below. Animals were maintained according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Bioethics and Animal Welfare Committee of the University of Torino and the procedures were performed in accordance with EU Council directive (2010/63/EU) and with the Italian DL 116/1992.

Treatments

Finasteride (3 mg/kg/day, Sigma-Aldrich, Milan, Italy) was suspended in a vehicle solution of sesame oil and ethanol (5% v/v). Either this solution, or vehicle, was administrated to the animals subcutaneously, at a volume of 100 µl/day for 20 days.

Finasteride and control rats were sacrificed at 24 hours and 1 month after last treatment in order to investigate the effects of the treatment and the effects of the withdrawal. Body weight and relative testis and prostate weights were assessed at the end of the treatment and at the end of the withdrawal period.

Liquid chromatography tandem mass spectrometry analysis

The cerebral cortex, cerebellum, hippocampus, plasma and CSF were collected and stored at -80°C until analyzed. In particular, blood samples were first collected in heparin tubes, then placed in centrifuge tubes and centrifuged at 2500 *g* for 15 min at 4°C to obtain plasma. CSF was obtained by puncture of *cisterna magna* as previously described [31]. Extraction and purification of the samples were performed according to Caruso and colleagues [32].

Briefly, samples were spiked with $^{13}C_3$-17β-E (1 ng/sample), D_9-PROG (0.2 ng/sample) and D_4-PREG (5 ng/sample), as internal standards and homogenized in 2 ml of MeOH/acetic acid (99:1 v/v) using a tissue lyser (Qiagen, Italy). After an overnight extraction at 4°C, samples were centrifuged at 12,000 rpm for 5 min and the pellet was extracted twice with 1 ml of MeOH/acetic acid (99:1 v/v). The organic phases were combined and evaporated to dryness. The organic residues were resuspended with 3 ml of MeOH/$H_2$0 (10:90 v/v) and passed through SPE cartridges, previously activated with MeOH (5ml) and MeOH:H_2O 1:9 v/v (5ml). The steroids were eluted in MeOH, concentrated and transferred in autosampler vials before liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Quantitative analysis was performed on the basis of calibration curves daily prepared and analyzed as previously described [32]. Briefly, blank samples (6% albumin in PBS) or cortex homogenate (obtained by control rats) were spiked with $^{13}C_3$-17β-E (1 ng/sample), D_9-PROG (0.2 ng/sample) and D_4-PREG (5 ng/sample), as internal standards. Increasing amounts (0.05 - 5 ng/sample) of each steroid were added. Calibration curves were extracted and analyzed as described for experimental samples.

Positive atmospheric pressure chemical ionization (APCI+) experiments were performed with a linear ion trap - mass spectrometer (LTQ, ThermoElectron Co, San Jose, CA, USA) using nitrogen as sheath, auxiliary and sweep gas. The instrument was equipped with a Surveyor liquid chromatography (LC) Pump Plus and a Surveyor Autosampler Plus (ThermoElectron Co, San Jose, CA, USA). The mass spectrometer (MS) was employed in tandem mode (MS/MS) using helium as collision gas.

The LC mobile phases were previously described [32]. The Hypersil Gold column (100x3 mm, 3 μm; ThermoElectron Co, San Jose, CA, USA) was maintained at 40°C. Peaks of the LC-MS/MS were evaluated using a Dell workstation by means of the software Excalibur® release 2.0 SR2 (ThermoElectron Co, San Jose, CA, USA). Samples were analyzed using the transitions previously reported [32].

Real-time polymerase chain reaction (RT-PCR)

Total RNA from tissues was extracted using the standard Trizol protocol based on the method developed by Chomczynski and Sacchi [33]. RNA was subsequently extracted in accordance with the manufacturer's protocol (Invitrogen, San Giuliano Milanese, Italy) and prepared by using Direct-zol™ RNA MiniPrep kit (Zymo Research, Irvine, CA, USA). RNA was analyzed using a TaqMan quantitative real-time PCR instrument (CFX96 real time system; Bio-Rad Laboratories, Segrate, Italy) using the iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories, Segrate, Italy). Samples were run in 96 well formats in duplicate as multiplexed reactions with a normalizing internal control, 18s rRNA (Applera, Monza, Italy). Specific TaqMan MGB probes (Applera, Monza, Italy) were: 5α-R (Rn00567064_m1); GABA-A subunit α2 (Rn01413643_m1); GABA-A subunit α4 (Rn00589846_m1); GABA-A subunit β3 (Rn00567029_m1); GABA-A subunit δ (Rn01517017_m1); GABA-A subunit γ2 (Rn00788325_m1).

Western blotting

Tissues were homogenized on ice in excess of lysis buffer (phosphate-buffered saline (PBS), pH 7.4, added with 1% Nonidet P-40) supplemented with protease cocktail inhibitor (Roche Diagnostic spa, Monza, Italy), with a tissue lyser II (Qiagen; Hilden, Germany). Homogenate was sonicated and centrifuged at 400 g at 4°C to remove particulate matter. The protein concentration of each sample was assayed relative to the bovine serum albumin standard according to the method of Bradford [34]. In the case of steroid receptors, equal amounts of each sample were solubilized in 0.1% sodium

decylsulphate (SDS) sample buffer, left for 5 min at 100°C, resolved on a 10% SDS-polyacrylamide gel, and electroblotted overnight to a nitrocellulose membrane (Trans-blot; Bio-Rad, Milan, Italy). The membranes were blocked at room temperature in PBS with added 0.1% Tween 20 and 5% nonfat dried milk. Successively, the filters were cut and the top was incubated overnight at 4°C with rabbit polyclonal antibody against ERα and ERβ (Santa Cruz, Heidelberg, Germany) or over three-days at 4°C with either mouse monoclonal antibody against PR (Thermo Scientific; Rockford, IL, USA) or rabbit monoclonal antibody against AR (Abcam; Cambridge, UK). Primary antibodies were diluted in PBS buffer - 0.1% Tween 20 – 3% nonfat dried milk. The filters were then washed for 1h and incubated with an anti-mouse or an anti-rabbit horseradish peroxidase–conjugated secondary antibody. In parallel, the bottom filters were incubated with a primary monoclonal anti-tubulin antibody (Sigma-Aldrich, St. Louis, MO, USA) diluted in PBS buffer-0.1% Tween 20–3% nonfat dried milk, as an internal control, then washed and incubated with an anti-mouse horseradish peroxidase–conjugated secondary antibody. After washing the filters, bound antibodies were detected with the ECL method (Bio-Rad, Milan, Italy). Chemiluminescent signals were acquired with a ChemiDoc™ XRS+ system (Bio-Rad, Milan, Italy) and analyzed with Image Lab™ software version 3.0 (Bio-Rad, Milan, Italy). The mean control value within a single experiment was set to 100 and all the other values were expressed as a percentage. Values of controls from different experiments were all within 10%.

Statistical analysis

The quantitative data obtained by the experiments were analyzed by inferential statistical analysis in accordance with the experimental protocols and the nature of the data (i.e. Student's t-test). $P \leq 0.05$ was considered significant. Analyses were performed using GRAPHPAD PRISM, version 4.00 (GraphPad Inc, La Jolla, CA).

Results

<u>Body weight and prostate and testis weights</u>

At the sacrifice body weight, relative prostate and relative testis weights were assessed. Body weight [Control (n=8): 346.3 ± 9.8 g vs. Finasteride (n=8): 351.3 ± 8.3 g] and relative testis weight [Control (n=8): 0.8920 ± 0.0213 g vs. Finasteride (n=8): 0.8560 ± 0.0297 g] were unaffected by finasteride at the end of the treatment. On the contrary, a significant decrease of relative prostate weight was detected [Control (n=8): 0.1111 ± 0.0116 g vs. Finasteride (n=8): 0.0786 ± 0.0072 g; $p \leq 0.05$]. At the end of the withdrawal period, body weight [Control (n=8): 445.6 ± 11.9 g vs. Finasteride (n=8): 443.6 ± 13.6 g], relative prostate weight [Control (n=8): 0.1259 ± 0.0061 g vs. Finasteride (n=8): 0.1113 ± 0.0107 g] and relative testis weight [Control (n=8): 0.7434 ± 0.0148 g vs. Finasteride (n=8): 0.7071 ± 0.0196 g] were not significantly different between the two experimental groups.

<u>Assessment of neuroactive steroid levels after subchronic treatment with finasteride</u>

Table 1 shows neuroactive steroid levels, assessed by LC-MS/MS, in the cerebral cortex, cerebellum, hippocampus, CSF and plasma of the animals treated with finasteride or vehicle. PREG levels in the CSF were significantly upregulated by finasteride. In contrast, PREG levels in plasma and in the brain structures analyzed were not significantly affected by the treatment. The levels of the PREG product (i.e., PROG) were increased in the hippocampus after finasteride treatment, but were not affected in the cerebral cortex, cerebellum, plasma and CSF. The levels of the first PROG metabolite (i.e., DHP) were significantly upregulated in the cerebellum by finasteride. In agreement with the upregulation of this 5α-R product, a significant increase of 5α-R gene expression was also detected in the same brain area [Control (n=5): 0.796±0.036 vs. Finasteride (n=5): 0.992±0.063, $p \leq 0.05$]. In the case of the further metabolites of PROG, only isopregnanolone levels were significantly affected by finasteride, with an increase in the cerebellum and plasma (Table 1).

The levels of DHEA and its metabolites (i.e., androgens and estrogens) were also affected by finasteride. As reported in Table 1, DHEA levels in the cerebral cortex were significantly decreased in

finasteride treated rats. DHT levels (i.e., metabolite of T) were reduced in plasma, but not in the CSF and brain tissue after finasteride treatment. In the case of the further metabolites of DHT (i.e., 3α-diol and 3β-diol), their levels were significantly decreased and enhanced, respectively, in the cerebellum and CSF of finasteride treated animals. In the case of estrogens, only the levels of 17β-E were significantly affected by finasteride. Indeed, the levels of this T metabolite were significantly increased in the cerebellum and plasma of finasteride treated rats.

Assessment of neuroactive steroid levels at the end of the withdrawal period

Table 2 shows neuroactive steroid levels, assessed by LC-MS/MS, in the cerebral cortex, cerebellum, hippocampus, CSF and plasma of the animals treated for 20 days with finasteride or vehicle and sacrificed 1 month after the last treatment. Finasteride withdrawal induced a different regulation of PREG levels in the cerebellum and the hippocampus. Thus, compared to control animals an upregulation of PREG levels was observed in the cerebellum, while in the hippocampus this neuroactive steroid was decreased at the end of the withdrawal period. PROG levels were significantly decreased in the cerebral cortex, hippocampus and plasma after withdrawal. Finasteride withdrawal also affected the levels of the PROG metabolite DHP, which were decreased in the cerebral cortex and upregulated in the cerebellum and hippocampus compared to control animals (Table 2). In agreement with these changes, 5α-R gene expression was significantly decreased in the cerebral cortex [Control (n=5): 1.428±0.105 vs. Finasteride (n=5): 1.038±0.093, $p \leq 0.05$] and increased in the cerebellum [Control (n=5): 1.722±0.076 vs. Finasteride (n=5): 2.192±0.145, $p \leq 0.05$]. The levels of the further metabolites of DHP were also affected (Table 2). In particular, the levels of THP in the cerebral cortex and plasma and the levels of isopregnanolone in the cerebral cortex were decreased after finasteride withdrawal.

The levels of DHEA and its metabolites (i.e., androgens and estrogens) were also affected at the end of the withdrawal period. Thus, as reported in Table 2, DHEA levels were significantly decreased in the cerebral cortex and upregulated in CSF. In the hippocampus, a tendency to decrease, which

however did not reach statistical significance (p=0.07), was observed. T levels were significantly increased in the cerebellum as well as in CSF, while the levels of its first metabolite (i.e., DHT) were significantly decreased not only in the cerebellum and CSF but also in plasma. The levels of the further metabolites of DHT were also affected by finasteride withdrawal. In particular, the levels of 3α-diol and 3β-diol were significantly decreased in plasma and the cerebellum, respectively. In case of estrogens, only the levels of 17β-E were significantly affected by finasteride withdrawal. The levels of this neuroactive steroid were significantly reduced in the cerebral cortex and upregulated in the CSF. In the hippocampus, a tendency to decrease, which however did not reach the statistical significance (p=0.063), was observed.

Effects of the subchronic treatment with finasteride and its withdrawal on the expression of classical and non-classical steroid receptors

On the basis of the observed changes in the levels of neuroactive steroids, we evaluated whether finasteride treatment and its withdrawal did also affect the expression of classical (i.e., AR, PR, ERα, ERβ) and non-classical (GABA-A receptor α2, α4, β3, δ and γ2 subunits) steroid receptors. These receptors were analyzed only in the brain regions in which changes in corresponding neuroactive steroid levels were detected.

As reported in Figure 2 panel A, after finasteride treatment we observed a significant upregulation of AR protein levels in the cerebral cortex. On the contrary, the expression levels of PR (panel B), ERα (panel C), and ERβ (panel D) in cerebellum as well as PR (panel E) in the hippocampus were unaffected. As reported in Figure 3, at the end of the withdrawal period the protein levels of AR (panel A) and ERα (panel C) were upregulated in the cerebral cortex while a downregulation of ERβ (panel D) was observed. The expression levels of PR in the cerebral cortex (panel B), the cerebellum (panel E) and the hippocampus (panel G) as well as of AR in the cerebellum (panel F) were unaffected.

After finasteride treatment, an increase in the gene expression of the β3 subunit of the GABA-A receptor was observed in the cerebellum (Figure 4). At the end of the withdrawal period the mRNA levels of the α4 and β3 subunits of the GABA-A receptor in the cerebral cortex were decreased (Figure 5).

Discussion

In this study we have assessed whether a subchronic treatment with a low dose of finasteride was able to affect neuroactive steroid levels and their receptors in the brain of male rats. Indeed, to our knowledge the effects of finasteride have been generally explored so far after the administration of very high doses [2, 8-18]. We demonstrated that, in agreement with the well-known effect of finasteride in the prostate [35], a significant decrease in its relative weight was observed at the end of the subchronic treatment with the drug. In addition, subchronic treatment with finasteride did also affect neuroactive steroid levels and expression of classical and non-classical steroid receptors in the brain.

Since finasteride inhibits 5α-R activity, we hypothesized that the subchronic treatment with this drug would affect the levels of 5α-R substrates, PROG and T and the levels of the 5α-R products, DHP and DHT. As mentioned above, T and PROG may be converted by different 5α-R isozymes in several brain areas [2, 3]. For instance, type 1 is expressed in olfactory bulb [36, 37], cerebellum [37, 38], prefrontal and cerebral cortex [37, 39, 40], hippocampus, striatum, thalamus and amygdala [37]. As recently observed, enzyme type 2 shows a very similar brain distribution [41]. However, a possible difference may be the cellular localization of these two isozymes. Indeed, type 1 is typically localized in glial cells [1] while type 2 is consistently expressed in neurons [41]. Therefore, this dichotomy may reflect differential roles in the modulation of brain function. Finasteride did not significantly affect the levels of PROG and T in plasma, CSF and brain tissues, with the exception of the hippocampus, in which, as expected, the 5α-R inhibitor caused a significant increase in the levels of PROG, the substrate of the enzyme. However, the increase in the levels of PROG was not accompanied by a

decrease in DHP levels. Concerning the 5α-R products, we detected a decrease in DHT levels in plasma after finasteride treatment, in agreement to what is observed in patients treated with this drug [42]. However, DHT levels in the CSF and brain tissue were not affected by the subchronic finasteride treatment. Surprisingly, in the cerebellum finasteride treatment resulted in a significant increase in DHP levels. This was unexpected since DHP is a product of 5α-R. The cause for this increase in DHP levels remains unexplained. Finasteride treatment did not significantly affect DHP levels in the other brain areas considered. In general, we may conclude that the subchronic treatment with a low dose of finasteride do not have major effects on the levels of the 5α-R substrates and products in the brain of male rats but it is only able to affect peripheral DHT production. Indeed, in this context it is important to recall that in periphery 5α-R type 1 gene is predominantly expressed in the skin (e.g., pubic skin and scalp) while 5α-R type 2 is mainly expressed in the prostate and gonads [1]. Whether the effect we observed might be due to an impairment of a specific isozyme is still unclear. Indeed, even if with a different affinity, both isozymes are able to convert T into DHT [1].

However, finasteride caused important changes in the levels of other neuroactive steroids, such as isopregnanolone, DHT and 17β-E in plasma, PREG, 3α-diol and 3β-diol in the CSF, DHEA in the cerebral cortex and isopregnanolone, 3α-diol, 3β-diol and 17β-E in the cerebellum. These changes, involving secondary metabolites of PROG and T, as well as PROG and T precursors, show regional specificity and suggest that 5α-R inhibition impact on other steroidogenic steps. Additional studies should determine whether subchronic finasteride treatments affect the expression or activity of other steroidogenic enzymes and alternative steroidogenic pathways. For instance, it has been reported that 20α-reduction of PROG is enhanced in the brain when 5α-reduction is inhibited by finasteride [43]. Furthermore, changes in neurotransmission caused by modifications in steroids with activity on GABA-A receptor may affect general steroidogenic activity. For instance, aromatase activity and therefore 17β-E synthesis is regulated by excitatory neurotransmission [44]. Interestingly, 17β-E levels are increased in the cerebellum in parallel to increased levels of

isopregnanolone. Since isopregnanolone antagonizes the effects of THP on the GABA-A receptor [1, 45, 46], the altered isopregnanolone levels may cause a modification in the activity of GABA-A and consequently in the balance in excitation/inhibition.

The analysis of neuroactive steroids one month after finasteride withdrawal revealed long-term consequences. In plasma, we detected a significant decrease in the levels of PROG, THP, DHT and 3α-diol compared to control animals. In the CSF, the levels of T and 17β-E were increased, while the levels of DHT were decreased, suggesting an increased accumulation of T and an increased conversion of T in 17β-E, due to the inhibition of the conversion of T in DHT. Interestingly, post-finasteride patients with persistent symptomatology showed significant decreased THP levels in plasma, as well as increased T levels in CSF that were associated with a decrease in DHT levels [30]. Stronger effects at the end of the withdrawal period were detected in the cerebral cortex, in which the levels of PROG, DHP, isopregnanolone, THP, DHEA and 17β-E were significantly decreased. In the cerebellum, the levels of PREG, DHP and T were increased while the levels of DHT and 3β-diol were decreased. In the hippocampus, the levels of PREG and PROG were decreased while the levels of DHP were increased. These findings indicate that finasteride treatment has long-term effects in the levels of neuroactive steroids, even after a long period of withdrawal. In fact, the effects of finasteride withdrawal were stronger than the effects of finasteride treatment *per se*, in particular in brain regions like for instance the cerebral cortex and the hippocampus. This suggests that the steroidogenic system is able to partially adapt and compensate for the chronic inhibition of 5α-R activity. However, the system, after this adaptation, may be less flexible to compensate for a new alteration and could be further disrupted when 5α-R activity is recovered.

Changes in the levels of neuroactive steroids could imply changes in the expression of their receptors. Therefore, we hypothesized that finasteride treatment and its withdrawal could also affect the expression of AR (i.e., the receptor able to bind DHEA, T and DHT), PR (i.e., the receptor able to bind PROG and DHP) ERα and ERβ (i.e., the receptor isoforms able to bind estrogens) and/or GABA-A receptor α2, α4, β3, δ and γ2 subunits (i.e., the receptor able to bind THP and 3α-diol). This

hypothesis has been confirmed, but only in some cases. For instance, in the cerebral cortex, in which we detected decreased levels of DHEA immediately after finasteride treatment and at the end of the withdrawal period, we detected an upregulation of AR expression. Even if the mechanism of action of DHEA in the nervous system has not yet been fully characterized, some observations suggest an interaction with AR [47-49]. It is interesting to note that the upregulation of AR has been also reported in the prostate of patients treated with finasteride for benign prostatic hyperplasia [50] as well as in the prepuce of patients treated with the drug for male androgenetic alopecia and showing persistent side effects [51]. In addition, in the cerebral cortex, the decrease in the levels of 17β-E after finasteride withdrawal was associated with the upregulation of ERα. In contrast, the expression of ERβ was decreased. This finding suggests that the expression of ERα and ERβ is regulated by different mechanisms in the cerebral cortex. Indeed, a differential regulation of ERα and ERβ in the cerebral cortex has been previously observed in aged male rats [52]. In addition, low sex steroid levels were associated with high expression of classical steroid receptors (i.e., AR and ER) in the brain of tropical male passerine birds [53]. In the brain of rodents, modifications in 17β-E levels are known to affect the expression and localization of ERs with regional specificity [54-56]. Moreover, it is interesting to note that finasteride treatment induces intersex animals in Western claved frogs showing high expression in the brain of AR and ER [57].

On the contrary, as we here reported for the cerebellum at the end of the withdrawal period, changes in the levels of other ligands of AR, such as T or DHT, were not associated with a significant change in the expression of this steroid receptor, suggesting a different regional regulation of AR by the neuroactive steroid environment. Regional regulation of AR is also suggested by a previous study showing that aging induced a decreased expression of brain androgen synthesis enzymes in both the cerebral cortex and cerebellum, but the expression of AR was decreased only in the cerebral cortex and was not affected in the cerebellum [52].

Similarly, changes in the levels of other neuroactive steroids, like for instance the increase in the levels of 17β-E in the cerebellum at the end of the withdrawal period, were not associated with

changes in the expression of ERα. Another example is provided by the changes of neuroactive steroids able to interact with PR, like for instance DHP [1]. Indeed, its upregulation in the cerebellum at the end of the withdrawal period was not associated with significant changes in the expression of PR.

The GABA-A receptor has a pentameric structure formed by multiple subunits. Up to now, 19 subunits have been identified and among these, α2, α4, β3, δ and γ2 subunits are more widely expressed and are targets for neuroactive steroids [58, 59]. THP is one of the neuroactive steroids able to interact directly with GABA-A receptors. The decrease in its levels in the cerebral cortex at the end of the withdrawal period was associated with the decrease in the gene expression of α4 and β3 subunits. The levels of isopregnanolone, which in contrast to THP does not bind directly to the GABA-A receptor, but, as mentioned before can antagonize the effects of THP on the GABA-A receptor [1, 45, 46], were also decreased in the cerebral cortex at the end of the withdrawal period and this was also associated with the decrease in the gene expression of α4 and β3 subunits of GABA-receptors. The mRNA levels of β3 subunit were also affected in the cerebellum after the subchronic treatment with finasteride. The increased gene expression of this subunit was associated with increased isopregnanolone levels and with decreased levels of 3α-diol (i.e., a T metabolite that interacts with GABA-A receptors). Data available in the literature indicate that THP treatment as well as its withdrawal (i.e., 24 hours) transiently increases the expression of α4 subunit in the hippocampus [60]. A decrease of this GABA-A subunit has been reported after neonatal THP treatment [61] or following the development of acute THP tolerance [62]. Usually, the effect of THP on the expression of α4 subunit is associated with changes in the expression of δ subunit [60], which was not affected in our experimental model. This indicates that THP treatment and THP withdrawal affect the expression of different GABA-A subunits than those affected by finasteride treatment and withdrawal, suggesting that the effect of finasteride on GABA-A subunits is mediated by other mechanisms in addition to the modification of THP levels.

In summary, our findings indicate that finasteride treatment causes modifications in the levels of neuroactive steroids and their receptors in brain tissue. These changes are even more prominent after a long time of drug withdrawal. Given the pleiotropic regulatory functions exerted by neuroactive steroids in the nervous system, these persistent changes in their levels may have multiple consequences for brain function. Indeed, alterations of neuroactive steroid levels have been reported to be associated with neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, traumatic brain injury and stroke as well as with psychiatric disorders, like for instance autism, depression, anxiety, posttraumatic stress and schizophrenia [63]. Moreover, as recently reviewed [64], emerging evidence in the literature suggests that 5α-R inhibitors, such as finasteride and dutasteride, may have important and serious adverse effects, like for instance on sexual function, insulin resistance and diabetes, high grade prostate cancer, cardiovascular disease, bone metabolism, neurobehavioral functions and neuropsychiatric disturbances. Further studies should explore the possible behavioral consequences of the modifications in neuroactive steroid levels caused by finasteride treatment and its withdrawal.

References

1   Melcangi RC, Garcia-Segura LM, Mensah-Nyagan AG: Neuroactive steroids: state of the art and new perspectives. Cell Mol Life Sci 2008; 65:777-797.

2   Finn DA, Beadles-Bohling AS, Beckley EH, Ford MM, Gililland KR, Gorin-Meyer RE, Wiren KM: A new look at the 5alpha-reductase inhibitor finasteride. CNS Drug Rev 2006; 12:53-76.

3   Traish AM: 5alpha-reductases in human physiology: an unfolding story. Endocr Pract 2012; 18:965-975.

4   Cantagrel V, Lefeber DJ, Ng BG, Guan Z, Silhavy JL, Bielas SL, Lehle L, Hombauer H, Adamowicz M, Swiezewska E, De Brouwer AP, Blumel P, Sykut-Cegielska J, Houliston S, Swistun D, Ali BR, Dobyns WB, Babovic-Vuksanovic D, van Bokhoven H, Wevers RA, Raetz CR, Freeze HH, Morava E, Al-Gazali L, Gleeson JG: SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder. Cell 2010; 142: 203-217.

5   Melcangi RC, Panzica G, Garcia-Segura LM: Neuroactive steroids: focus on human brain. Neuroscience 2011; 191: 1-5.

6   Panzica GC, Balthazart J, Frye CA, Garcia-Segura LM, Herbison AE, Mensah-Nyagan AG, McCarthy MM, Melcangi RC: Milestones on Steroids and the Nervous System: 10 years of basic and translational research. J Neuroendocrinol 2012; 24: 1-15.

7   Melcangi RC, Panzica GC: Allopregnanolone: state of the art. Prog Neurobiol 2014; 113: 1-5.

8   Ciriza I, Carrero P, Frye CA, Garcia-Segura LM: Reduced metabolites mediate neuroprotective effects of progesterone in the adult rat hippocampus. The synthetic progestin medroxyprogesterone acetate (Provera) is not neuroprotective. J Neurobiol 2006; 66: 916-28.

9   Ford MM, Yoneyama N, Strong MN, Fretwell A, Tanchuck M, Finn DA: Inhibition of 5alpha-reduced steroid biosynthesis impedes acquisition of ethanol drinking in male C57BL/6J mice. Alcohol Clin Exp Res 2008; 32: 1408-1416.

10   Biagini G, Longo D, Baldelli E, Zoli M, Rogawski MA, Bertazzoni G, Avoli M: Neurosteroids and epileptogenesis in the pilocarpine model: evidence for a relationship between P450scc induction and length of the latent period. Epilepsia 2009; 50 Suppl 1:53-58.

11   Atif F, Sayeed I, Yousuf S, Ishrat T, Hua F, Wang J, Brat DJ, Stein DG: Progesterone inhibits the growth of human neuroblastoma: in vitro and in vivo evidence. Mol Med 2011; 17: 1084-1094.

12   Bortolato M, Devoto P, Roncada P, Frau R, Flore G, Saba P, Pistritto G, Soggiu A, Pisanu S, Zappala A, Ristaldi MS, Tattoli M, Cuomo V, Marrosu F, Barbaccia ML: Isolation rearing-induced reduction of brain 5alpha-reductase expression: relevance to dopaminergic impairments. Neuropharmacology 2011; 60: 1301-1308.

13   Paris JJ, Brunton PJ, Russell JA, Walf AA, Frye CA: Inhibition of 5alpha-reductase activity in late pregnancy decreases gestational length and fecundity and impairs object memory and central progestogen milieu of juvenile rat offspring. J Neuroendocrinol 2011; 23: 1079-1090.

14   Darbra S, Modol L, Vallee M, Pallares M: Neonatal neurosteroid levels are determinant in shaping adult prepulse inhibition response to hippocampal allopregnanolone in rats. Psychoneuroendocrinology 2013; 38: 1397-1406.

15   Handa RJ, Kudwa AE, Donner NC, McGivern RF, Brown R: Central 5-alpha reduction of testosterone is required for testosterone's inhibition of the hypothalamo-pituitary-adrenal axis response to restraint stress in adult male rats. Brain Res 2013; 1529: 74-82.

16   Izumi Y, Svrakic N, O'Dell K, Zorumski CF: Ammonia inhibits long-term potentiation via neurosteroid synthesis in hippocampal pyramidal neurons. Neuroscience 2013; 233: 166-173.

17   Modol L, Darbra S, Vallee M, Pallares M: Alteration of neonatal Allopregnanolone levels affects exploration, anxiety, aversive learning and adult behavioural response to intrahippocampal neurosteroids. Behav. Brain Res 2013; 241: 96-104.

18   Jeffrey M, Lang M, Gane J, Chow E, Wu C, Zhang L: Novel anticonvulsive effects of progesterone in a mouse model of hippocampal electrical kindling. Neuroscience 2014; 257: 65-75.

19   Kaufman KD, Olsen EA, Whiting D, Savin R, DeVillez R, Bergfeld W, Price VH, Van Neste D, Roberts JL, Hordinsky M, Shapiro J, Binkowitz B, Gormley GJ: Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. J Am Acad Dermatol 1998; 39: 578-589.

20   McClellan KJ, Markham A: Finasteride: a review of its use in male pattern hair loss. Drugs 1999; 57: 111-126.

21   Olsen EA, Hordinsky M, Whiting D, Stough D, Hobbs S, Ellis ML, Wilson T, Rittmaster RS: The importance of dual 5alpha-reductase inhibition in the treatment of male pattern hair loss: results of a randomized placebo-controlled study of dutasteride versus finasteride. J Am Acad Dermatol 2006; 55: 1014-1023.

22   Traish AM, Hassani J, Guay AT, Zitzmann M, Hansen ML: Adverse side effects of 5alpha-reductase inhibitors therapy: persistent diminished libido and erectile dysfunction and depression in a subset of patients. J Sex Med 2011; 8: 872-884.

23   Irwig MS: Safety concerns regarding 5alpha reductase inhibitors for the treatment of androgenetic alopecia. Curr Opin Endocrinol Diabetes Obes 2015; 22: 248-253

24   Altomare G, Capella GL: Depression circumstantially related to the administration of finasteride for androgenetic alopecia. J Dermatol 2002; 29: 665-669.

25   Rahimi-Ardabili B, Pourandarjani R, Habibollahi P, Mualeki A: Finasteride induced depression: a prospective study. BMC Clin Pharmacol 2006; 6: 7.

26   Irwig MS, Kolukula S: Persistent sexual side effects of finasteride for male pattern hair loss. J Sex Med 2011; 8: 1747-1753.

27   Irwig MS: Depressive symptoms and suicidal thoughts among former users of finasteride with persistent sexual side effects. J Clin Psychiatry 2012; 73: 1220-1223.

28   Irwig MS: Persistent sexual side effects of finasteride: could they be permanent? J Sex Med 2012; 9: 2927-2932.

29   Melcangi RC, Caruso D, Abbiati F, Giatti S, Calabrese D, Piazza F, Cavaletti G: Neuroactive Steroid Levels are Modified in Cerebrospinal Fluid and Plasma of Post-Finasteride Patients Showing Persistent Sexual Side Effects and Anxious/Depressive Symptomatology. J Sex Med 2013; 10: 2598-2603.

30   Caruso D, Abbiati F, Giatti S, Romano S, Fusco L, Cavaletti G, Melcangi RC: Patients treated for male pattern hair with finasteride show, after discontinuation of the drug, altered levels of neuroactive steroids in cerebrospinal fluid and plasma. J Steroid Biochem Mol Biol 2015; 146: 74-79.

31   Lebedev SV, Blinov DV, Petrov SV: Spatial characteristics of cisterna magna in rats and novel technique for puncture with a stereotactic manipulator. Bull Exp Biol Med 2004; 137: 635-638.

32   Caruso D, Pesaresi M, Abbiati F, Calabrese D, Giatti S, Garcia-Segura LM, Melcangi RC: Comparison of plasma and cerebrospinal fluid levels of neuroactive steroids with their brain, spinal cord and peripheral nerve levels in male and female rats. Psychoneuroendocrinology 2013; 38: 2278-2290.

33   Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156-159.

34   Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254.

35   Zhang MG, Wu W, Zhang CM, Wang XJ, Gao PJ, Lu YL, Shen ZJ: Effects of oral finasteride on erectile function in a rat model. J Sex Med 2012; 9: 1328-1336.

36   Kiyokage E, Toida K, Suzuki-Yamamoto T, Ishimura K: Localization of 5alpha-reductase in the rat main olfactory bulb. J Comp Neurol 2005; 493:381-395.

37   Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A: Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proc Natl Acad Sci U S A 2006; 103:14602-14607.

38   Kiyokage E, Toida K, Suzuki-Yamamoto T, Ishimura K: Cellular localization of 5alpha-reductase in the rat cerebellum. J Chem Neuroanat 2014; 59-60:8-16.

39   Sanchez P, Torres JM, Gavete P, Ortega E: Effects of swim stress on mRNA and protein levels of steroid 5alpha-reductase isozymes in prefrontal cortex of adult male rats. Neurochem Int 2008; 52:426-431.

40   Agis-Balboa RC, Guidotti A, Pinna G: 5alpha-reductase type I expression is downregulated in the prefrontal cortex/Brodmann's area 9 (BA9) of depressed patients. Psychopharmacology (Berl). 2014; 231:3569-3580.

41   Castelli MP, Casti A, Casu A, Frau R, Bortolato M, Spiga S, Ennas MG: Regional distribution of 5alpha-reductase type 2 in the adult rat brain: an immunohistochemical analysis. Psychoneuroendocrinology 2013; 38:281-293.

42   Duskova M, Hill M, Starka L: Changes of metabolic profile in men treated for androgenetic alopecia with 1 mg finasteride. Endocr Regul 2010; 44: 3-8.

43   Mukai Y, Higashi T, Nagura Y, Shimada K: Studies on neurosteroids XXV. Influence of a 5alpha-reductase inhibitor, finasteride, on rat brain neurosteroid levels and metabolism. Biol Pharm Bull 2008; 31: 1646-1650.

44   Charlier TD, Cornil CA, Patte-Mensah C, Meyer L, Mensah-Nyagan AG, Balthazart J: Local modulation of steroid action: rapid control of enzymatic activity. Front Neurosci 2015; 9:83.

45   Hedstrom H, Bixo M, Nyberg S, Spigset O, Zingmark E, Backstrom T: Studies of pharmacokinetic and pharmacodynamic properties of isoallopregnanolone in healthy women. Psychopharmacology (Berl) 2009; 203: 85-98.

46   Bengtsson SK, Nyberg S, Hedstrom H, Zingmark E, Jonsson B, Backstrom T, Bixo M: Isoallopregnanolone antagonize allopregnanolone-induced effects on saccadic eye velocity and self-reported sedation in humans. Psychoneuroendocrinology 2015; 52: 22-31.

47   Lu SF, Mo Q, Hu S, Garippa C, Simon NG: Dehydroepiandrosterone upregulates neural androgen receptor level and transcriptional activity. J Neurobiol 2003; 57: 163-171.

48   Mo Q, Lu SF, Simon NG: Dehydroepiandrosterone and its metabolites: differential effects on androgen receptor trafficking and transcriptional activity. J Steroid Biochem Mol Biol 2006; 99: 50-58.

49   Mo Q, Lu S, Garippa C, Brownstein MJ, Simon NG: Genome-wide analysis of DHEA- and DHT-induced gene expression in mouse hypothalamus and hippocampus. J Steroid Biochem Mol Biol 2009; 114: 135-143.

50   Hsieh JT, Chen SC, Yu HJ, Chang HC: Finasteride upregulates expression of androgen receptor in hyperplastic prostate and LNCaP cells: implications for chemoprevention of prostate cancer. Prostate 2011; 71: 1115-1121.

51   Di Loreto C, La Marra F, Mazzon G, Belgrano E, Trombetta C, Cauci S: Immunohistochemical evaluation of androgen receptor and nerve structure density in human prepuce from patients with persistent sexual side effects after finasteride use for androgenetic alopecia. PLoS One 2014; 9: e100237.

52   Munetomo A, Hojo Y, Higo S, Kato A, Yoshida K, Shirasawa T, Shimizu T, Barron A, Kimoto T, Kawato S: Aging-induced changes in sex-steroidogenic enzymes and sex-steroid receptors in the cortex, hypothalamus and cerebellum. J Physiol Sci 2015; 65:253-263.

53   Canoine V, Fusani L, Schlinger B, Hau M: Low sex steroids, high steroid receptors: Increasing the sensitivity of the nonreproductive brain. Dev Neurobiol 2007; 67: 57-67.

54   Yamada S, Noguchi D, Ito H, Yamanouchi K: Sex and regional differences in decrease of estrogen receptor alpha-immunoreactive cells by estrogen in rat hypothalamus and midbrain. Neurosci Lett 2009; 463: 135-139.

55   Mitterling KL, Spencer JL, Dziedzic N, Shenoy S, McCarthy K, Waters EM, McEwen BS, Milner TA: Cellular and subcellular localization of estrogen and progestin receptor immunoreactivities in the mouse hippocampus. J Comp Neurol 2010; 518: 2729-2743.

56   Reyes-Guerrero G, Guzman C, Garcia DE, Camacho-Arroyo I, Vazquez-Garcia M: Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett 2010; 471: 109-113.

57   Langlois VS, Duarte-Guterman P, Ing S, Pauli BD, Cooke GM, Trudeau VL: Fadrozole and finasteride exposures modulate sex steroid- and thyroid hormone-related gene expression in Silurana (Xenopus) tropicalis early larval development. Gen Comp Endocrinol 2010; 166: 417-427.

58   Belelli D, Lambert JJ: Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 2005; 6: 565-575.

59   Gunn BG, Cunningham L, Mitchell SG, Swinny JD, Lambert JJ, Belelli D: GABA receptor-acting neurosteroids: A role in the development and regulation of the stress response. Front. Neuroendocrinol. 2014; 36: 28-48.

60   Smith SS, Shen H, Gong QH, Zhou X: Neurosteroid regulation of GABA(A) receptors: Focus on the alpha4 and delta subunits. Pharmacol Ther 2007; 116: 58-76.

61   Darbra S, Modol L, Llido A, Casas C, Vallee M, Pallares M: Neonatal allopregnanolone levels alteration: effects on behavior and role of the hippocampus. Prog Neurobiol 2014; 113: 95-105.

62   Turkmen S, Backstrom T, Wahlstrom G, Andreen L, Johansson IM: Tolerance to allopregnanolone with focus on the GABA-A receptor. Br J Pharmacol 2011; 162: 311-327.

63   Giatti S, Garcia-Segura LM, Melcangi RC. New steps forward in the neuroactive steroid field. J Steroid Biochem Mol Biol 2015; doi: 10.1016/j.jsbmb.2015.03.002.

64   Traish AM, Melcangi RC, Bortolato M, Garcia-Segura LM, Zitzmann M. Adverse effects of 5alpha-reductase inhibitors: What do we know, don't know, and need to know? Rev Endocr Metab Disord. 2015. DOI 10.1007/s11154-015-9319-y
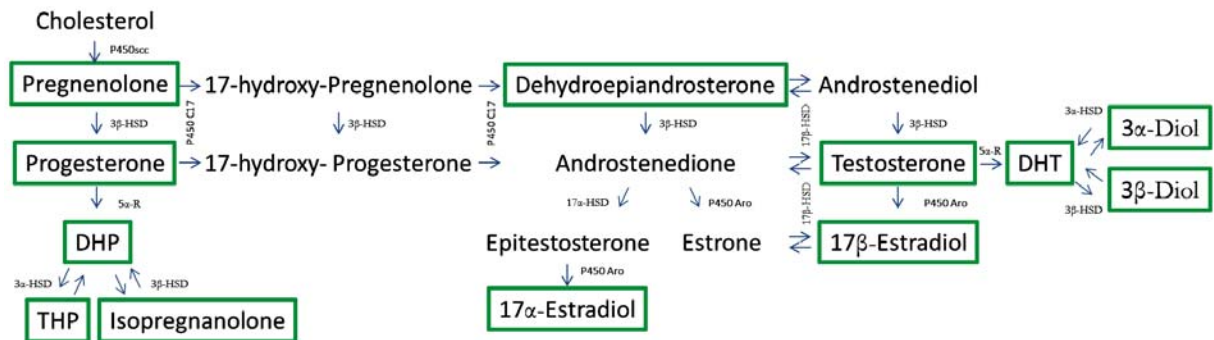
Figure 1

Schematic representation of neurosteroidogenesis. Framed neuroactive steroids reported have been

assessed by LC-MS/MS.
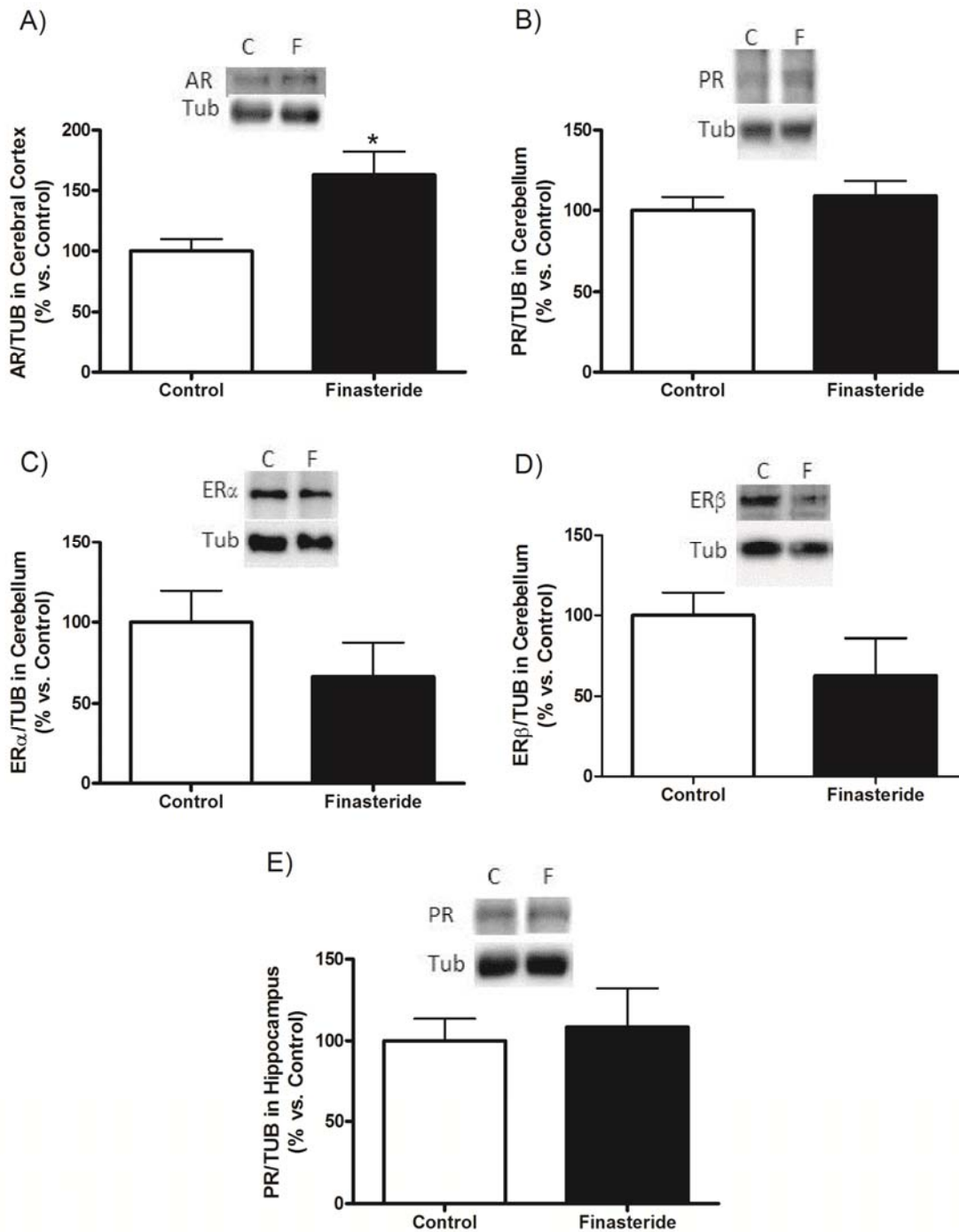
Figure 2

Figure 2

Protein levels of classical steroid receptors detected by Western blotting in control (n=5) and subchronically treated finasteride animals (n=5). Expression of androgen receptor (AR; panel A) in cerebral cortex, progesterone receptor (PR; panel B), estrogen receptor α (ERα; panel C) and estrogen receptor β (ERβ; panel D) in cerebellum, and PR (panel E) in hippocampus. In each panel a

representative blot is reported. The columns represent the mean±SEM after normalization with α-tubulin. Data were analyzed by Student's t-test. *$p \leq 0.05$ vs. control.
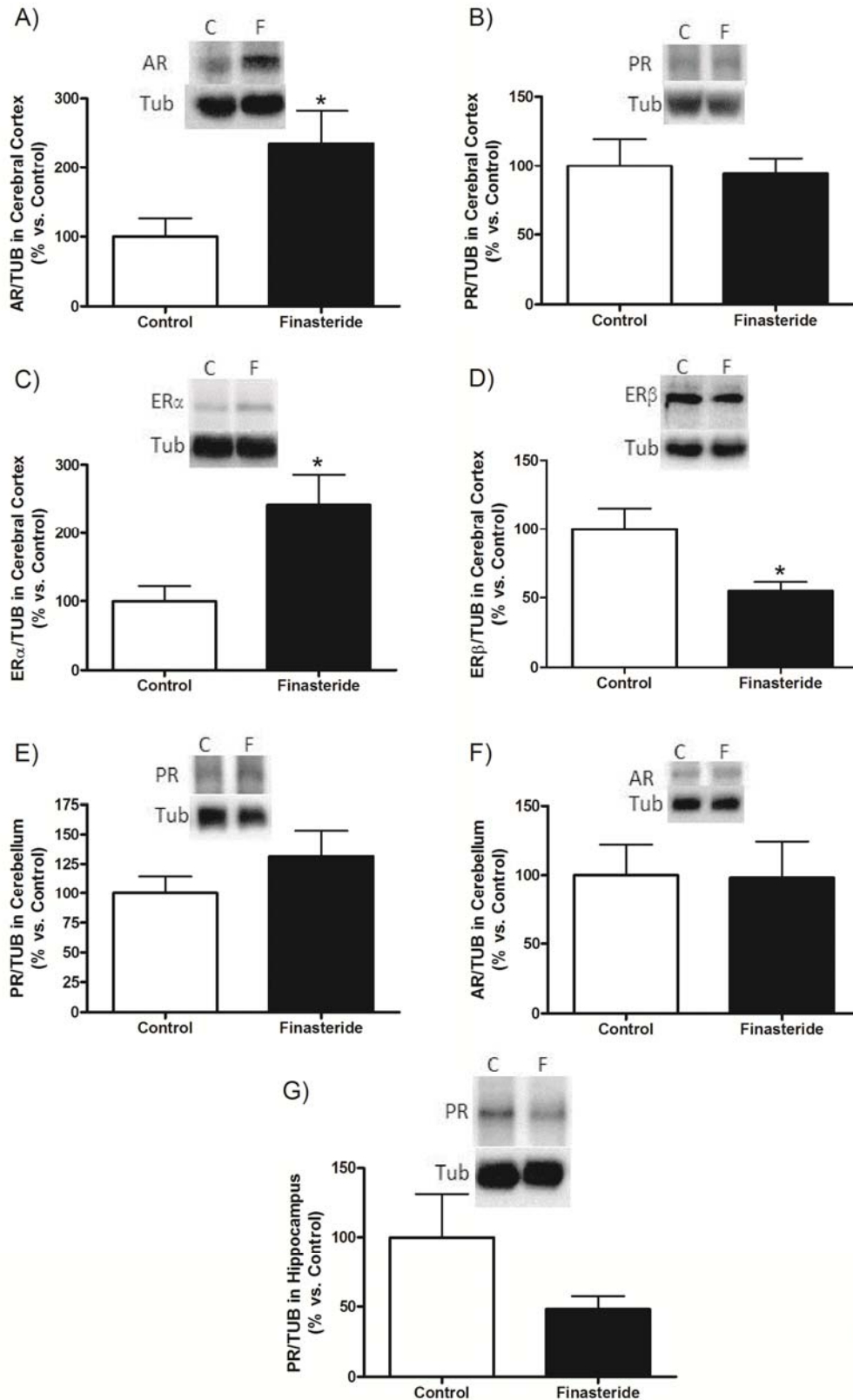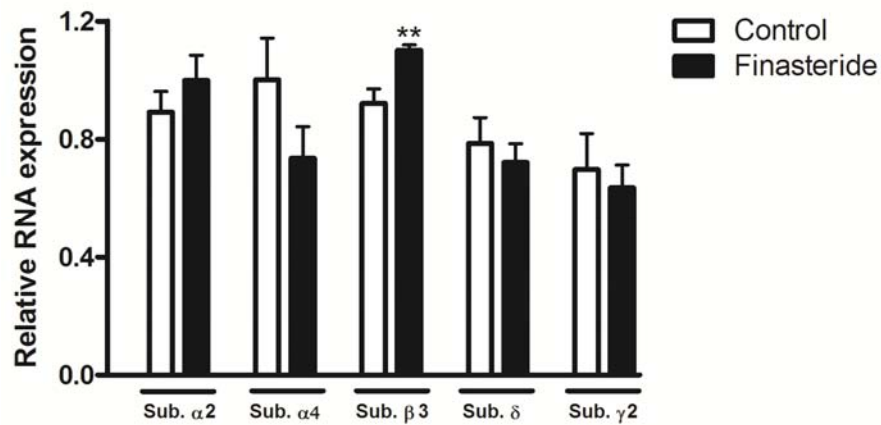
Figure 3

Protein levels of classical steroid receptors detected by Western blotting at the end of the

withdrawal period in control (n=5) and finasteride-treated animals (n=5). Expression of androgen

receptor (AR; panel A), progesterone receptor (PR; panel B), estrogen receptor α (ERα; panel C) and estrogen receptor β (ERβ; panel D) in cerebral cortex, PR (panel E) and AR (panel F) in cerebellum, and PR (panel G) in hippocampus. In each panel a representative blot is reported. The columns represent the mean±SEM after normalization with α-tubulin. Data were analyzed by Student's t-test. *p≤0.05 vs. control.
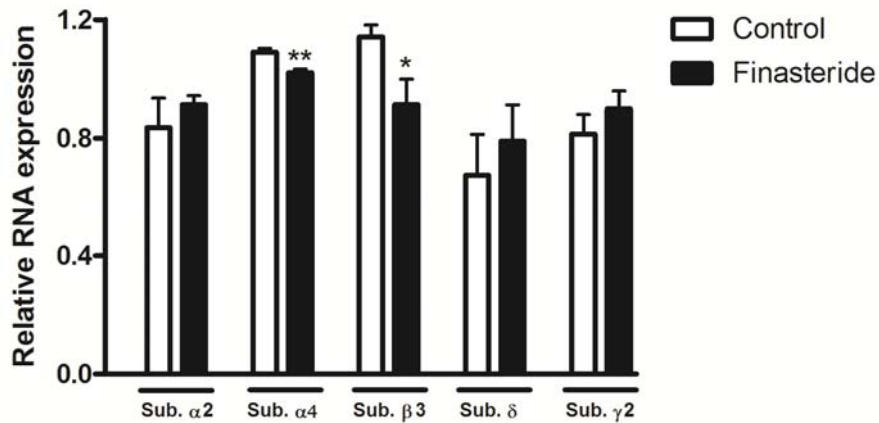
Figure 4

Gene expression of α2, α4, β3, δ, γ2 GABA-A receptor subunits detected by Real Time PCR in cerebellum after chronic treatment. The columns represent the mean±SEM after normalization with 18s rRNA in control (n=5) and chronically treated finasteride animals (n=5). Data were analyzed by Student's t-test. **$p \leq 0.01$ vs. control.

Figure 5

Gene expression of α2, α4, β3, δ, γ2 GABA-A receptor subunits detected by Real Time PCR in cerebral cortex at the end of the withdrawal period. The columns represent the mean±SEM after normalization with 18s rRNA in control (n=5) and chronically treated finasteride animals (n=5). Data were analyzed by Student's t-test. *p≤0.05 and **p≤0.01 vs. control.

Table 1. Levels of neuroactive steroids in plasma, cerebrospinal fluid and brain areas of control and finasteride treated male rats: effect after subchronic treatment

	Plasma		CSF		Cerebral Cortex		Cerebellum		Hippocampus	
	control	Finasteride	control	Finasteride	control	Finasteride	control	Finasteride	control	Finasteride
PREG	0.20±0.046	0.19±0.03	0.31±0.091	0.53±0.07*	1.70±0.24	2.01±0.23	1.60±0.45	1.08±0.29	5.61±1.25	8.68±1.63
PROG	1.31±0.218	0.94±0.12	0.62±0.07	0.53±0.035	2.61±0.68	2.94±0.31	0.58±0.16	0.98±0.24	6.26±0.44	9.64±1.45*
DHP	0.56±0.07	0.52±0.14	0.49±0.12	0.31±0.06	5.18±0.81	5.49±0.76	2.94±1.06	5.56±0.89*	9.40±2.53	10.3±1.48
ISOPREG	u.d.l.	0.14±0.01*	0.13±0.022	0.11±0.007	2.54±0.62	3.48±1.03	0.51±0.06	1.16±0.20**	0.84±0.11	0.84±0.27
THP	0.25±0.07	0.14±0.04	2.31±0.15	2.23±0.15	15.6±1.75	19.0±1.95	5.34±0.48	9.34±2.33	u.d.l.	u.d.l.
DHEA	0.11±0.03	0.11±0.04	0.096±0.02	0.060±0.004	0.81±0.11	0.48±0.06**	0.51±0.08	0.75±0.19	0.29±0.09	0.30±0.08
T	4.23±1.19	3.16±0.91	0.10±0.01	0.12±0.037	2.38±0.37	1.71±0.32	1.86±0.26	1.89±0.26	3.21±0.56	1.97±0.78
DHT	0.79±0.09	0.30±0.02***	1.54±0.12	1.65±0.20	17.5±1.89	16.9±1.55	0.83±0.50	1.08±0.11	3.87±0.63	4.00±0.41
3α-diol	0.84±0.10	1.04±0.28	0.076±0.014	u.d.l.*	0.57±0.06	0.60±0.11	0.54±0.07	0.34±0.07*	0.26±0.07	0.30±0.05
3β-diol	u.d.l.	u.d.l.	0.056±0.004	0.10±0.02*	0.79±0.17	0.60±0.08	0.25±0.034	0.72±0.21*	u.d.l.	u.d.l.
17α-E	u.d.l.	u.d.l.	u.d.l.	u.d.l.	0.076±0.02	0.053±0.01	0.054±0.02	0.077±0.02	u.d.l.	u.d.l.
17β-E	0.022±0.001	0.048±0.01*	0.06±0.02	0.04±0.012	0.15±0.03	0.10±0.01	0.032±0.006	0.066±0.02*	0.03±0.007	0.05±0.008

Levels of pregnenolone (PREG), progesterone (PROG), dihydroprogesterone (DHP), tetrahydroprogesterone (THP), isopregnanolone (ISOPREG), dehydroepiandrosterone (DHEA), testosterone (T), dihydrotestosterone (DHT), 5alpha-androstane-3alpha, 17beta-diol (3α-diol), 5alpha-androstane-3beta, 17beta-diol (3β-diol), 17alpha-estradiol (17α-E) and 17beta-estradiol (17β-E) in plasma, cerebrospinal fluid (CSF), cerebral cortex, cerebellum and hippocampus of male rats. Data (n = 8 for each group) are expressed as pg/μl ± SEM in case of plasma and CSF and pg/mg ± SEM in case of brain areas. Student's t test analysis: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus control. Under detection limit (u.d.l.). Detection limit were 0.05 pg/μl or pg/mg for 3β-diol; 0.02 pg/μl or pg/mg for 17α-E; 0.1 pg/μl or pg/mg for ISOPREG and THP.

Table 2. Levels of neuroactive steroids in plasma, cerebrospinal fluid and brain areas of control and finasteride treated male rats: effect at the end of the withdrawal period

	Plasma		CSF		Cerebral Cortex		Cerebellum		Hippocampus	
	control	Finasteride	control	Finasteride	control	Finasteride	control	Finasteride	control	FInasteride
PREG	0.19±0.03	0.18±0.04	0.20±0.051	0.27±0.04	1.07±0.48	1.24±0.18	0.66±0.03	1.06±0.12**	7.44±1.07	4.37±1.35*
PROG	2.12±0.35	1.32±0.21*	0.64±0.06	0.57±0.05	3.08±0.44	1.83±0.31*	1.48±0.30	1.52±0.57	8.44±1.07	5.27±0.56*
DHP	0.32±0.03	0.34±0.04	0.45±0.12	0.56±0.07	12.03±1.57	3.52±0.21***	2.59±0.61	4.38±0.70*	6.67±0.72	9.88±1.27*
ISOPREG	u.d.l.	0.13±0.04	0.11±0.004	0.11±0.004	2.93±0.35	1.89±0.32*	0.65±0.08	0.88±0.16	0.47±0.06	0.59±0.10
THP	0.50±0.09	0.14±0.03**	2.49±0.19	2.29±0.19	17.8±1.75	7.5±0.47***	4.12±0.37	4.58±0.63	u.d.l.	u.d.l.
DHEA	u.d.l.	u.d.l.	0.064±0.007	0.14±0.03*	1.26±0.31	0.30±0.04**	0.53±0.10	0.40±0.09	0.95±0.43	0.29±0.08
T	3.30±0.7	3.31±0.78	0.15±0.03	0.30±0.07*	3.89±0.61	3.65±0.63	1.22±0.27	2.26±0.25**	7.56±2.70	4.84±1.14
DHT	0.44±0.04	0.33±0.04*	1.35±0.04	0.98±0.10**	9.73±1.38	9.00±0.59	0.60±0.16	0.23±0.12*	3.03±0.43	4.00±0.37
3 -diol	1.63±0.29	1.00±0.19*	u.d.l.	u.d.l.	0.64±0.18	0.52±0.041	0.49±0.14	0.31±0.08	0.26±0.03	0.23±0.04
3 -diol	u.d.l.	u.d.l.	u.d.l.	u.d.l.	0.52±0.21	0.53±0.09	0.63±0.18	0.19±0.03*	u.d.l.	u.d.l.
17 -E	u.d.l.	u.d.l.	u.d.l.	u.d.l.	0.046±0.009	0.045±0.009	0.064±0.03	0.041±0.03	u.d.l.	u.d.l.
17 -E	0.028±0.004	0.024±0.002	u.d.l.	0.04±0.009**	0.12±0.02	0.06±0.01*	0.037±0.01	0.055±0.01	0.074±0.02	0.034±0.01

Levels of pregnenolone (PREG), progesterone (PROG), dihydroprogesterone (DHP), tetrahydroprogesterone (THP), isopregnanolone (ISOPREG), dehydroepiandrosterone (DHEA), testosterone (T), dihydrotestosterone (DHT), 5alpha-androstane-3alpha, 17beta-diol (3α-diol), 5alpha-androstane-3beta, 17beta-diol (3βα-diol), 17alpha-estradiol (17α-E) and 17beta-estradiol (17β-E) in plasma, cerebrospinal fluid (CSF), cerebral cortex, cerebellum and hippocampus of male rats. Data (n = 8 for each group) are expressed as pg/μl ± SEM in case of plasma and CSF and pg/mg ± SEM in case of brain areas. Student's t test analysis: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus control. Under detection limit (u.d.l.). Detection limit were 0.05 pg/μl or pg/mg for DHEA, 3α-diol and 3β-diol; 0.02 pg/μl or pg/mg for 17α-E and 17β-E; 0.1 pg/μl or pg/mg for ISOPREG and THP.