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THE EFFECT OF FINASTERIDE IN TOURETTE SYNDROME:

RESULTS OF A CLINICAL TRIAL

Settore scientifico disciplinari di afferenza

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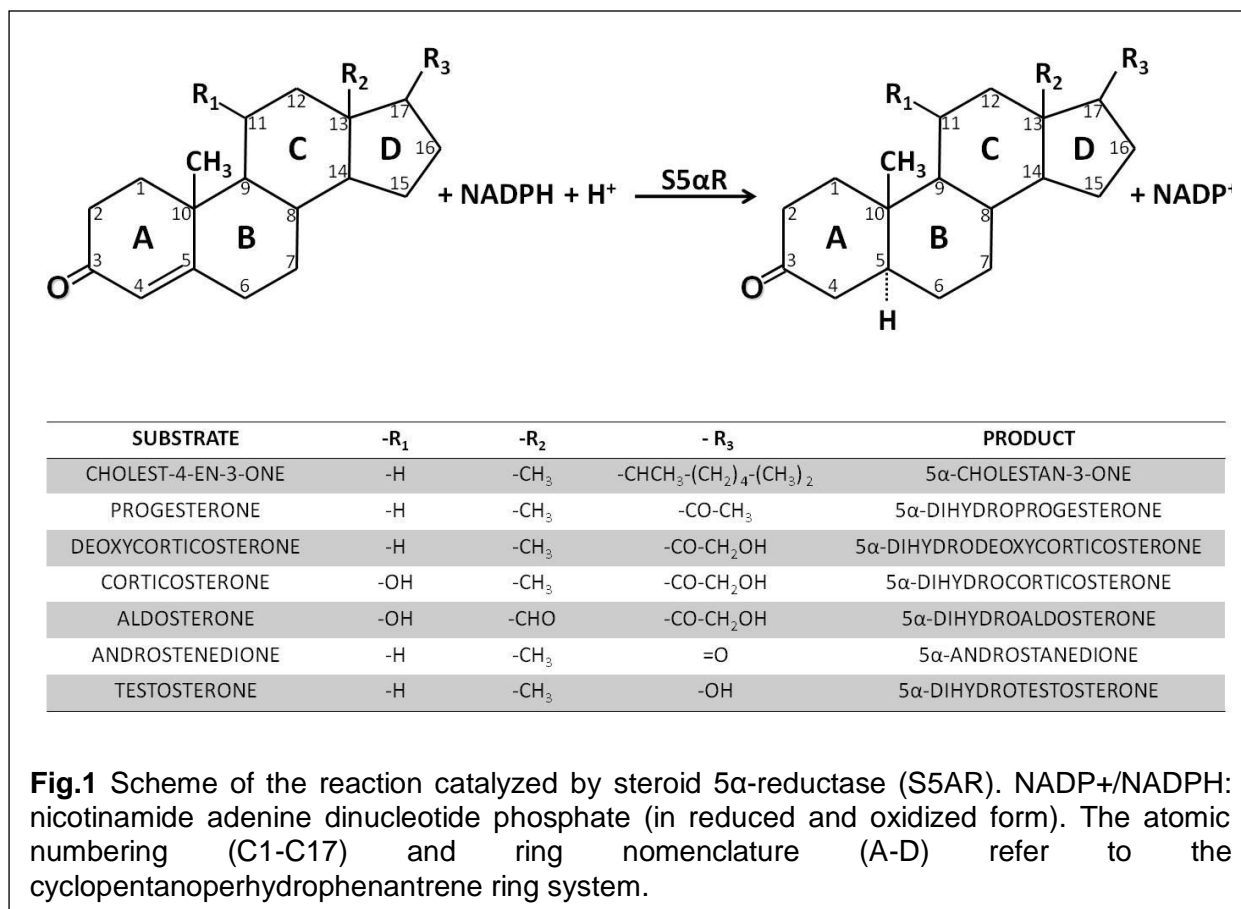Esame finale anno accademico 2009 - 2010

1. INTRODUCTION

The enzyme steroid 5α reductase (S5αR) catalyzes the conversion of Δ^4-3-ketosteroid precursors - such as testosterone, progesterone and androstenedione - into their 5α-reduced metabolites. Although the current nomenclature assigns five enzymes to the S5αR family, only the types 1 and 2 appear to play an important role in steroidogenesis, mediating an overlapping set of reactions, albeit with distinct chemical characteristics and anatomical distribution. The discovery that the 5α-reduced metabolite of testosterone, 5α-dihydrotestosterone (DHT), is the most potent androgen and stimulates prostatic growth led to the development of S5αR inhibitors with high efficacy and tolerability. Two of these agents, finasteride and dutasteride, have received official approval for the treatment of benign prostatic hyperplasia and are being tested for prevention of prostate cancer. Finasteride is also approved for male-pattern alopecia and has been shown to induce very limited side effects. Over the last decade, converging lines of evidence have highlighted the role of both 5α-reduced steroids and their precursors in brain neurotransmission and behavioral regulation. Capitalizing on these premises, we and other groups have recently investigated the role of S5αR in neuropsychiatric disorders. Our preliminary data suggest that S5αR inhibitors may elicit therapeutic effects in a number of disorders associated to dopaminergic hyperreactivity, including Tourette syndrome, psychotic disorders and impulse control disorders.

In this thesis I introduce emerging preclinical and clinical evidences related to these effects, including an open label study of efficacy and tolerability of 5-alpha reductase inhibitors in a sample of patients affected by Tourette Syndrome. It further discusses

some of the potential mechanisms underlying the role of S5αR in the pathophysiology of mental disorders

1.1 General characteristics of steroid 5α-reductase

Steroid 5α-reductases (S5αRs; 3-oxo-5-α-steroid-4-dehydrogenases; E.C.1.3.99.5) are a family of enzymes catalyzing the saturation of the 4,5 double bond of the A ring of several Δ^4-3-ketosteroid substrates, including progesterone, deoxycorticosterone, corticosterone, aldosterone, androstenedione and testosterone (Fig.1).

SUBSTRATE	-R_1	-R_2	- R_3	PRODUCT
CHOLEST-4-EN-3-ONE	-H	-CH_3	-$CHCH_3$-$(CH_2)_4$-$(CH_3)_2$	5α-CHOLESTAN-3-ONE
PROGESTERONE	-H	-CH_3	-CO-CH_3	5α-DIHYDROPROGESTERONE
DEOXYCORTICOSTERONE	-H	-CH_3	-CO-CH_2OH	5α-DIHYDRODEOXYCORTICOSTERONE
CORTICOSTERONE	-OH	-CH_3	-CO-CH_2OH	5α-DIHYDROCORTICOSTERONE
ALDOSTERONE	-OH	-CHO	-CO-CH_2OH	5α-DIHYDROALDOSTERONE
ANDROSTENEDIONE	-H	-CH_3	=O	5α-ANDROSTANEDIONE
TESTOSTERONE	-H	-CH_3	-OH	5α-DIHYDROTESTOSTERONE

Fig.1 Scheme of the reaction catalyzed by steroid 5α-reductase (S5AR). NADP+/NADPH: nicotinamide adenine dinucleotide phosphate (in reduced and oxidized form). The atomic numbering (C1-C17) and ring nomenclature (A-D) refer to the cyclopentanoperhydrophenantrene ring system.

This process, which requires nicotinamide adenine dinucleotide phosphate (NADPH) as a co-factor, consists in the formation of an enolate intermediate on the C3 position of the substrate ketosteroid, which consequently results in the protonation of C4 and direct transfer of a hydride ion from NADPH to C5 [1-3]. The reaction catalyzed by S5αR increases the susceptibility of the carbonyl group in C3 to be reduced by 3α-oxysteroid dehydrogenase (3α-HSD) (Fig. 2) or 3β-hydroxysteroid dehydrogenase (3β-HSD), or conjugated with hydrophilic moieties, such as sulfate and glucuronyl groups, in order to facilitate their elimination. In addition to its role in the regulation of steroid catabolism, 5α reduction is the rate-limiting step in the metabolism of non-aromatic steroids, in view of its irreversibility under physiological conditions [4-6].

Several S5αR products play a pivotal role in a large number of physiological processes. For example, the metabolite of testosterone, 5α-dihydrotestosterone (DHT), is the most potent androgen hormone *in vivo*, and orchestrates the development of male external genitalia and secondary sex traits, as well as the trophism of the prostate [7-9] . The role of S5αR in male hormone metabolism also encompasses the conversion of the adrenal androgen androstenedione into its 5α-reduced metabolite androstanedione, which is further converted into androsterone by 3α-HSD [10] (Fig.2).

The functions of S5αRs are not limited to endocrine regulation. Several 5α-reduced steroids play central roles in the modulation of key physiological functions across different organs and systems. In the eye, the metabolite of cortisol, 5α-dihydrocortisol, plays a role in the production of aqueous humor [11]. In the kidney, 5α-dihydroaldosterone exerts a potent natriuretic function [12]. In the liver, the S5αR-mediated conversion of cholestenone into cholestanone is a fundamental step for the synthesis of cholestanol [13, 14]; moreover, the glucocorticoids 5α-dihydrocorticosterone (3α,5α-THB) and 3α,5α-tetrahydrocorticosterone (3α,5α-THB)

(see Fig.2) are posited to regulate glucose metabolism [15]. Finally, in the brain, the 5α-reduced metabolites of progesterone and deoxycorticosterone, 5α-dihydroprogesterone (DHP) and 5α-dihydrodeoxycorticosterone (5α-DHDOC), are respectively converted by 3α-HSD in 3α,5α-tetrahydroprogesterone (allopregnanolone, AP) and 3α,5α-tetrahydro-deoxycorticosterone (3α,5α-THDOC), which modulate the behavioral reaction to environmental stress by activating the γ-amino-butyric acid (GABA)$_A$ receptor [16, 17].
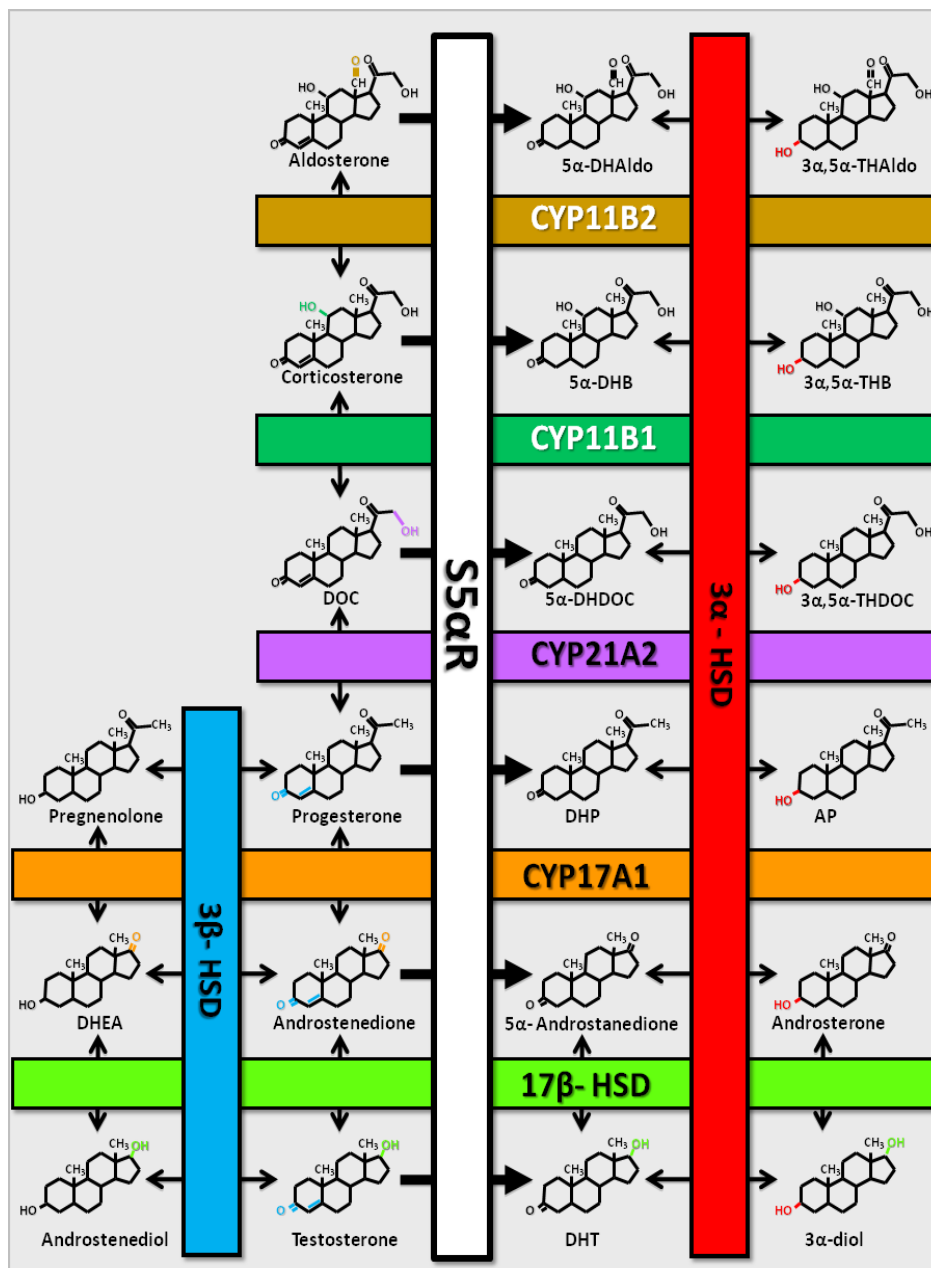
Fig. 2 Schematization of the role of S5αR (steroid 5α-reductase) in steroidogenesis. Metabolic changes in steroid configurations are represented in the same color as the enzymes (boxes) catalyzing the reactions. Enzymes: 3β-HSD: 3β-hydroxysteroid dehydrogenase; 3α-HSD; 3α-hydroxysteroid dehydrogenase; 17β-HSD: 17β-hydroxysteroid dehydrogenase; CYP11B2: Aldosterone synthase; CYP11B1: Steroid 11- β-hydroxylase; Steroid 11-β-hydroxylase; CYP21A2: Steroid 21-hydroxylase; CYP17A1: 17α-hydroxylase/17,20 lyase. Steroids: 5α-DHAldo, 5α-dihydroaldosterone; 3α,5α-THAldo, 3α,5α-tetrahydroaldosterone; 5α-DHB, 5α-dihydrocorticosterone; 3α,5α-THB, 3α,5α-tetrahydrocorticosterone; DOC, deoxycorticosterone; 5α-DHDOC, 5α-dihydro deoxycorticosterone; 3α,5α-THDOC, 3α,5α-tetrahydrodeoxycorticosterone; DHP, 5α-dihydroprogesterone; AP, 3α,5α-tetrahydroprogesterone (allopregnanolone); DHEA,

The first functional characterization of 5α-reduction processes was reported in the early 1950s by several independent groups of researchers, in reference to the conversion of deoxycorticosterone and androgenic 3-ketosteroids into their 5α-reduced metabolites [18, 20]. The enzyme responsible for this reaction was therefore termed Δ^{4-5} α-hydrogenase [21]. In the following years, the identification of similar reactions for other steroids, such as cortisone [22], cholestenone [23] and progesterone [24] led to a plethora of alternative nomenclatures and classifications, based on the conceptual premise that each metabolic process could be catalyzed by a different substrate-specific enzyme [25].

The current genetic, molecular and enzymological evidence on S5αR, however, has greatly challenged this hypothesis; while multiple types of S5αR enzymes have actually been discovered, the two main types subserving a key role in steroidogenesis (named S5αR1 and S5αR2) appear to mediate an overlapping spectrum of reactions [26]. The characterization of different S5αR types proved an extremely difficult task, in view of the numerous technical problems posed by the molecular analysis of S5αR, which requires denaturing detergents for its purification from cell membranes (for a review, see [26]). The identification of S5αR1 and S5αR2 was finally allowed by the cloning of their cDNA [27-30], which paved the way for a number of investigations on the biochemical characteristics and anatomical distribution of these enzymes (for a review, see [26]).

Our knowledge on the morphological, functional and evolutionary relations among different genes and proteins is currently undergoing a general reassessment, in view of novel information provided by high-resolution sequence maps of human genome [31, 32] and the introduction of novel technologies for large-scale, high-throughput cloning [33, 34] and characterization/ modeling of proteins [35, 36]. The employment of these tools has recently allowed to enlarge the family of S5αRs, initially limited to *SRD5A1*

and *SRD5A2* genes and their products (S5αR1 and S5αR2, respectively), to three additional homologous genes: *SRD5A3* (previously known as *SRD5A2*-like), which encodes for a polyprenol reductase conventionally termed S5αR3 [37]; *TECR* (also called synaptic glycoprotein 2 or *GPSN2* in relation to its ortholog first identified in zebrafish), encoding for the enzyme trans-2,3-enoyl-CoA reductase; and *TECR*-like (*TECRL*, alternatively named *GPSN2*-like or *SRD5A2*-like 2).

Although the apparently negligible contribution of S5αR3 and TECR to steroidogenesis has prompted some authors to question the meaning of the current nomenclature [38], phylogenetic analyses have revealed that all the genes in the *SRD5A* family derive from a common ancestor, which underwent duplication in very early evolutionary stages (eukaryotic organisms), leading to the generation of three subfamilies: *SRD5A1/2*, *SRD5A3* and *TECR/TECRL* [39].

In contrast, the differentiation of S5αR1 and S5αR2 (as well as TECR and TECRL) from a common progenitor occurred much later, likely in early chordates [39]. In fact, both enzymes are found in tunicates, fish, amphibians, birds and mammals - including rodents and non-human primates -, but not in nematodes [39-43]. The characteristics of these genes and their products are described below and partially schematized in Tables 1 and 2 for humans and rats, respectively.

S5αR1

The first isolated S5αR isoenzyme, S5αR1 is encoded by the gene *SRD5A1*, located in the locus 5p15 of the human genome. The gene is organized in 5 exons and 4 introns, and encodes for a highly lipophilic, non-glycosilated protein, featuring 5 predicted transmembrane helices. Although the enzyme has not been crystallized to date,

mutagenesis studies have ascertained that the specific characteristics of substrate and inhibitor affinity are conferred by an internal segment between amino acids 26 and 29 [44, 45], located in the first transmembrane segment of the molecule. The protein is typically found in the lipidic bilayer of the endoplasmic reticulum membrane, frequently in perinuclear localization [46, 47].

	S5αR1	S5αR2	S5αR3	TECR	TECRL
Gene	SRD5A1	SRD5A2	SRD5A3	TECR	TECRL
Location	5p15	2p23	4q12	19p13	4q13
Length (b)	36,173	56,385	25,458	36,411	131,002
Protein size (aa)	259	254	319	308	363
Transmembrane helices	5	4	6	3	3
Protein weight (Da)	29,459	28,393	36,521	36,034	42,009
Optimal pH	6-8.5	5-5.5	6.9	7	?
Affinity for testosterone	Km = 1.7 µM	Km = 0.2 µM	?	?	?
Affinity for progesterone	Km = 1.3 µM	Km = 0.2 µM	?	?	?
Affinity for NADPH	Km = 3-5 µM	Km =7-10 µM	?	?	?

Table 1. Characteristics of genes and proteins of the human S5AR family. Molecular and enzymological details unknown to date are indicated with a question mark. The number of transmembrane helices is predicted based on the primary sequence, but not based on actual experimental findings. Data are taken from OMIM, Uniprot, Unigene, Proteobank and BRENDA databases.

	S5αR1	S5αR2	S5αR3	TECR	TECRL
Gene	Srd5a1	Srd5a2	Srd5a3	Tecr	Tecrl
Location	17p14	6q13	14p11	19q11	14p21
Length (b)	33,976	35,888	15,044	26,688	73,848
Protein size (aa)	259 (long isoform); 255 (short isoform)	254	330	308	361
Homology with human gene	72.5%	77.6%	82.4%	98.7%	85.1%
Transmembrane helices	5	4	6	3	3
Protein weight (Da)	29,780 (l) - 29,339 (s)	28,772	38,092	36,123	42,057

Table 2. Characteristics of genes and proteins of the S5AR family in rat (*Rattus norvegicus*). The number of transmembrane helices is predicted based on the primary sequence, but not based on actual experimental findings. Data are taken from OMIM, Uniprot, Unigene, Proteobank and BRENDA databases.

S5αR1 is expressed in cells and structures of ectodermal origin, such as epidermal keratynocytes, melanocytes, sebaceous and sweat glands, neurons of central and peripheral nervous systems and adrenal glands [44, 48-51]. In addition, the enzyme has been detected in fibroblasts, hepatocytes, muscle fibers [44, 52, 53] and several other organs, including prostate, lung, colon and kidney [26, 54, 55].

In the adult brain, S5αR1 is the prevalent isoenzyme [26, 42, 56-61], and is therefore conjectured to account for the largest part of the 5α-reduction reactions occurring within neurosteroidogenic pathways [57, 62-64]. Nonetheless, the pattern of localization of S5αR1 in the central nervous system remains partially elusive, in view of divergent results on its cell expression across different species. In mice, the transcript of the gene has been primarily detected in neurons across the mitral cell layer of the olfactory bulb; the pyramidal cells of the layers 2,3 and 5 in the neocortex; the pyramidal and granule cells in CA1, CA3 and dentate gyrus of the hippocampus; the output neurons of

basolateral amygdala; the medium spiny neurons in the striatum; the dorsomedial and reticular nuclei neurons in the thalamus; the Purkinje cells of the cerebellar cortex. Conversely, no expression in glial cells was detected [65]. In preliminary studies, the authors reportedly found a virtually identical pattern of expression in the rat brain. In striking contrast with these findings, other groups of authors found S5αR1 immunoreactivity only in glial and ependymal cells of the rat [48, 66, 67], with high expression in the cortex, thalamus, hypothalamus, circumventricular organs and cerebellum.

Post-mortem studies in humans have revealed the presence of S5αR1 in the temporal cortex, hippocampus, hypothalamus, pons and cerebellum [57, 68]. However, a complete analysis of the total cerebral expression of S5αR enzymes in the human brain is still lacking.

Few data are known on the transcriptional and translational regulation of *SRD5A1* and its product. In rats, the promoter of the gene has been identified in the 5′ upstream region of the gene, and consists of a bidirectional sequence with Sp1-dependent activity [69]. In cell cultures, expression of the gene products has been shown to be dependent on a number of factors, such as nerve growth factor [70], stimulation of adrenergic and 5-HT$_{2A}$ serotonin receptors [71, 72]. and inhibition of histone deacetylase [73]. Of note, DHT, but not testosterone or progesterone, has been shown to enhance S5αR1 expression [74, 75]. This unusual mechanism of feed-forward stimulation may reflect specific developmental requirements [26], possibly in relation to mechanisms of male sexual maturation.

Our current insight into the specific functions of S5αR1 is limited. Two different polymorphisms of the *SRD5A1* gene associated with lower enzymatic activity have

been linked to higher risk of peripheral arterial disease [76] and polycystic ovary syndrome [77]. Nevertheless, no pathological phenotype has been associated to a nonsense mutation of this gene in humans. Indeed, the congenital deficiency of S5αR1 may not result in major abnormalities, as suggested by the lack of overt physical and sexual impairments in transgenic male mice with a knockout (KO) mutation for the *Srd5a1* gene [41]. Female mice with this mutation, however, were reported to display parturition defects and reduced litter size, plausibly due to their high expression of estrogens, which may have caused deficits in the ripening process of the cervix during late pregnancy [78, 79]. Interestingly, recent preclinical evidence has highlighted a key role for S5αR1 in behavioral regulation and stress response. Short-term stressors have been shown to enhance the synthesis of neurosteroids, such as AP and 3α,5α-THDOC [80-84]. This phenomenon may be partially linked to the increases in brain 5AR1 observed following acute stress [85-87] or as a long-term consequence of prenatal stress [88]. Conversely, prolonged, inescapable stress has been shown to induce a reduction in brain S5αR1 expression [89-91]. These findings suggest that 5AR1 activity may play a physiological role in stress response through regulation of neurosteroid synthesis. In consideration of the role of AP and 3α,5α-THDOC in the modulation of the signaling of (GABA)$_A$ receptor [16, 17], it is possible that changes in S5αR1 may have profound repercussions on the functional regulation of this receptor and its broad set of function in emotional regulation. Accordingly, changes in neurosteroid levels in the brain of rats subjected to stress have been associated with plastic changes in GABA$_A$ receptors activity [92]. In relation to this concept, it is worth noting that S5αR1 KO male mice fail to exhibit a reduction in anxiety- and depression-like behavior following administration of progesterone [93]; likewise, they display no aggressive responses to testosterone [93].

S5αR2

The gene *SRD5A2*, which encodes for S5αR2, is located in chromosome 2p23 and results from the duplication of a common ancestor with *SRD5A1*, as denoted by the common exon-intron organization (5 exons and 4 introns) and a relatively high level of identity shared by both genes [39]. This similarity is also reflected in other matching aspects of S5αR1 and S5αR2, such as the common localization in the membrane of endoplasmic reticulum and the overlapping enzymatic actions.

The elements of divergence between the two isoenzymes are related to their biochemical and physiological characteristics, such as optimal pH, substrate specificity and localization [26, 28, 30, 42, 94-96]. For example, S5αR2 has a higher affinity for testosterone and progesterone than S5αR1 (see Table 1), and is responsible for the synthesis of about two thirds of circulating DHT [53]. The substrate specificity for S5αR2 has been linked to the amino acid sequence 15-17 [45], probably located in the first of the four predicted transmembrane helices. Another key element that has been extensively used to differentiate the two isoenzymes is their pH optimum in vitro (see Table 1). This criterion, however, may not reflect different properties of the enzymes in their native states; in fact, several lines of immunocytochemical and enzymological evidence suggest that both enzymes may have neutral pH optima [28, 29, 42, 57].

The pattern of distribution of S5αR2 is also remarkably distinct from that of S5αR1. In general, S5αR2 is predominantly expressed in tissues and organs of the male urogenital tract (including prostate, epididymis, testicles and seminal vesicles), as well as genital skin, hair follicles and liver [53]. In the brain, S5αR2 transcript has been mainly found in early developmental stages [97]. In the adults, S5αR2 expression is posited to be dependent on androgen signaling [98]. Although a complete

characterization of S5αR2 expression in brain regions has never been reported to date, the enzyme (or its transcript) has been detected in several regions, including hypothalamus, prefrontal cortex and nucleus accumbens [91, 99, 100].

The main physiological function of SRD5A2 has been linked to the development of male genitalia. Indeed, the congenital deficiency of SRD5A2 results in pseudovaginal perineoscrotal hypospadia, a rare form of male pseudohermaphroditism characterized by normal urogenital tracts but underdeveloped external male genitalia [101] and prostate. In mice, the knockout of this enzyme resulted in a different phenotypical presentation, with fully formed internal and external genitalia, but a reduced size of the prostate and seminal vesicle [102]. This interesting finding underscores the relative importance of S5αR1 and 2 in the synthesis and metabolism of androgens is likely to be variable across different species, and advocates caution on the translational value of research on S5AR isoforms in murine models (a thorough analysis of this important concept can be found [26]).

Further evidence about the role of SRD5A2 gene comes from association studies between some of its polymorphic variants and the risk for certain disorders. Of the 28 different polymorphic mutations of this gene, one of the most prevalent is the substitution of valine with leucine in position 89 (V89L). This haplotype (presumably resulting in higher enzymatic activity) has been associated with higher vulnerability for polycystic ovary syndrome as well as higher risk (and/or aggressiveness) of several malignant tumors, including prostate, ovarian and early-onset breast cancer [103-107]. Furthermore, this polymorphic variant has been linked to increased sperm motility , as well as reduced risk of hypospadia [109].

The substitution of alanine with threonine at codon 49 (A49T) has also been associated with clinical conditions typically linked to higher enzyme activity, such as increased risk of prostate cancer [69, 110, 111] and increased sperm concentration [108]. However, the same mutation has also been found in association to alterations indicative of reduced enzymatic function, such as low-severity hypospadia and decreased risk of vertex balding [112]. Finally, recent evidence suggests that this polymorphic variant may also be associated with higher risk of autism in boys [113].

S5αR3

S5αR3 was discovered in 2007 through the analysis of the genome-wide gene expression profile of hormone-refractory prostate cancer cells [114]. While this enzyme is reportedly able to slowly convert testosterone into 5α–dihydrotestosterone [115], its main physiological function has been revealed to be linked to the reduction of polyprenol to dolichol [116], a key process that enables the N-glycosylation of proteins. The localization of S5αR3 has been described as ubiquitous throughout the organism [116], but very little is currently known on the cell- and tissue-specificity of its expression.

In mice, SDR5A3 knockout has been shown to lead to embryonic death by day E12.5. The mutation is often conducive to cardiac and neural malformations [116]. In humans, the congenital deficiency of this enzyme results in a complex syndrome featuring mental retardation, ocular abnormalities (such as optic disc atrophy and coloboma, with loss of vision), cerebellar malformations and coagulation deficits [116, 117]. Nevertheless, these patients do not feature apparent alterations in their steroid levels, suggesting that this enzyme may only play an accessory role in steroidogenesis [116].

TECR and TECRL

TECR is posited to catalyze the last step for the elongation of fatty acids, consisting in the reduction of the double bond of a trans-enoyl coA [118]. A second, shorter isoform of TECR (157 aa) has been reported, featuring the absence of the sequence between aa 20 and 170, but its functional significance remains unknown. While it is likely that TECRL may catalyze a similar enzymatic reaction, its substrate remains unknown to date. No information is available on the ability of these enzymes to process the 5α reduction of ketosteroids.

To the best of our knowledge, the phenotype resulting from the deficiencies of TECR and TECRL enzymes remains unknown. Should such mutations be viable, it is logical to envision that their phenotypic consequences may be akin to those observed in other syndromes related to the deficiencies of other enzymes implicated in the fatty acid synthesis cascade, such as the Acyl-Co A dehydrogenases. These conditions typically result in hypoglycemia, muscle and heart problems and are often lethal [119-122].

1.2   S5αR inhibitors

The development of inhibitors for S5αR was prompted by the discoveries that DHT regulates prostate trophism and S5αR2 deficiency resulted in a lower prevalence of male-pattern alopecia. The first class of inhibitors developed for clinical use, 4-azasteroids, featured a cyclopentanoperhydrophenanthrene nucleus with the substitution of the carbon atom in C4 position with nitrogen. Among these compounds, the compound 4-MA (17β-N,N-diethylacarbamoyl-4-methyl-4-aza-5α-androstan-3-one) was initially selected in view of its high potency and affinity for both S5αR1 and S5αR2

[123]. However, the hepatotoxicity, micromolar affinity for the androgen receptor, and lack of selectivity over 3β-HSD [124] of this agent led to its rapid withdrawal from human studies. Subsequent research on the characteristics of 4-azasteroids paved the way for the development of finasteride [MK906; 17β-(N-tert-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one] (Fig.3), an unsaturated derivative of 4-MA, with relatively high selectivity for S5αR2 over S5αR1 [125, 126]. This compound proved to be highly effective in the control of DHT levels and the progression of benign prostatic hyperplasia (BPH), and was therefore approved by the US Food and Drug Administration (FDA) for this disorder in 1992. Five years later, the same agency approved low-dose finasteride for the therapy of male-pattern hair loss.

The inhibition of S5αR mediated by finasteride is based on the transfer of a hydride group from NADPH to the Δ^1-double bond of the drug. This process results in the formation of a covalent NADP-dihydrofinasteride adduct, which potently binds to the free S5αR enzyme, competing with its endogenous substrates [127]. The extremely low dissociation constant of this compound for S5αR2 (K_i = 3–5 nM), however, results in a very slow turnover of the enzyme-adduct complex ($T_{1/2}$ ≈ 30 days), rendering the inhibition virtually irreversible [26]. In the 1990s, the quest for novel S5αR inhibitors led to the development of dutasteride (GG745; (5α,17β)-N-{2,5-bis(trifluoromethyl)-phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide) [128; 129] (Fig.3), a 4-azasteroid with much higher potency than finasteride in inhibiting both S5αR1 and S5αR2 (100 and 3 times greater, respectively). Dutasteride has been approved for BPH in 2002 and has been shown to induce therapeutic outcomes comparable to finasteride in this disorder [130, 131].

Fig.3 Structure of the FDA-approved S5αR inhibitors finasteride and dutasteride
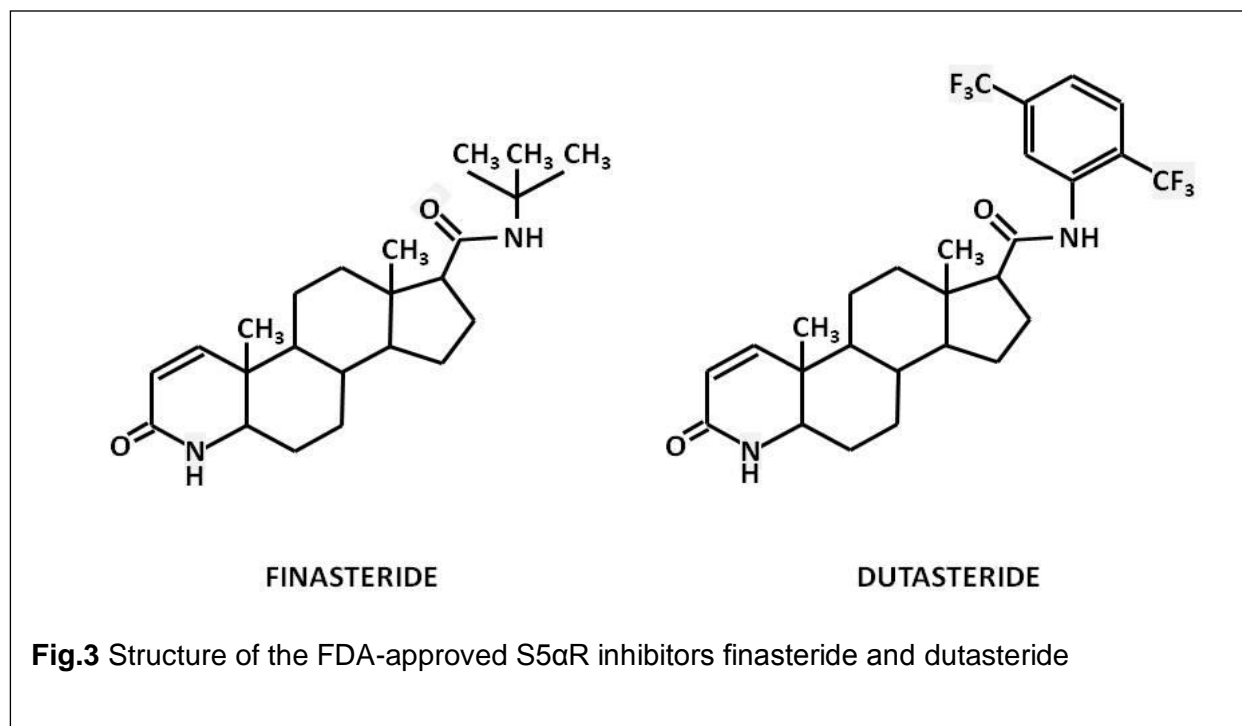
Although finasteride and dutasteride are the only approved S5αR inhibitors, novel promising classes of drugs are emerging, such as the steroidal 3-carboxylic acid epristeride, a potent, irreversible inhibitor of S5αR2 [43]; the selective S5αR1 inhibitor MK-386 [132, 133]; novel non-steroidal molecules, such as FR146687 [134] and FK-143 [135] (for a complete review on the topic see [136]).

In addition, the extract of the berries from the Saw palmetto (*Serenoa repens*), a palm tree native to the Southern Atlantic coast, has recently emerged as a popular treatment for prostate enlargement both in the United States and Europe. The extract is a purified mixture of fatty acids (such as lauric, oleic, myristic, palmitic and linoleic acid) and sterols. As certain polyunsaturated fatty acid have been shown to inhibit S5αRs [137], it has been hypothesized that the therapeutic properties of the extract may be contributed by a blockade of these enzymes [10, 138].

Therapeutic indications.

A large body of data from randomized clinical trials has demonstrated the efficacy of S5αR inhibitors in the treatment of BPH. Several clinical trials have shown the long-term efficacy of finasteride (5 mg/day) in alleviating symptoms, improving urinary flow rates, reducing prostate volume and decreasing the risk of acute urinary retention and the need for surgical intervention [139-141].

Additionally, finasteride has been shown to significantly reduce the risk of prostate cancer (24.4% vs 18.8% in placebo group), irrespective of age, ethnicity, family history and baseline PSA levels [142, 143]. It is worth noting that a higher incidence of high-grade prostate cancer (Gleason scores 7-10) (37% vs 22.2% with placebo) has also been found in finasteride-treated patients [144], although it is currently unknown whether such an increase may reflect confounding factors rather than an actual biological phenomenon. In view of the well-established growth stimulatory potential of DHT for the prostate, as well as the documented role of S5αR1 overexpression in the progression of prostate cancer [145], the dual inhibition mediated by dutasteride is expected to elicit greater beneficial effects than those observed with finasteride on prostate cancer, by slowing its progression or even causing its regression [141, 146]; this putative advantage, albeit supported by preclinical data [145], has yet to be corroborated by conclusive clinical evidence based on large-scale, long-term comparative studies.

Lower doses of finasteride are also indicated for the treatment of androgenetic alopecia, both orally (1 mg/day) or locally (by gel). The drug significantly diminishes the progression of the baldness and often stimulates new growth. The mechanism of action is based on the reduction of DHT levels, which play a key role in male-pattern hair loss

[147, 148]. Interestingly, however, the drug has shown no beneficial effect in treating the disorder in postmenopausal women. Discontinuation of finasteride results in loss of any positive effects on hair growth within 12 months [149]. As increased S5αR activity is also conducive to female hirsutism [150], finasteride therapy has been proposed for this condition. The results have been promising, with efficacy comparable to antiandrogen agents, but no appreciable side effects [140, 151, 152]. It should be noted that finasteride is contraindicated in pregnant women, in view of the high risk of birth defects. Although no data are currently available on the excretion of S5αR inhibitors in milk, lactating mothers should also refrain from using finasteride, to avoid potential side effects on the sex development of their children.

Tolerability and peripheral side effect

Since its development and first clinical studies, finasteride was reported to elicit very limited side effects in volunteers and patients [153 ,154]. The high tolerability and safety of finasteride has been confirmed by several randomized clinical trials over very long follow-up periods [142, 155-157]. The most common side effects induced by finasteride, at the daily dose of 5 mg (recommended dosage for BPH) include decreased libido, ejaculatory disorders and erectile dysfunction, and are ascribed to the reduction in DHT levels as well as the increased estrogen synthesis (due to enhanced availability of testosterone and androstenedione for aromatization). The reported incidence of these adverse effects is variable across different studies, but is generally low (around 5%) and mainly reported during the initial stages of treatment. None of these side effects has been found to significantly interfere with therapeutic compliance, as indicated by comparable rates of treatment withdrawal between finasteride- and placebo-treated groups [149, 158]. Of note, in the PLESS (Proscar Long-Term Efficacy and Safety) trial

(on 3040 male BPH patients over 4 years), the drop-out rate was higher in the placebo group (42%) in comparison with the patients treated with finasteride (34%) [140].

In a small percentage of patients, finasteride has been shown to induce gynecomastia and breast tenderness [159, 160], likely due to enhancements in estrogen levels. The time of onset of gynecomastia (2-4 months) observed in the most of finasteride users is generally delayed relative to other sexual-related adverse events. Interestingly, gynecomastia tends to be more frequently unilateral in individuals treated with low doses of finasteride [161, 162]. Unlike other side effects, breast enlargement has reported to be long-lasting in several cases, even following finasteride withdrawal [159].

Other very rare side effects include hypersensitivity reactions, such as itch, skin rash, lips swelling or lid edema [163]. The cutaneous rash may be linked to the increased photosensitivity produced by certain steroids (such as 5β-reduced steroids), which would be disproportionately produced in the liver as a result of S5αR inhibition [164]. No significant side effects have been reported on other steroid-regulated metabolic processes, such as bone turnover, lipoprotein formation or hemoglobin production. Similarly, finasteride treatment does not result in any increase in the rate of serious adverse events [155].

Despite its higher potency and efficacy in blocking both S5αR1 and S5αR2 isotypes, dutasteride has been shown to induce a spectrum of side effects strikingly akin to those observed with finasteride. A 2-year study of dutasteride for BPH treatment revealed low rates of impotence, decreased libido, ejaculation disorder, and gynecomastia. Similarly to finasteride, these side effects were transient, with no statistically significant difference noted at 2 years [130]. The tolerability of dutasteride appears comparable to that of finasteride during long-term treatments [142, 155, 165-167].

Psychological effects.

In addition to its endocrine and metabolic effects, inhibition of S5αR results in a broad array of effects in emotional and cognitive regulation in rodents, which stem from the ensuing alterations in the profile of neuroactive steroids within cortical and limbic brain regions. While only few clinical studies have analyzed the psychotropic effects of S5αR inhibitors, numerous investigations in experimental animals have focused on the behavioral implication of finasteride treatment.

The best-characterized and most frequently investigated behavioral effect of pharmacological S5αR blockade in rodents is the suppression of the synthesis of AP and 3α,5α-THDOC, which leads to perturbed modulation of $GABA_A$ receptor function and altered responsiveness to environmental stimuli and stress [168-170]. Indeed, both AP and 3α,5α-THDOC have been shown to elicit anxiolytic-like actions in different animal models [171-173]. Furthermore, AP has been shown to regulate aggression and fear responses in male mice [174-177].

Systemic administration of finasteride has been shown to increase indices of anxiety-like behaviors in the open field and elevated plus maze paradigms and depression-like behavior in the forced swim test [178, 179]. Several brain regions, including hippocampus and amygdala, have been shown to play a role in these effects [178, 180, 181].

Finasteride may exert its effects on anxiety and mood by decreasing the levels of other 5α-reduced neurosteroids, such as androsterone and 3αdiol, which have been shown to exert anxiolytic and antidepressant properties [182-185].

The translational value of the preclinical investigations on finasteride's behavioral effects is partially limited by the fact that, in rats, finasteride has a high affinity for both S5αR1 and S5αR2 [44, 186]. This characteristic is strikingly at variance with the relative selectivity of finasteride for S5αR2 in humans, suggesting possible differences in the neuropsychological outcomes of this drug. Accordingly, several studies have even reported potential beneficial effects of finasteride on anxiety and mood in BPH patients, but this outcome is arguably influenced by their enhancement in quality of life due to the reduction of urinary symptoms [187].

Few studies have actually shown an association between finasteride administration and increased anxiety and depression in patients with BPH and androgenetic alopecia [188, 189]. In line with this concept, several studies have reported an inverse relationship between AP levels and anxiety and depression symptoms in patients [52, 190]; furthermore, antidepressant administration has been shown to significantly increase the low levels of AP found in patients with major depression [191-195].

The role of neurosteroids in mediating antidepressant effects, however, has been recently challenged [196, 197] in consideration of the lack of changes in neurosteroid concentrations in relation to the mood-enhancing effects of non-pharmacological antidepressant treatments, such as electroconvulsive therapy or repetitive transcranial magneticstimulation[198-200].

1.3.   S5αR inhibitors as putative therapeutic agents for some neuropsychiatric disorders.

Multiple lines of evidence suggest the implication of steroidal mediators in the pathophysiology of schizophrenia and other psychiatric disorders.

The conceptual premises for the employment of S5αR inhibitors in schizophrenia lie in several lines of preclinical and clinical evidence highlighting a role for neuroactive steroids in psychotic disorders. One of the most compelling evidence in favor of this link is the observation that the well-characterized gender differences in schizophrenia may be supported by different hormonal profiles between males and females [201-203]. In men, the onset of psychotic symptoms occurs generally earlier (in late adolescence / young adulthood), and with a more insidious presentation than in women; furthermore, men tend to display higher severity of psychotic manifestations, higher rate of negative symptoms and worse clinical outcome and functioning [201, 204-208]. This phenomenon has been largely ascribed to the protective role of estrogens.

Accordingly, women have been shown to have a higher vulnerability to psychotic manifestations during estrogen withdrawal, such as after the delivery of babies [209] or during menopause. In addition, several lines of evidence have recently shown that androgen precursors, such as testosterone and dehydroepiandrosterone (DHEA), may modulate the expression of psychotic phenomena. Despite a number of initial contradictory findings on the efficacy of these steroids as adjunctive therapies to standard antipsychotic drugs (see [203]), recent studies have highlighted both androgens as potential targets for schizophrenia therapy. Following preliminary studies reporting low levels of testosterone in patients with predominantly negative symptoms [210], recent clinical evidence has shown that serum total and free testosterone levels

are inversely correlated with the negative score of the Positive and Negative Syndrome Scale (PANSS) [211, 212]. In addition, testosterone augmentation has been documented to induce improvements in negative symptoms in a recent placebo-controlled, double blind trial on schizophrenia male patients [213].

High levels of DHEA have often been reported in schizophrenia [214], and have been highlighted as a potential predictor for poor cognitive function [215]. Of note, cortisol/DHEA ratio has been associated with a number of cognitive functions [216] and may serve as an index for responsiveness to antipsychotic treatment [217]. Moreover, DHEA was found to significantly attenuate antipsychotic-induced extrapyramidal symptoms and negative symptoms, as assessed through the Scale for the Assessment of Negative Symptoms (SANS), but not the PANSS scale [215, 218, 219]. Another promising neurosteroid target is pregnenolone (as well as its derivative pregnenolone sulfate). These molecules have been repeatedly proposed for the management of psychotic symptoms [220-227]. Indeed, in a recent double-blind, randomized study on 58 schizophrenia and schizoaffective disorder patients, addition of low doses of pregnenolone (30 mg/day) to standard antipsychotic treatments was found to significantly ameliorate positive symptoms, as assessed by the PANSS. In contrast, higher doses of pregnenolone (200 mg/day) did not elicit any significant effect vs placebo [228]. Both pregnenolone and DHEA levels were found to be higher in the cingulate and parietal cortex of subject with schizophrenia [229]. The effects of pregnenolone and DHEA (and their sulfates) may be related to their effects on neuronal excitability and synaptic plasticity, as well as neuroprotection (reviewed in [228, 230]).

In contrast with these findings, levels of the 5α-reduced neurosteroid AP were found significantly decreased in post-mortem samples from parietal cortex as compared to control subjects [229]. This complex scenario, featuring increased levels of certain

steroid precursors and possibly reduced concentrations of some of their metabolites may signify that certain steroidogenic enzymes are functionally altered in schizophrenia and other psychotic disorders. In line with this hypothesis, environmental stress, a major factor in the etiology of psychosis [231] has been shown to affect the synthesis and metabolism of several neurosteroids [80, 88, 232]. As stated above, for example, cogent preclinical evidence has documented that S5αR expression and function are affected by short-term and chronic stress. Neuroactive steroids have also been shown to modulate the responses of all the key neurobiological targets implicated in schizophrenia, such as dopamine, GABA, as well as the glutamate N-methyl-*d*-aspartate (NMDA) and σ receptors [16, 85, 233, 234].

Preclinical evidences.

Based on these premises it has been hypothesized that, in view of the central role of S5αR in the metabolism of testosterone as well as the synthesis of AP and other neurosteroids, the blockade of this enzyme may elicit therapeutic effects in schizophrenia. In particular, inhibition of S5αR may have resulted in decreased levels of 5α-reduced steroids and potential increase in the concentrations of their precursors, which may elicit beneficial effects in psychotic disorders. Furthermore, it has been speculated that, by increasing the availability of testosterone and androstenedione for aromatization, the effects of S5αR blockade may also include the neuroprotective and beneficial action of estrogens in psychosis [235-237].

The first studies on S5αR inhibitors were performed in rat models of schizophrenia, based on the stimulation of dopamine receptors with direct and indirect agonists. In addition to hyperlocomotion and stereotyped behavior (two well-validated assays to measure behavioral outcomes of dopaminergic activation, with high predictive validity

for typical and atypical antipsychotics), it has been focused on the model of prepulse inhibition (PPI) of the acoustic startle reflex, and its disruption mediated by dopamine agonists and NMDA antagonists [238].
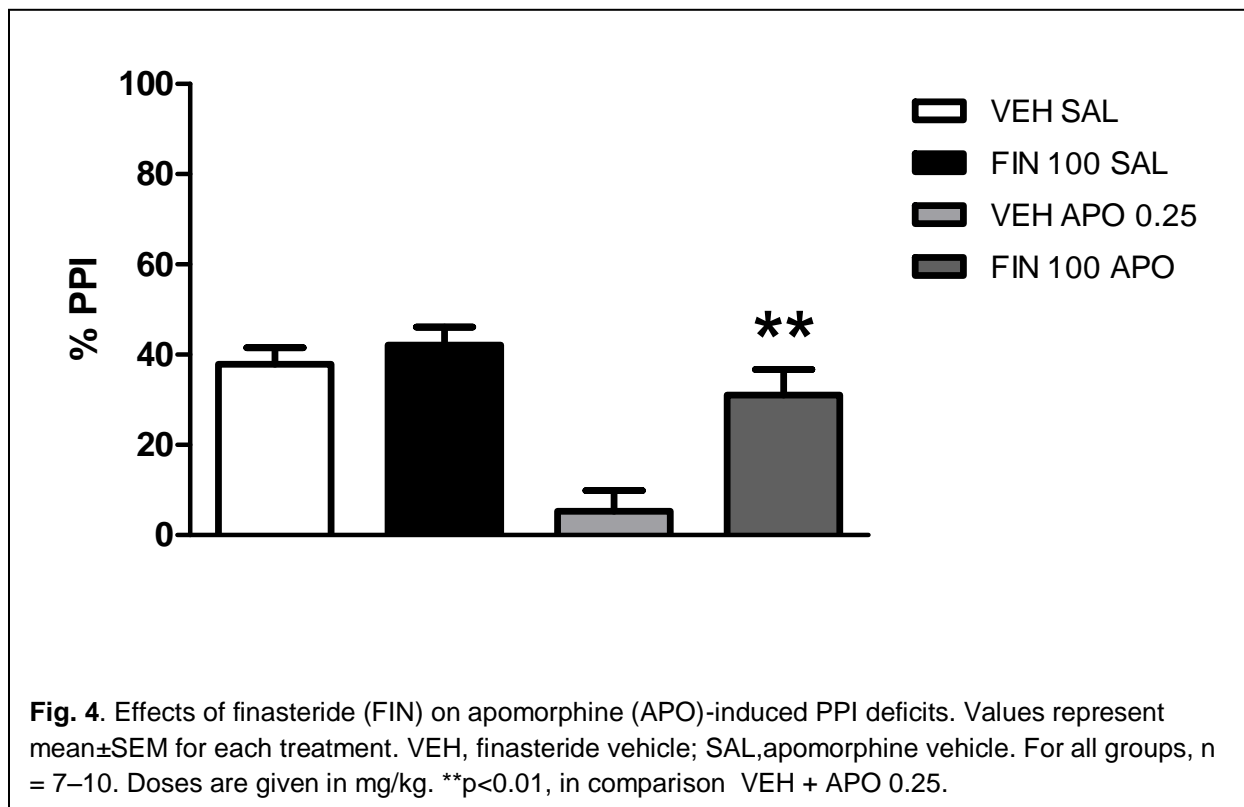
PPI is the normal reduction in startle amplitude occurring when a startling stimulus is preceded by a weaker prepulse and functions as a measure of preattentional sensorimotor gating. This endophenotype has been extensively employed to model the well-documented gating deficits in schizophrenia and other deficits, also in view of the highly remarkable finding that psychotic patients display deficits in PPI of the blink reflex (reviewed in [239]). The high face and predictive validity of PPI as a tool for the assessment of neurobehavioral deficits related to schizophrenia is highlighted by the fact that, in animals, administration of dopaminergic agonists as well as other psychotomimetic agents significantly reduces this index, in a fashion sensitive to typical and atypical antipsychotic drugs [240].

In addition to schizophrenia, PPI deficits have also been found in other neuropsychiatric disorders related to dopamine dysregulation, such as mania [241] and Tourette syndrome [242, 243].

Recent results of preclinical studies showed that S5αR inhibitors such as finasteride and dutasteride, prevented the PPI deficits and other behavioral changes induced in rats by both d-amphetamine, a dopamine release enhancer, and apomorphine, a potent dopamine receptor agonist [238].

The potent antidopaminergic properties of S5αR inhibitors were not accompanied by the extrapyramidal symptoms commonly triggered by neuroleptics, such as haloperidol

[238]. In fact, even very high doses (1000 mg/kg) failed to produce cataleptic reactions in either the bar or the paw tests.

Fig. 4. Effects of finasteride (FIN) on apomorphine (APO)-induced PPI deficits. Values represent mean±SEM for each treatment. VEH, finasteride vehicle; SAL,apomorphine vehicle. For all groups, n = 7–10. Doses are given in mg/kg. **p<0.01, in comparison VEH + APO 0.25.

All the behavioral effects mediated by finasteride and other S5αR inhibitors were observed within a short time after administration, suggesting that the NSs responsible may function through non-genomic interactions. Indeed, many neuroactive steroids are known to influence behavioral and cognitive functions through fast-acting interplay with numerous neurotransmitter systems [244].

Subsequent studies showed that the effects of finasteride were observed in both male and female rats (Fig 4) and were not affected by orchiectomy in adult males (Fig 5 and Fig.6), suggesting that the mechanisms mediating the antipsychotic-like effects did not depend on gonadal androgens [Soc. Neurosci. 2010, abstract n° 665.23].
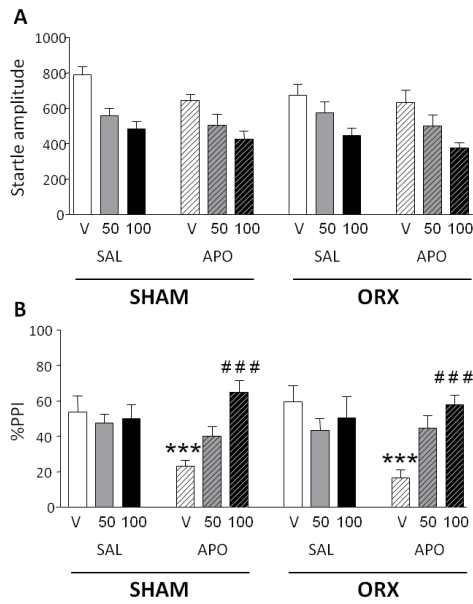
Fig. 5. Effects of systemic finasteride on startle reflex (A) and PPI (B) in sham-operated (SHAM) and orchiectomized (ORX) rats. Orchiectomy did non alter the ability of systemic FIN to prevent the PPI deficits induced by the dopamine (DA) receptor agonists apomorphine (Fig. 5a). Finasteride doses are indicated in mg/kg (I.P.). V, vehicle of finasteride; SAL, saline; APO, apomorphine (0.25 mg/kg, S.C.). Values are expressed as mean ± S.E.M. ***, P<0.001 vs rats treated with vehicle and saline (pre-treatment x treatment interaction); ###, P<0.001 vs rats treated with vehicle and APO. Main statistical effects are not indicated.
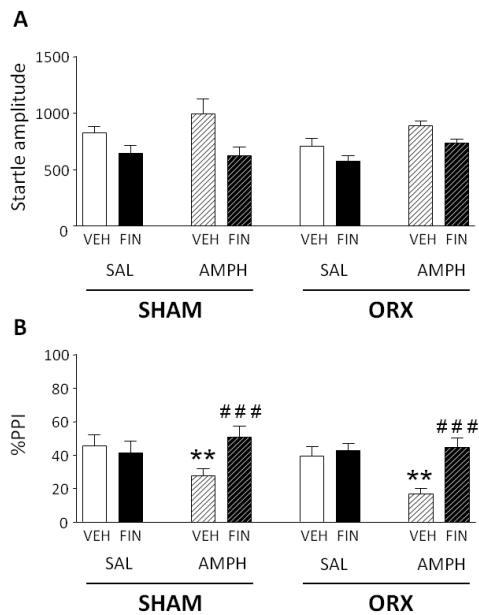

Fig. 6. Effects of systemic finasteride on startle reflex (A) and PPI (B) in sham-operated (SHAM) and orchiectomized (ORX) rats. Orchiectomy did non alter the ability of systemic FIN to prevent the PPI deficits induced by the dopamine (DA) receptor agonists d-amphetamine. VEH, vehicle of finasteride; SAL, saline; AMPH, d-amphetamine (2.5 mg/kg, S.C.). Values are expressed as mean ± S.E.M. **, P<0.01 vs rats treated with vehicle and saline (pre-treatment x treatment interaction); ###, P<0.001 vs rats treated with vehicle and AMPH. Main statistical effects are not indicated.

In view of the important contributions of the dopaminergic system in psychotic disorders, it has been investigated whether antipsychotic-like effects of finasteride were mediated by this system. This research revealed that local injection of finasteride into nucleus accumbens e medial prefrontal cortex - two key terminals of the mesolimbic and mesocortical pathways of the dopaminergic system - attenuated apomorphine induced PPI deficit. (fig 7) [Devoto et al submitted].

These findings are in agreement with previous data, showing that both regions may be central in the behavioral actions of finasteride and S5αR products [178, 245-247]. Moreover, both the nucleus accumbens and the medial prefrontal cortex are key substrates for the dopaminergic regulation of PPI (reviewed in [243]) and play a well-established role in the pathophysiology of schizophrenia. The impact of S5αR on the dopaminergic modulation of sensorimotor gating, however, does not appear to involve the direct participation of other forebrain regions, such as the dorsal caudate, basolateral amygdala and ventral hippocampus (fig 7) [Devoto et al submitted].
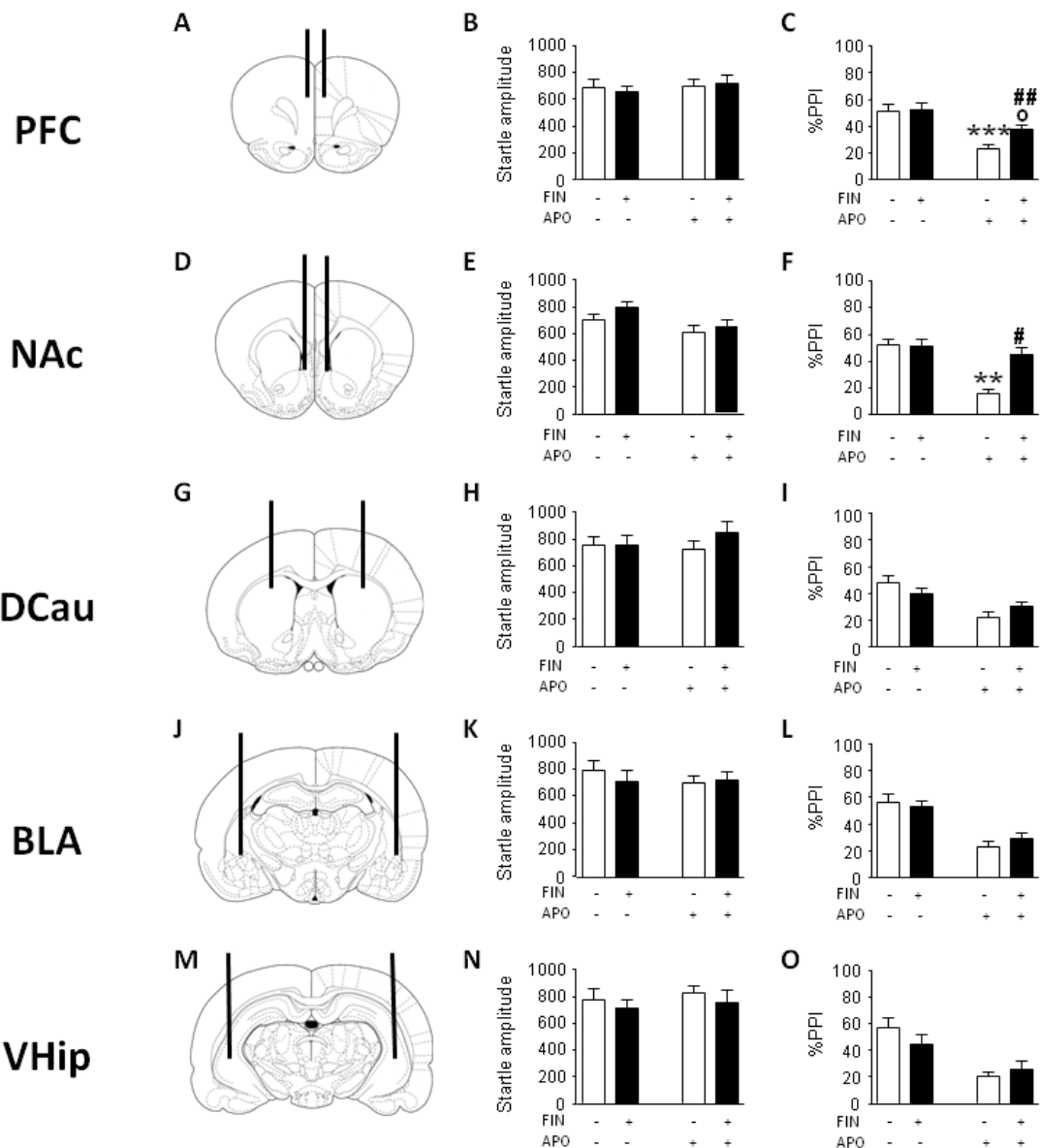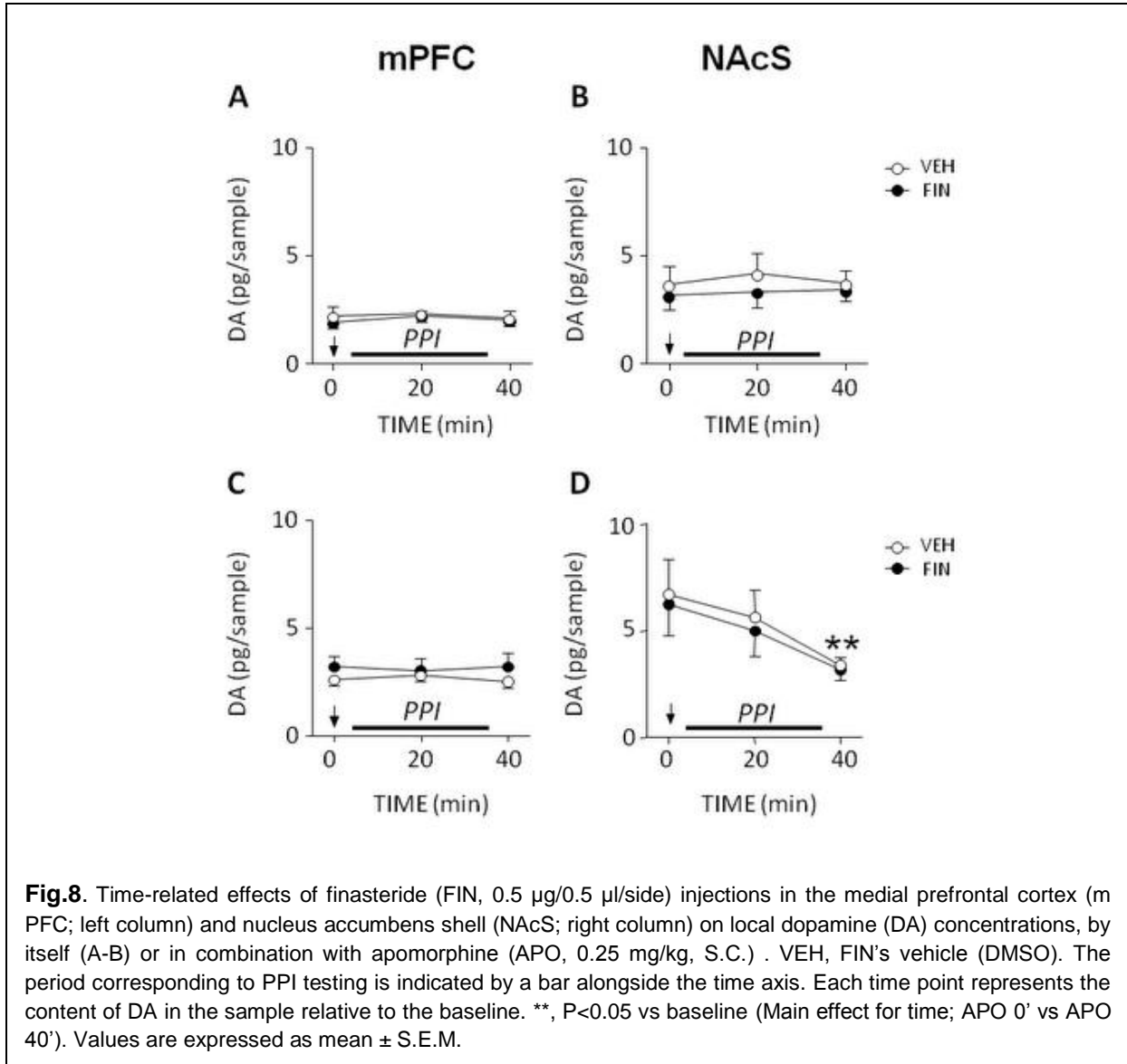
Fig. 7 APO-induced PPI deficits were reversed by local lateral FIN injections in medial prefrontal cortex (mPFC) and nucleus accumbens shell (nAcS), but not other regions involved in gating regulation, such as dorsal caudate, ventral hippocampus and basolateral amygdala. Effects of local finasteride (FIN) on startle reflex and PPI across medial prefrontal cortex (mPFC) (A-C), nucleus accumbens shell (NAcS) (D-F), dorsal caudate (DCau) (G-I), basolateral amygdala (J-L) and ventral hippocampus (VHip) (M-O). Localization of the cannulae is schematized in the left column. APO, apomorphine (0.25 mg/kg, S.C.). + and – refer to the presence of a drug or its vehicle (Ringer solution-DMSO for FIN, saline for APO). Values are expressed as mean ± S.E.M. *, P<0.05; **, P<0.01; ***, P<0.001 vs rats treated with Ringer-DMSO and saline (pre-treatment x treatment interaction); #, P<0.05; ##, P<0.01 vs rats treated with vehicle and APO; °, P<0.05 vs rats treated with FIN and saline.

Intracerebral administration of finasteride was not accompanied by changes in extracellular concentrations of dopamine in either region (Fig 8). These results suggest that the antipsychotic-like properties of finasteride are mainly related to post-synaptic processes [Devoto et al. submitted].

Fig.8. Time-related effects of finasteride (FIN, 0.5 µg/0.5 µl/side) injections in the medial prefrontal cortex (m PFC; left column) and nucleus accumbens shell (NAcS; right column) on local dopamine (DA) concentrations, by itself (A-B) or in combination with apomorphine (APO, 0.25 mg/kg, S.C.) . VEH, FIN's vehicle (DMSO). The period corresponding to PPI testing is indicated by a bar alongside the time axis. Each time point represents the content of DA in the sample relative to the baseline. **, $P<0.05$ vs baseline (Main effect for time; APO 0' vs APO 40'). Values are expressed as mean ± S.E.M.

Clinical evidences.

To date, there are only few reports on the effect of 5-alpha reductase inhibitors in neurological and psychiatric disorders. In a recent case report finasteride was tested in a male patient affected by chronic schizophrenia and unresponsive to standard antipsychotic therapies [248]. Over the first 24 days of experimental treatment with finasteride (5 mg/day), the patient exhibited a mild improvement in positive and negative symptoms, with no reported side effects. Following discontinuation of the medication, the patient experienced a precipitation of his psychotic symptoms, which led him to request the continuation of finasteride regimen [248].

Another recently documented case of a male adult affected by benign essential blepharospasm (BEB) responded poorly to botulinum toxin but exhibited a significant improvement of his dystonic symptoms in response to finasteride (5 mg/day) prescribed for concomitant BPH [249]. Nevertheless, the same therapy failed to induce noticeable improvements in other patients, underscoring that S5αR inhibition may address only a select subset of BEB patients [249]. Interestingly, this possibility mirrors the equivocal role of dopamine in this disorder, as activation of its receptors has been shown to either exacerbate or attenuate the severity of blepharospasm [250-255].

Finasteride-mediated effects on alcohol response were investigated in a placebo-controlled study on male and female "social drinkers" (i.e. reportedly drinking at least three times a week and three times on one occasion during the month prior the testing) found that a high dose of finasteride (200 mg over 24 h) reduced several self-reported stimulant effects of alcohol, as measured by the Central-Stimulant and Dynamic-Peripheral Subscales of the Alcohol Sensation Scale [256]. The biological mechanisms involved in these clinical implications are currently unknown.

Potential therapeutic role of S5αR inhibitors has been found also in impulse-control disorders as pathological gambling [Bortolato et al, submitted] occurring in response to dopamine-replacement therapies in Parkinson's disease (PD). [257; 258]. Interestingly, both the prefrontal cortex and the ventral striatum have been shown to play a key role in pathological gambling [259] and in the behavioral outcomes of dopamine-replacement therapy [260].

Finasteride in Gilles de La Tourette Syndrome

Tourette syndrome (TS) is a chronic neuropsychiatric disorder, characterized by motor and one or more phonic tics although not necessarily concurrently, lasting longer than 1 year [261, 262]. It affects between 1% and 3% of the general population [263]. The onset of TS manifestations occurs typically in childhood (6-7 years of age), and the severity and frequency of tics typically tends to diminish during adolescence in most patients [264; 265; 266]. Tics are defined as sudden, rapid, recurrent, non-rhythmic, stereotyped movement or vocalization [267] that wax and wane in severity over weeks and months [268]. The motor patterns of tics can involve individual muscles or small groups of muscles (simple tics: eye blinking, nose twitching, mouth opening, sniffing) or more muscle acting in a coordinated pattern to produce movements that can resemble purposeful voluntary movements (complex tics: head shaking, scratching, touching, hitting, gestures or uttering phrases). Extreme forms of this disorder present as paroxysms or continuous orchestrated displays of simple and complex motor tics, such as self-injurious motor tics, hitting or biting, and socially unacceptable coprolalic utterances – e.g., shouting obscenities, racial slurs– and gestures. [269].

The majority of adult TS patients also display comorbid psychiatric disorders, such as mood and personality and anxiety disorders, including obsessive-compulsive disorder (OCD) [270, 271, 266].

The only medications currently approved for TS therapy are haloperidol and pimozide; however, the number of side effects induced by these medications, such as extrapyramidal symptoms [272, 268, 273] has led to the quest for alternative therapies. Atypical antipsychotics and α-adrenergic agonists are interesting options for TS therapy, but they also often lead to adverse metabolic and cardiovascular effects [274, 271].

While rich evidence has shown that hyperactivation of dopamine signaling plays a central role in the pathophysiology of TS [275, 276] several findings point to androgens in the pathophysiology and clinical course of TS [277-280].

In contrast with evidence supporting an autosomal dominant transmission for TS, this disorder affects men much more commonly than women [281]. This background indicates that androgens may facilitate the phenotypical expression of TS in men. Accordingly, TS symptoms are exacerbated by exogenous androgens [282]. Furthermore, the severity of tic symptoms in children affected by TS is correlated to the degree of preference for masculine play, suggesting that the disorder might be underpinned by elevations in androgen levels [280]. Finally, TS features a number of symptoms strongly reminiscent of androgen-mediated behaviors, such as impulsivity, aggressiveness, rage, increased sex drive and premature erotic urges [283, 284].

The hypothesis that androgens might precipitate TS symptoms prompted researchers to test the effects of androgen receptor antagonists, such as flutamide and cyproterone, in TS patients [285- 287]. Although both compounds were shown to attenuate TS symptoms, two main factors discourage their use in TS: first, their therapeutic effects

seem to be modest and short-lived; second, they induce a number of severe side effects, including liver toxicity, dysmetabolic symptoms, cardiovascular and gastrointestinal disorders and severe sexual dysfunctions [288].

Compelling evidence suggests that TS is underpinned by alterations in dopaminergic signaling. Specifically, while dopamine agonists exacerbate tics, dopaminergic blockade has been previously shown to suppress tic severity [289]. Notably, PPI deficits mediated by dopaminergic agonists are likely the best validated model for the gating impairments in TS [290]. Indeed, TS patients typically report that tics are accompanied by an inner tension that intensifies until the discomfort becomes so great that they feel compelled to enact the tic and are preceded by the perception of intrusive, distressing stimuli in their perceptual domain [264; 291-293]. These gating disturbances termed ''premonitory urges' are reflected in the PPI deficits exhibited by TS patients [241-243; 294].

A recent evidence shows that finasteride exerted significant therapeutic properties in a severe, treatment-refractory case of TS [295]. It was a severe case of male TS patient with explosive vocalizations, stereotyped coprolalic utterances, ritual behaviors, self-injuring motor tics, aggressive and contamination-theme obsessions and excessive sexual drive. Previous therapeutic attempts with typical antipsychotics (pimozide - 4 mg/day - clomipramine - 37.5 mg/day- chlorpromazine - 25 mg/day), had resulted in transient improvements, but the high rate of extrapyramidal and cognitive side effects had led him to repeated withdrawals. Finasteride (5 mg/day) led to a gradual improvement of his motor and vocal tics, as assessed by standardized scales with no reported side effects. The discontinuation of the regimen after 18 weeks, however, resulted in an abrupt, dramatic exacerbation of the symptoms, which was countered by reinstatement of the S5αR inhibitor. Over the last 2 years of follow-up treatment, the patient has shown a stable improvement of his tics, with a marked reduction in severity

of his vocal and motor tics (about 30% and 50% of his pre-treatment scores, respectively). The treatment has not led to any overt sign of toxicity or side effects; while a reduction in sex drive was reported, this effect was described by the patient as "beneficial", in consideration of his excessive libido before initiation of the therapy [295].

In line with these clinical and preclinical premises, we investigate in this report the therapeutic potential effect of finasteride, the prototypical inhibitor of steroid 5-α-reductase (5AR), in a population of unresponsive severe male adult TS patients.

2   AIMS OF THE STUDY

The pharmacological armamentarium for Tourette syndrome (TS) is still extremely limited and unsatisfactory, in view of the suboptimal therapeutic efficacy and the serious side effects of the currently available medications.

We report here the first prospective, open-label study on treatment response, safety and tolerability of finasteride (FIN) in 10 adult males with TS.

The aim of this study was to investigate the efficacy and tolerability of FIN as a treatment for tics in adult males with TS.

3 METHODS

3.1 Subjects.

The trial was a prospective open-label study with 10 male adult TS patients, recruited through the Tourette Syndrome Center at the University of Cagliari, referrals from local professionals and the AST-SIT Association (*Associazione Sindrome di Tourette "Siamo in tanti"*).

Recruitment of subjects occurred according to the following inclusion criteria: (1) Age included between 18–and 43 years at the time of release of informed consent; (2) male gender; (3) full diagnostic criteria for TS as established by the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Text Revision (DSM-IV-TR) (American Psychiatric Association 2000), on examination by a neurologist and/or neuropsychiatrist investigator; (4) previous failure to respond or inability to tolerate antipsychotic or other medications, as determined by the investigators; (5) no changes in TS drug therapy over the last two months prior to the beginning of the trial; (6) significant distress or impairment induced by tics, as reported by patients; (7) Yale Global Severity Scale (YGTSS) [296] score ≥ 50 (mild-moderate) at the beginning of the trial; (8) no significant abnormalities in hematochemical, endocrine, and urinalytical parameters (9) normal intelligence and lack of concomitant neurological disorders; (10) ability to swallow pills.

Subjects were allowed to maintain previous medications, but their doses were left unmodified throughout the study. All patients provided written informed consent for use of FIN. The study protocol was approved by the Institutional Review Board (IRB) of the University of Cagliari.

3.2   Procedures.

All subjects underwent medical, neurological and psychiatric assessment at baseline. All laboratory data (including serum hematology, chemistry evaluation, urinalysis and testosterone and progesterone serum levels) were within normal limits before initiation of treatment. FIN was always administered at 5 mg/day, as the common dosage used for benigne prostatic hyperplasia (BPH) and this regimen was held constant throughout the trial.

TS symptoms and tic severity were assessed and monitored at pretreatment (baseline) and at 2, 6, 12 and 18 weeks of treatment, both with physical and neuropsychiatric examinations and using the Yale Global Tic Severity Scale (YGTSS) [296]. At the baseline and at the end of the trial (18th week of treatment), obsessive-compulsive symptoms were assessed with the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) [297].

For the safety analysis, investigators assessed patient well-being, incidence of adverse events and treatment compliance through an open-ended interview at each visit. An

adverse event was defined as any unexpected and unfavourable change in the patient's health during the study period, whether considered to be related to treatment or not.

3.3   Data analysis.

Data were expressed as means ± SEM (analyzed as absolute values and in relation to the baseline). Normality was tested by Lilliefors' test for each time point. Analyses of the temporal changes in YGTSS scores was performed by Friedman's test for repeated measures, followed by Dunn's post-hoc analyses. Effect size was assessed by Kendall's W coefficient. Y-BOCS scores were evaluated by Wilcoxon pair-matched test, followed by Dunn's multiple comparisons test for post-hoc analysis.

4   RESULTS

4.1   Description of sample.

Sociodemographic characteristics of the study sample are described in Table 3. Psychiatric comorbidity was observed in seven patients that met criteria of DSM-IV TR; four patients met criteria for Obsessive Compulsive Disorder (OCD); two patients for Obsessive Compulsive Personality Disorder (OCP-D) generalized anxiety disorder; one patient for Major Depressive Disorder (MDD).

Four subjects took other psychotropic medications: one subject was under aripiprazole and clordemetildiazepam; one subject was under amitriptyline and clotiapine; one subject was under pimozide; one subject was under sertraline) for their comorbid psychiatric disorders during the study; the dosages of these medications remained stable during the study.

4.2   Dosing, range, and compliance.

Treatment duration was approximately 18 weeks. No dosing titration occurred. In all the sample population, 2 subjects were treated with FIN while taking their previous tic medications (one patient with aripiprazole and another one with pimozide respectively).

The remaining 8 subjects were on no medication and received FIN during the trial. In addition to assuming FIN, the MDD patient assumed amitriptyline 40 mg/day and clotiapine 15 mg/day, instead one patient with comorbid OCD was treated with sertraline 50 mg/day through the three last weeks of the trial (See Table 3).

Patients	Age of TS onset (years)	TS duration (years)	Comorbid disorders axis I (DSM-IV TR)	Previous medications to FIN	Reasons for therapeutic failure of previous medications	Concomitant medications to FIN
1	14 y	22y	None	Olanzapine 5 mg/die; Risperidone 2 mg/die; Tetrabenazine 25 mg/die; Clonazepam 0.6 mg/die.	Drowsiness; muscle pain; restlessness	none
2	15y	10y	None	Haloperidol 2mg/die	No side effects; ineffectiveness	none
3	11y	24y	None	Thioridazine chloridrate 10 mg/die; Haloperidol 2 mg/die	Drowsiness; muscle pain and fatigue	none
4	11y	8y	OCD	Haloperidol 1 mg/die; Risperidone 1 mg/die; Pimozide 8 mg/die	Drowsiness; confusion and decreased concentration; muscle pain ; ineffectiveness	Pimozide 4 mg/die
5	15y	31y	OCD	Lorazepam 1.5 mg/die	Ineffectiveness	none
6	28y	3y	OCD	Alprazolam 1 mg/die	Drowsiness	Sertraline 50 mg/die
7	6y	19y	OCP-D	Diazepam 5 mg/die; Clonazepam 3 mg/die Alprazolam 0.5 mg/die; Paroxetine 10 mg/die	Ineffectiveness	none
8	8y	21y	OCD	Haloperidol 1.5 mg/die; Aripiprazole 15 mg/die; Pimozide 2 mg/die; Sertraline 50 mg/die	Drowsiness; sleep disorders; confusion; akathisia; tremors	Aripiprazole 15 mg/die , Clordemetildiazepam 1.5 mg/die
9	12 y	21y	MDD	Risperidone 4-6 mg/die	Restlessness; muscle tension; increased salivation and appetite; insomnia.	Amitriptyline 40 mg/die; Clotiapine 15 mg/die
10	7 y	14y	OCP-D	Haloperidol 2 mg/die; Risperidone 2.5 mg/die; Olanzapine 10 mg/die; Clordemetildiazepam 0.5 mg/die	Drowsiness; decreased concentration.	none

Table 3. Characteristics of study population (n = 10)

TS: Tourette Syndrome; OCD: Obsessive-Compulsive Disorder. OCP-D: Obsessive Compulsive Personality Disorder. MDD: Major Depressive Disorder. DSM IV-TR: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision; APA 2000.

4.3   Effects of FIN on TS and tic severity.

Preliminary evaluations by Lilliefors' test for YGTSS scores found significant deviations from normality for global severity (at 6 weeks; $p<0.05$) and motor tics (at 12 weeks; $p<0.01$) (data not shown); thus, calculation of the effects of FIN was performed with Friedman's test.

This analysis revealed a significant, time-dependent decline in YGTSS global severity score ($\chi^2(4) = 31.12$, $p<0.0001$). Post-hoc comparisons (performed by Dunn's test) revealed that the severity was significantly reduced at 6, 12 and 18 weeks of treatment (Fig. 9 A). The effect size, estimated by the Kendall's coefficient of concordance, was W=0.78.

A significant, time-dependent reduction was also found in total tic score ($\chi^2(4) = 34.16$, $p<0.0001$). Post-hoc comparisons (performed by Dunn's test) revealed that the severity was significantly reduced at 12 and 18 weeks of treatment in comparison to baseline values (Fig. 9B). Kendall's W coefficient of concordance for these parameters was 0.85.

The analyses of motor and phonic tics confirmed a dramatic reduction in both scores (motor: $\chi^2(4) = 34.16$, $p<0.0001$; phonic: $\chi^2(4) = 34.62$, $p<0.0001$; Friedman's test) (Fig. 9 C-D), with effect sizes of 0.76 and 0.87, respectively (Kendall's W coefficient).
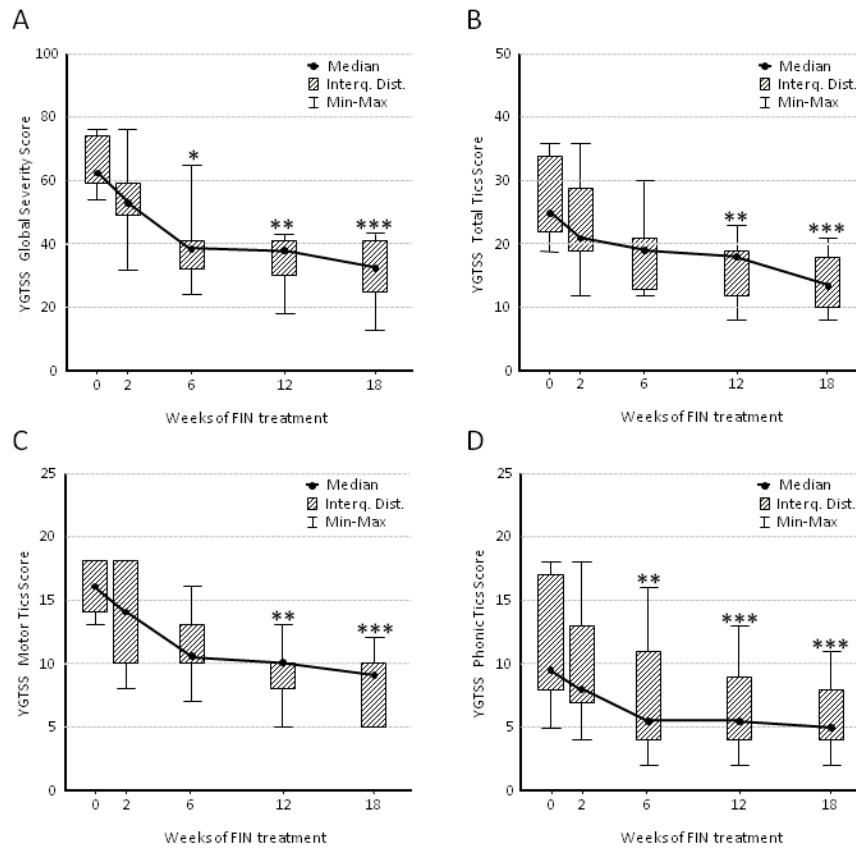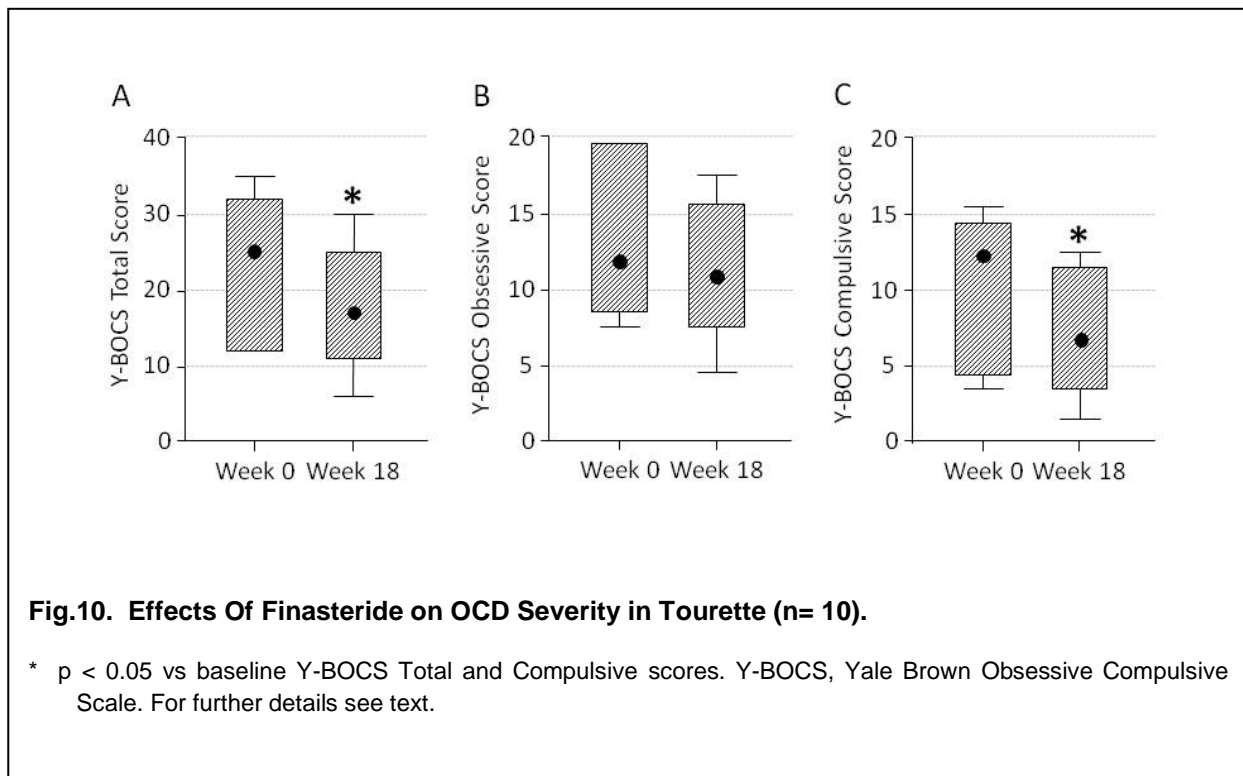
Fig. 9. Effects of finasteride on tic severity and tic in Tourette patients (n= 10).

*** p < 0.0001; ** p < 0.001; * p < 0.05 vs baseline YGTSS scores. YGTSS, Yale Global Tic Severity Scale. For further details see text.

4.4 Effects of FIN on obsessive-compulsive symptoms.

Exploratory analyses were conducted to assess effects of FIN on obsessive and compulsive traits (Y-BOCS) in the 6 patients with these features (Fig. 10). There was a significant reduction in Y-BOCS total scores (Z=2.20 $p<0.05$, *Wilcoxon matched-pairs test; effect size: r=0.90)* and Y-BOCS Compulsion subscale scores (Z=2.20 $p<0.05$, *Wilcoxon matched-pairs test; effect size: r=0.90)* but no significant reduction in Y-BOCS Obsession scores (Z=1.83, NS*, Wilcoxon matched-pairs test; effect size: r=0.75).*

Fig.10. Effects Of Finasteride on OCD Severity in Tourette (n= 10).

* p < 0.05 vs baseline Y-BOCS Total and Compulsive scores. Y-BOCS, Yale Brown Obsessive Compulsive Scale. For further details see text.

4.5   Adverse effects.

The majority of subjects tolerated FIN well; no patients of this sample dropped out before 18 weeks. Most common adverse effects were reported to be mild and included decreased libido in two patients of the sample and occasional difficulty in achieving erection in one of them. No depressive symptoms worsened in MDD patient or severe anxious features occurred in the sample, despite FIN has been recently reported to induce depression in a subset of patients [304].

No significant differences in body weight, vital signs or other clinical and lab parameters were found between baseline and end point.

5.   DISCUSSION

Results of this prospective, open-label, safety and tolerability study suggest the efficacy of FIN (5 mg/day) in reducing global severity, total and motor tics within 6 weeks from treatment initiation in a small sample of male adults with TS who had failed to respond or had been unable to tolerate previous tic treatment. Our exploratory analysis also revealed a mild, yet significantly beneficial effect on compulsive, but not obsessive symptoms.

To the best of our knowledge, this is the first open-label report of therapeutic effects of FIN in TS. The present results confirm and extend our previous finding on the therapeutic effects of FIN in a severe case of TS, refractory to antipsychotic treatment [295]. Of note, the patient described in that original report (not included in the present open label study) is still under FIN treatment and has reported a stable improvement of its symptoms over the last 3 years, with a general improvement of his quality of life and no noticeable adverse effects. This premise suggests that FIN therapy may be a viable long-term therapeutic option for TS male adult patients unresponsive to antipsychotics or other treatments. Furthermore, our present findings complement previous reports documenting the therapeutic potential of 5AR in chronic schizophrenia [248] and perseverative and idiosyncratic personality traits in adult male patients [249; Bortolato et al. submitted].

The enzyme 5AR catalyzes numerous processes, including the conversion of testosterone into the potent psychotropic androgen dihydrotestosterone (DHT), as well as the synthesis of neurosteroids from progesterone [16;17].

The mechanism of FIN-induced reduction in TS symptoms may reflect its effects on metabolism of testosterone, consisting in inhibiting DHT synthesis and favouring the aromatase-mediated conversion of testosterone in β-estradiol. Accordingly, several lines of evidence point to androgens in the pathophysiology and clinical course of TS [see 277- 280]. For example, this disorder affects men more commonly than women [281] and its symptoms are exacerbated by exogenous anabolic androgens [282] TS features a number of symptoms strongly reminiscent of androgen-mediated behaviors, such as impulsivity, aggressiveness, rage and increased sex drive [283].

Previous trials have found that androgen receptor antagonist, such as flutamide and cyproterone, attenuate TS symptoms [285, 286, 287]. However, the therapeutic effects of these compounds were reportedly modest, short-lived, and countered by important side effects, including liver toxicity, dysmetabolic symptoms, cardiovascular and gastrointestinal disorders and sexual dysfunctions [288].

The theoretical framework supporting these trials was based on the idea that TS symptoms may be exacerbated by the direct signaling of testosterone.

Our current results suggest that tic severity may be compounded not by this steroid, but rather by its 5-alpha-reduced androgenic metabolites, such as DHT, which are not susceptible to aromatization. Indeed, FIN may exert protective effects in TS by increasing testosterone's availability for its conversion into β-estradiol. This premise suggests that alterations in the brain-based metabolic fates of testosterone (and/or other androgen precursors, such as androstenedione) may play a role in the pathogenesis of TS. Indeed, previous studies have reported low LH and testosterone in some TS patients [298, 299]. It is possible that an exaggerated conversion of

testosterone into its androgenic (rather than estrogenic) metabolites in the brain may underpin some of the neurochemical imbalances featured in TS. Alternatively, FIN may have elicited its effect by inhibiting the conversion of progesterone in neurosteroids, thereby enhancing its brain levels [300]. Interestingly, progesterone acts as a potent antagonist for σ_1 receptors, which have been shown to modulate dopaminergic signaling [233, 301].

In preclinical studies, FIN and other 5AR inhibitors exerted profound antipsychotic-like effects in animal models of TS [238]. These effects appear to be supported by the prefrontal cortex and ventral striatum [Devoto et al, submitted], two dopaminergic regions that play a central role in the pathophysiology of TS [302] as well as in the behavioural outcomes of dopamine-replacement therapy [258-260, Bortolato et al. submitted] Further studies are warranted to investigate the possible interactions between steroids and dopamine receptors in relation to the psychotropic effects of FIN in TS and related disorders. Preclinical studies are in progress in our laboratory to identify which S5αAR substrate or metabolite is primarily involved in FIN antipsychotic-like action.

Extensive clinical literature shows that FIN is very well tolerated and induces limited side effects [303, 304]. Accordingly, FIN was very well tolerated in our study, and adverse effects were mild and manageable [305].

Despite the neutral side-effect profile of FIN in this and other studies, the generalizability of our findings to other groups of TS patients is obviously limited by the inapplicability of this therapy to children (who represent the greatest target population in this disorder) and pre-menopausal female adults (in view of the teratogenic effects of FIN) [306, 307]. Nevertheless, the identification of the neurobiological underpinnings of the effects of FIN

and other 5AR inhibitors may open to novel, well-tolerated therapeutic options for this disorder.

Some limitations of this study design must be taken into account. All subjects in our sample were treatment-refractory; thus, we cannot rule out that the observed effects may reflect some unique mechanism not observed in the majority of TS patients. Furthermore, the open-label nature of the study and the lack of treatment-blinding in the design pose important limits in the value of these results, in view of the possibility of placebo effects, as well as ascertainment and observer bias. Finally, the presence of concomitant medications implies that some of the observed effects may be related to drug interaction and pharmacodynamic synergisms, rather than to the inherent effects of FIN. Future controlled, comparative studies are warranted to properly evaluate the effectiveness of the therapeutic action of FIN in adult males with TS.

6.   CONCLUSION

The therapeutic effects of finasteride as an adjunctive treatment in TS have been confirmed in a first open-label study with ten adult male TS patients. In these patients, finasteride was found to elicit significant reduction of the severity of tics and associated compulsive (but not obsessive) manifestations within six weeks from initiation of the therapy.

S5αR is the key enzyme in the synthesis of androgens and neurosteroids, and its regulation is fundamental in the management of steroid signaling in the brain. By means of several complementary approaches, research has provided a great deal of knowledge about the function of S5αR isoenzymes and their role in the hormonal balance in periphery. Preliminary information on the role of S5αR in several mental disorders is paving the way to a new series of future investigations which will further our understanding of the role of these enzymes in the pathophysiology of Tourette Syndrome and other mental disorders, and examine the specific role of neurosteroids in the behavioral outcomes of S5αR inhibition. S5αR inhibitors have many desirable characteristics for the pharmacotherapy of mental illnesses: they are clinically approved, have been extensively characterized in patients and are very well-tolerated so might provide a useful complement to the current pharmacological armamentarium of novel agents for some of the most challenging psychiatric disorders, such as Tourette Syndrome, schizophrenia, impulse-control disorders, as well as alcohol- and substance-related disorders. Despite promising clinical results, it is important to stress that efficacy of finasteride for treatment of TS and other neuropsychiatric disorders cannot be evaluated from case reports and observations, but double-blind, placebo-controlled

clinical trials should confirm the actual therapeutic potential of S5αR blockers in these disorders.

REFERENCES

[1] Berseus O. Conversion of cholesterol to bile acids in rat: purification and properties of a delta-4-3-ketosteroid 5-beta-reductase and a 3-alpha-hydroxysteroid dehydrogenase. Eur J Biochem, 1967; 2: 493-502.

[2] Bjorkhem I. Mechamism and stereochemistry of the enzymatic conversion of a delta 4-3-oxosteroid into a 3-oxo-5alpha-steroid. Eur J Biochem, 1969; 8: 345-51.

[3] Wilton DC. Is a Schiff base involved in the mechanism of the delta4-3-oxo steroid 5alpha- or 5beta-reductases from mammalian liver? Biochem J, 1976; 155: 487-91.

[4] Mellon SH, Vaudry H. Biosynthesis of neurosteroids and regulation of their synthesis. Int Rev Neurobiol, 2001; 46: 33-78.

[5] Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. Endocr Rev, 2004; 25: 947-70.

[6] Miller WL. Steroidogenic enzymes. Endocr Dev, 2008; 13: 1-18.

[7] Liao S, Fang S. Receptor-proteims for androgens and the mode of action of androgens on gene transcription in ventral prostate. Vitam Horm, 1969; 27: 17-90.

[8] Gomez EC, Frost P. Testosterone metabolism and androgenic effect. Adv Biol Skin, 1972; 12: 367-79.

[9] Mooradian AD, Morley JE, Korenman SG. Biological actions of androgens. Endocr Rev, 1987; 8: 1-28.

[10] Weisser H, Tunn S, Behnke B, Krieg M. Effects of the sabal serrulata extract IDS 89 and its subfractions on 5 alpha-reductase activity in human benign prostatic hyperplasia. Prostate, 1996; 28: 300-6.

[11] Weinstein BI, Kandalaft N, Ritch R, Camras CB, Morris DJ, Latif SA, Vecsei P, Vittek J, Gordon GG, Southren AL. 5 alpha-dihydrocortisol in human aqueous humor and metabolism of cortisol by human lenses in vitro. Invest Ophthalmol Vis Sci, 1991; 32: 2130-5.

[12] Morris DJ, Souness GW, Saccoccio NA, Harnik M. The effects of infusions of ring-A-reduced derivatives of aldosterone on the antinatriuretic and kaliuretic actions of aldosterone. Steroids, 1989; 53: 21-36.

[13] Bjorkhem I, Karlmar KE. Biosynthesis of cholestanol: conversion of cholesterol into 4-cholesten-3-one by rat liver microsomes. Biochim Biophys Acta, 1974; 337: 129-31.

[14] Seyama Y. Cholestanol metabolism, molecular pathology, and nutritional implications. J Med Food, 2003; 6: 217-24.

[15] McInnes KJ, Kenyon CJ, Chapman KE, Livingstone DE, Macdonald LJ, Walker BR, Andrew R. 5alpha-reduced glucocorticoids, novel endogenous activators of the glucocorticoid receptor. J Biol Chem, 2004; 279: 22908-12.

[16] Barbaccia ML, Affricano D, Purdy RH, Maciocco E, Spiga F, Biggio G. Clozapine, but not haloperidol, increases brain concentrations of neuroactive steroids in the rat. Neuropsychopharmacology, 2001; 25: 489-97.

[17] Girdler SS, Klatzkin R. Neurosteroids in the context of stress: implications for depressive disorders. Pharmacol Ther, 2007; 116: 125-39.

[18] Samuels AJ, Hecht HH, Tyler F, Carlisle R. Leukocyte changes following intra-muscular injection of epinephrine and epinephrine congeners, with observations on the alterations induced by adrenergic blocking agents. Am J Med, 1950; 8: 533-4.

[19] Schneider JJ. Conversion of desoxycorticosterone to four allopregnane metabolites by rat liver in vitro. J Biol Chem, 1952; 199: 235-44.

[20] Schneider JJ. Conversion of desoxycorticosterone to four allopregnane metabolites by rat liver in vitro. J Biol Chem, 1952; 199: 235-44.

[21] Dorfman RI, Forchielli E. Separation of delta 4-5 alpha-hydrogenases from rat liver homogenates. J Biol Chem, 1956; 223: 443-8.

[22] McGuire JS, Hollis VW, Tomkins GM. Some Characteristics of the Microsomal Steroid Reductases (5α) of Rat Liver. Journal of Biological Chemistry, 1960; 235: 3112-3117.

[23] Shefer S, Milch S, Mosbach EH. Biosynthesis of 5-Alpha-Cholestan-3-Beta-Ol in Rat and Guinea Pig Liver in Vitro. J Lipid Res, 1965; 6: 33-6.

[24] Karavolas HJ, Herf SM. Conversion of progesterone by rat medial basal hypothalamic tissue to 5 -pregnane-3,20-dione. Endocrinology, 1971; 89: 940-2.

[25] Martini L, Zoppi S, Motta M. Studies on the possible existence of two 5 alpha-reductases in the rat prostate. J Steroid Biochem, 1986; 24: 177-82.

[26] Russell DW, Wilson JD. Steroid 5 alpha-reductase: two genes/two enzymes. Annu Rev Biochem, 1994; 63: 25-61.

[27] Andersson S, Bishop RW, Russell DW. Expression cloning and regulation of steroid 5 alpha-reductase, an enzyme essential for male sexual differentiation. J Biol Chem, 1989; 264: 16249-55.

[28] Andersson S, Berman DM, Jenkins EP, Russell DW. Deletion of steroid 5 alpha-reductase 2 gene in male pseudohermaphroditism. Nature, 1991; 354: 159-61.

[29] Andersson S, Russell DW. Structural and biochemical properties of cloned and expressed human and rat steroid 5 alpha-reductases. Proc Natl Acad Sci U S A, 1990; 87: 3640-4.

[30] Jenkins EP, Andersson S, Imperato-McGinley J, Wilson JD, Russell DW. Genetic and pharmacological evidence for more than one human steroid 5 alpha-reductase. J Clin Invest, 1992; 89: 293-300.

[31] Kidd JM, Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T, Hansen N, Teague B, Alkan C, Antonacci F, Haugen E, Zerr T, Yamada NA, Tsang P, Newman TL, Tuzun E, Cheng Z, Ebling HM, Tusneem N, David R, Gillett W, Phelps KA, Weaver M, Saranga D, Brand A, Tao W, Gustafson E, McKernan K, Chen L, Malig M, Smith JD, Korn JM, McCarroll SA, Altshuler DA, Peiffer DA, Dorschner M, Stamatoyannopoulos J, Schwartz D, Nickerson DA, Mullikin JC, Wilson RK, Bruhn L, Olson MV, Kaul R, Smith DR, Eichler EE. Mapping and sequencing of structural variation from eight human genomes. Nature, 2008; 453: 56-64.

[32] Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, Boutell JM, Bryant J, Carter RJ, Keira Cheetham R, Cox AJ, Ellis DJ, Flatbush MR, Gormley NA, Humphray SJ, Irving LJ, Karbelashvili MS, Kirk SM, Li H, Liu X, Maisinger KS, Murray LJ, Obradovic B, Ost T, Parkinson ML, Pratt MR, Rasolonjatovo IM, Reed MT, Rigatti R, Rodighiero C, Ross MT, Sabot A, Sankar SV, Scally A, Schroth GP, Smith ME, Smith VP, Spiridou A, Torrance PE, Tzonev SS, Vermaas EH, Walter K, Wu X, Zhang L, Alam MD, Anastasi C, Aniebo IC, Bailey DM, Bancarz IR, Banerjee S, Barbour SG, Baybayan PA, Benoit VA, Benson KF, Bevis C, Black PJ, Boodhun A, Brennan JS, Bridgham JA, Brown RC, Brown AA, Buermann DH, Bundu AA, Burrows JC, Carter NP, Castillo N, Chiara ECM, Chang S, Neil Cooley R, Crake NR, Dada OO, Diakoumakos KD, Dominguez-Fernandez B, Earnshaw DJ, Egbujor UC, Elmore DW, Etchin SS, Ewan MR, Fedurco M, Fraser LJ, Fuentes Fajardo KV, Scott Furey W, George D, Gietzen KJ, Goddard CP, Golda GS, Granieri PA, Green DE, Gustafson DL, Hansen NF, Harnish K, Haudenschild CD, Heyer NI, Hims MM, Ho JT, Horgan AM, Hoschler K, Hurwitz S, Ivanov DV, Johnson MQ, James T, Huw Jones TA, Kang GD, Kerelska TH, Kersey AD, Khrebtukova I, Kindwall AP, Kingsbury Z, Kokko-Gonzales PI, Kumar A, Laurent MA, Lawley CT, Lee SE, Lee X, Liao AK, Loch JA, Lok M, Luo S, Mammen RM, Martin JW, McCauley PG, McNitt P, Mehta P, Moon KW, Mullens JW, Newington T, Ning Z, Ling Ng B, Novo SM, O'Neill MJ, Osborne MA, Osnowski A, Ostadan O, Paraschos LL, Pickering L, Pike AC, Chris Pinkard D, Pliskin DP, Podhasky J, Quijano VJ, Raczy C, Rae VH, Rawlings SR, Chiva Rodriguez A, Roe PM, Rogers J, Rogert Bacigalupo MC, Romanov N, Romieu A, Roth RK, Rourke NJ, Ruediger ST, Rusman E, Sanches-Kuiper RM, Schenker MR, Seoane JM, Shaw RJ, Shiver MK, Short SW, Sizto NL, Sluis JP, Smith MA, Ernest Sohna Sohna J, Spence EJ, Stevens K, Sutton N, Szajkowski L, Tregidgo CL, Turcatti G, Vandevondele S, Verhovsky Y, Virk SM, Wakelin S, Walcott GC, Wang J, Worsley GJ, Yan J, Yau L, Zuerlein M, Mullikin JC, Hurles ME, McCooke NJ, West JS, Oaks FL, Lundberg PL, Klenerman D, Durbin R, Smith AJ. Accurate whole human genome sequencing using reversible terminator chemistry. Nature, 2008; 456: 53-9.

[33] Betton JM. High throughput cloning and expression strategies for protein production. Biochimie, 2004; 86: 601-5.

[34] Sievert V, Ergin A, Bussow K. High throughput cloning with restriction enzymes. Methods Mol Biol, 2008; 426: 163-73.

[35] Mirkovic N, Li Z, Parnassa A, Murray D. Strategies for high-throughput comparative modeling: applications to leverage analysis in structural genomics and protein family organization. Proteins, 2007; 66: 766-77.

[36] Lee H, Li Z, Silkov A, Fischer M, Petrey D, Honig B, Murray D. High-throughput computational structure-based characterization of protein families: START domains and implications for structural genomics. J Struct Funct Genomics, 2010; 11: 51-9.

[37] Cantagrel V, Lefeber DJ, Ng BG, Guan Z, Silhavy JL, Bielas SL, Lehle L, Hombauer H, Adamowicz M, Swiezewska E, De Brouwer AP, Blümel 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 Jul 23;142(2):203-17.

[38] Stiles AR, Russell DW. SRD5A3: A surprising role in glycosylation. Cell, 2010; 142: 196-8.

[39] Langlois VS, Zhang D, Cooke GM, Trudeau VL. Evolution of steroid-5alpha-reductases and comparison of their function with 5beta-reductase. Gen Comp Endocrinol, 2010; 166: 489-97.

[40] Berman DM, Russell DW. Cell-type-specific expression of rat steroid 5 alpha-reductase isozymes. Proc Natl Acad Sci U S A, 1993; 90: 9359-63.

[41] Mahendroo MS, Cala KM, Russell DW. 5 alpha-reduced androgens play a key role in murine parturition. Mol Endocrinol, 1996; 10: 380-92.

[42] Normington K, Russell DW. Tissue distribution and kinetic characteristics of rat steroid 5 alpha-reductase isozymes. Evidence for distinct physiological functions. J Biol Chem, 1992; 267: 19548-54.

[43] Levy MA, Brandt M, Sheedy KM, Dinh JT, Holt DA, Garrison LM, Bergsma DJ, Metcalf BW. Epristeride is a selective and specific uncompetitive inhibitor of human steroid 5 alpha-reductase isoform 2. J Steroid Biochem Mol Biol, 1994; 48: 197-206.

[44] Thigpen AE, Russell DW. Four-amino acid segment in steroid 5 alpha-reductase 1 confers sensitivity to finasteride, a competitive inhibitor. J Biol Chem, 1992; 267: 8577-83.

[45] Baxter FO, Trivic S, Lee IR. Structure-function studies of human 5-alpha reductase type 2 using site directed mutagenesis. J Steroid Biochem Mol Biol, 2001; 77: 167-75.

[46] Savory JG, May D, Reich T, La Casse EC, Lakins J, Tenniswood M, Raymond Y, Hache RJ, Sikorska M, Lefebvre YA. 5 alpha-Reductase type 1 is localized to the outer nuclear membrane. Mol Cell Endocrinol, 1995; 110: 137-47.

[47] Span PN, Sweep CG, Benraad TJ, Smals A. Differential subcellular distribution of rat prostatic steroid 5alpha-reductase isozyme activities. Eur J Endocrinol, 1996; 134: 386-92.

[48] Tsuruo Y, Miyamoto T, Yokoi H, Kitagawa K, Futaki S, Ishimura K. Immunohistochemical presence of 5 alpha-reductase rat type 1-containing cells in the rat brain. Brain Res, 1996; 722: 207-11.

[49] Chen W, Zouboulis CC, Fritsch M, Blume-Peytavi U, Kodelja V, Goerdt S, Luu-The V, Orfanos CE. Evidence of heterogeneity and quantitative differences of the type 1 5alpha-reductase expression in cultured human skin cells--evidence of its presence in melanocytes. J Invest Dermatol, 1998; 110: 84-9.

[50] Yokoi H, Tsuruo Y, Ishimura K. Steroid 5alpha-reductase type 1 immunolocalized in the rat peripheral nervous system and paraganglia. Histochem J, 1998; 30: 731-9.

[51] Yokoi H, Tsuruo Y, Miyamoto T, Ishimura K. Steroid 5 alpha-reductase type 1 immunolocalized in the adrenal gland of normal, gonadectomized, and sex hormone-supplemented rats. Histochem Cell Biol, 1998; 109: 127-34.

[52] Ellis JA, Panagiotopoulos S, Akdeniz A, Jerums G, Harrap SB. Androgenic correlates of genetic variation in the gene encoding 5alpha-reductase type 1. J Hum Genet, 2005; 50: 534-7.

[53] 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.

[54] Habib FK, Ross M, Bayne CW, Grigor K, Buck AC, Bollina P, Chapman K. The localisation and expression of 5 alpha-reductase types I and II mRNAs in human hyperplastic prostate and in prostate primary cultures. J Endocrinol, 1998; 156: 509-17.

[55] Titus MA, Gregory CW, Ford OH, 3rd, Schell MJ, Maygarden SJ, Mohler JL. Steroid 5alpha-reductase isozymes I and II in recurrent prostate cancer. Clin Cancer Res, 2005; 11: 4365-71.

[56] Lephart ED. Brain 5alpha-reductase: cellular, enzymatic, and molecular perspectives and implications for biological function. Mol Cell Neurosci, 1993; 4: 473-84.

[57] Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression. J Clin Invest, 1993; 92: 903-10.

[58] Melcangi RC, Poletti A, Cavarretta I, Celotti F, Colciago A, Magnaghi V, Motta M, Negri-Cesi P, Martini L. The 5alpha-reductase in the central nervous system: expression and modes of control. J Steroid Biochem Mol Biol, 1998; 65: 295-9.

[59] Steckelbroeck S, Watzka M, Reichelt R, Hans VH, Stoffel-Wagner B, Heidrich DD, Schramm J, Bidlingmaier F, Klingmuller D. Characterization of the 5alpha-reductase-3alpha-hydroxysteroid dehydrogenase complex in the human brain. J Clin Endocrinol Metab, 2001; 86: 1324-31.

[60] Stoffel-Wagner B. Neurosteroid biosynthesis in the human brain and its clinical implications. Ann N Y Acad Sci, 2003; 1007: 64-78.

[61] Torres JM, Ortega E. Differential regulation of steroid 5alpha-reductase isozymes expression by androgens in the adult rat brain. FASEB J, 2003; 17: 1428-33.

[62] Melcangi RC, Celotti F, Castano P, Martini L. Differential localization of the 5 alpha-reductase and the 3 alpha-hydroxysteroid dehydrogenase in neuronal and glial cultures. Endocrinology, 1993; 132: 1252-9.

[63] Martini L, Celotti F, Melcangi RC. Testosterone and progesterone metabolism in the central nervous system: cellular localization and mechanism of control of the enzymes involved. Cell Mol Neurobiol, 1996; 16: 271-82.

[64] Negri-Cesi P, Poletti A, Celotti F. Metabolism of steroids in the brain: a new insight into the role of 5alpha-reductase and aromatase in brain differentiation and functions. J Steroid Biochem Mol Biol, 1996; 58: 455-66.

[65] 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: 14602-7.

[66] Pelletier G, Luu-The V, Labrie F. Immunocytochemical localization of 5 alpha-reductase in rat brain. Mol Cell Neurosci, 1994; 5: 394-9.

[67] Kiyokage E, Toida K, Suzuki-Yamamoto T, Ishimura K. Localization of 5alpha-reductase in the rat main olfactory bulb. J Comp Neurol, 2005; 493: 381-95.

[68] Stoffel-Wagner B, Beyenburg S, Watzka M, Blumcke I, Bauer J, Schramm J, Bidlingmaier F, Elger CE. Expression of 5alpha-reductase and 3alpha-hydroxisteroid oxidoreductase in the hippocampus of patients with chronic temporal lobe epilepsy. Epilepsia, 2000; 41: 140-7.

[69] Blanchard Y, Seenundun S, Robaire B. The promoter of the rat 5alpha-reductase type 1 gene is bidirectional and Sp1-dependent. Mol Cell Endocrinol, 2007; 264: 171-83.

[70] Her S, Arimochi H, Morita K. Nerve growth factor induces elevation of steroid 5alpha-reductase mRNA levels in rat C6 glioma cells through expression of transcription factor Egr-1. Brain Res Mol Brain Res, 2004; 126: 157-64.

[71] Morita K, Arimochi H, Tsuruo Y. Adrenergic activation of steroid 5alpha-reductase gene expression in rat C6 glioma cells: involvement of cyclic amp/protein kinase A-mediated signaling pathway. J Mol Neurosci, 2004; 22: 205-12.

[72] Morita K, Arimochi H, Her S. Serotonergic 5-HT2A receptor stimulation induces steroid 5alpha-reductase gene expression in rat C6 glioma cells via transcription factor Egr-1. Brain Res Mol Brain Res, 2005; 139: 193-200.

[73] Her S, Lee MS, Morita K. Trichostatin A stimulates steroid 5alpha-reductase gene expression in rat C6 glioma cells via a mechanism involving Sp1 and Sp3 transcription factors. J Mol Neurosci, 2010; 41: 252-62.

[74] George FW, Russell DW, Wilson JD. Feed-forward control of prostate growth: dihydrotestosterone induces expression of its own biosynthetic enzyme, steroid 5 alpha-reductase. Proc Natl Acad Sci U S A, 1991; 88: 8044-7.

[75] Zhou Z, Speiser PW. Regulation of HSD17B1 and SRD5A1 in lymphocytes. Mol Genet Metab, 1999; 68: 410-7.

[76] Signorelli SS, Barresi V, Musso N, Anzaldi M, Croce E, Fiore V, Condorelli DF. Polymorphisms of steroid 5-alpha-reductase type I (SRD5A1) gene are associated to peripheral arterial disease. J Endocrinol Invest, 2008; 31: 1092-7.

[77] Goodarzi MO, Shah NA, Antoine HJ, Pall M, Guo X, Azziz R. Variants in the 5alpha-reductase type 1 and type 2 genes are associated with polycystic ovary syndrome and the severity of hirsutism in affected women. J Clin Endocrinol Metab, 2006; 91: 4085-91.

[78] Mahendroo MS, Cala KM, Landrum DP, Russell DW. Fetal death in mice lacking 5alpha-reductase type 1 caused by estrogen excess. Mol Endocrinol, 1997; 11: 917-27.

[79] Mahendroo MS, Porter A, Russell DW, Word RA. The parturition defect in steroid 5alpha-reductase type 1 knockout mice is due to impaired cervical ripening. Mol Endocrinol, 1999; 13: 981-92.

[80] Purdy RH, Morrow AL, Moore PH, Jr., Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci U S A, 1991; 88: 4553-7.

[81] Barbaccia ML, Roscetti G, Trabucchi M, Cuccheddu T, Concas A, Biggio G. Neurosteroids in the brain of handling-habituated and naive rats: effect of CO_2 inhalation. Eur J Pharmacol, 1994; 261: 317-20.

[82] Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, Biggio G. Time-dependent changes in rat brain neuroactive steroid concentrations and GABAA receptor function after acute stress. Neuroendocrinology, 1996; 63: 166-72.

[83] Torres JM, Ruiz E, Ortega E. Effects of CRH and ACTH administration on plasma and brain neurosteroid levels. Neurochem Res, 2001; 26: 555-8.

[84] Morrow AL, Devaud LL, Purdy RH, Paul SM. Neuroactive steroid modulators of the stress response. Ann N Y Acad Sci, 1995; 771: 257-72.

[85] Sanchez P, Torres JM, Olmo A, O'Valle F, Ortega E. Effects of environmental stress on mRNA and protein expression levels of steroid 5alpha-Reductase isozymes in adult rat brain. Horm Behav, 2009; 56: 348-53.

[86] Sanchez MG, Bourque M, Morissette M, Di Paolo T. Steroids-dopamine interactions in the pathophysiology and treatment of CNS disorders. CNS Neurosci Ther, 2010; 16: e43-71.

[87] Morita K, Kuwada A, Fujihara H, Morita Y, Sei H. Influence of sleep disturbance on steroid 5alpha-reductase mRNA levels in rat brain. Neuroscience, 2002; 115: 341-8.

[88] Ordyan NE, Pivina SG. Anxiety levels and neurosteroid synthesis in the brains of prenatally stressed male rats. Neurosci Behav Physiol, 2003; 33: 899-903.

[89] Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, Watanabe H, Costa E, Guidotti A. Brain 5alpha-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. Proc Natl Acad Sci U S A, 2001; 98: 2849-54.

[90] Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5alpha-dihydroprogesterone in psychiatric disorders. Brain Res Brain Res Rev, 2001; 37: 110-5.

[91] Bortolato M DP, Roncada P, Frau R, Flore G, Saba P, Pistritto G, Soggiu A, Pisanu S, Zappala' A, Tattoli M, Cuomo V, Marrosu F, Barbaccia ML. Isolation rearing results in decreased 5-α-reductase expression: relevance to dopaminergic impairments. Neuropharmacology, In Press.

[92] Biggio G, Concas A, Follesa P, Sanna E, Serra M. Stress, ethanol, and neuroactive steroids. Pharmacol Ther, 2007; 116: 140-71.

[93] Frye CA, Walf AA. Hippocampal 3alpha,5alpha-THP may alter depressive behavior of pregnant and lactating rats. Pharmacol Biochem Behav, 2004; 78: 531-40.

[94] Labrie F, Sugimoto Y, Luu-The V, Simard J, Lachance Y, Bachvarov D, Leblanc G, Durocher F, Paquet N. Structure of human type II 5 alpha-reductase gene. Endocrinology, 1992; 131: 1571-3.

[95] Wilson JD, Griffin JE, Russell DW. Steroid 5 alpha-reductase 2 deficiency. Endocr Rev, 1993; 14: 577-93.

[96] Celotti F, Negri-Cesi P, Poletti A. Steroid metabolism in the mammalian brain: 5alpha-reduction and aromatization. Brain Res Bull, 1997; 44: 365-75.

[97] Poletti A, Negri-Cesi P, Rabuffetti M, Colciago A, Celotti F, Martini L. Transient expression of the 5alpha-reductase type 2 isozyme in the rat brain in late fetal and early postnatal life. Endocrinology, 1998; 139: 2171-8.

[98] Sanchez P, Torres JM, Del Moral RG, Ortega E. Effects of testosterone on brain mRNA levels of steroid 5alpha-reductase isozymes in early postnatal life of rat. Neurochem Int, 2006; 49: 626-30.

[99] Poletti A, Coscarella A, Negri-Cesi P, Colciago A, Celotti F, Martini L. 5 alpha-reductase isozymes in the central nervous system. Steroids, 1998; 63: 246-51.

[100] 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: 426-31.

[101] Imperato-McGinley J, Zhu YS. Androgens and male physiology the syndrome of 5alpha-reductase-2 deficiency. Mol Cell Endocrinol, 2002; 198: 51-9.

[102] Mahendroo MS, Cala KM, Hess DL, Russell DW. Unexpected virilization in male mice lacking steroid 5 alpha-reductase enzymes. Endocrinology, 2001; 142: 4652-62.

[103] Wang C, Tao W, Chen Q, Hu H, Wen XY, Han R. SRD5A2 V89L polymorphism and prostate cancer risk: a meta-analysis. Prostate, 2010; 70: 170-8.

[104] Li J, Coates RJ, Gwinn M, Khoury MJ. Steroid 5-{alpha}-reductase Type 2 (SRD5a2) gene polymorphisms and risk of prostate cancer: a HuGE review. Am J Epidemiol, 2010; 171: 1-13.

[105] Nagle CM, Chenevix-Trench G, Webb PM, Spurdle AB. Ovarian cancer survival and polymorphisms in hormone and DNA repair pathway genes. Cancer Lett, 2007; 251: 96-104.

[106] Beesley J, Jordan SJ, Spurdle AB, Song H, Ramus SJ, Kjaer SK, Hogdall E, DiCioccio RA, McGuire V, Whittemore AS, Gayther SA, Pharoah PD, Webb PM, Chenevix-Trench G. Association between single-nucleotide polymorphisms in hormone metabolism and DNA repair genes and epithelial ovarian cancer: results from two Australian studies and an additional validation set. Cancer Epidemiol Biomarkers Prev, 2007; 16: 2557-65.

[107] Scorilas A, Bharaj B, Giai M, Diamandis EP. Codon 89 polymorphism in the human 5 alpha-reductase gene in primary breast cancer. Br J Cancer, 2001; 84: 760-7.

[108] Elzanaty S, Giwercman YL, Giwercman A. Significant impact of 5alpha-reductase type 2 polymorphisms on sperm concentration and motility. Int J Androl, 2006; 29: 414-20.

[109] Thai HT, Kalbasi M, Lagerstedt K, Frisen L, Kockum I, Nordenskjold A. The valine allele of the V89L polymorphism in the 5-alpha-reductase gene confers a reduced risk for hypospadias. J Clin Endocrinol Metab, 2005; 90: 6695-8.

[110] Makridakis NM, Ross RK, Pike MC, Crocitto LE, Kolonel LN, Pearce CL, Henderson BE, Reichardt JK. Association of mis-sense substitution in SRD5A2 gene with prostate cancer in African-American and Hispanic men in Los Angeles, USA. Lancet, 1999; 354: 975-8.

[111] Loukola A, Chadha M, Penn SG, Rank D, Conti DV, Thompson D, Cicek M, Love B, Bivolarevic V, Yang Q, Jiang Y, Hanzel DK, Dains K, Paris PL, Casey G, Witte JS. Comprehensive evaluation of the association between prostate cancer and genotypes/haplotypes in CYP17A1, CYP3A4, and SRD5A2. Eur J Hum Genet, 2004; 12: 321-32.

[112] Hayes VM, Severi G, Padilla EJ, Morris HA, Tilley WD, Southey MC, English DR, Sutherland RL, Hopper JL, Boyle P, Giles GG. 5alpha-Reductase type 2 gene variant associations with prostate cancer risk, circulating hormone levels and androgenetic alopecia. Int J Cancer, 2007; 120: 776-80.

[113] Schmidtova E, Kelemenova S, Celec P, Ficek A, Ostatnikova D. Polymorphisms in Genes Involved in Testosterone Metabolism in Slovak Autistic Boys. The Endocrinologist, 2010; 20: 245-249 10.1097/TEN.0b013e3181f661d2.

[114] Tamura K, Furihata M, Tsunoda T, Ashida S, Takata R, Obara W, Yoshioka H, Daigo Y, Nasu Y, Kumon H, Konaka H, Namiki M, Tozawa K, Kohri K, Tanji N, Yokoyama M, Shimazui T, Akaza H, Mizutani Y, Miki T, Fujioka T, Shuin T, Nakamura Y, Nakagawa H. Molecular features of hormone-refractory prostate cancer cells by genome-wide gene expression profiles. Cancer Res, 2007; 67: 5117-25.

[115] Uemura M, Tamura K, Chung S, Honma S, Okuyama A, Nakamura Y, Nakagawa H. Novel 5 alpha-steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. Cancer Sci, 2008; 99: 81-6.

[116] 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: 203-17.

[117] Al-Gazali L, Hertecant J, Algawi K, El Teraifi H, Dattani M. A new autosomal recessive syndrome of ocular colobomas, ichthyosis, brain malformations and endocrine abnormalities in an inbred Emirati family. Am J Med Genet A, 2008; 146: 813-9.

[118] Moon YA, Horton JD. Identification of two mammalian reductases involved in the two-carbon fatty acyl elongation cascade. J Biol Chem, 2003; 278: 7335-43.

[119] Bennett MJ, Hale DE. Medium chain acyl-coenzyme A dehydrogenase deficiency. N J Med, 1992; 89: 675-8.

[120] Pollitt RJ. Disorders of mitochondrial long-chain fatty acid oxidation. J Inherit Metab Dis, 1995; 18: 473-90.

[121] Rakheja D, Bennett MJ, Rogers BB. Long-chain L-3-hydroxyacyl-coenzyme a dehydrogenase deficiency: a molecular and biochemical review. Lab Invest, 2002; 82: 815-24.

[122] Jethva R, Bennett MJ, Vockley J. Short-chain acyl-coenzyme A dehydrogenase deficiency. Mol Genet Metab, 2008; 95: 195-200.

[123] Brooks JR, Baptista EM, Berman C, Ham EA, Hichens M, Johnston DB, Primka RL, Rasmusson GH, Reynolds GF, Schmitt SM, Arth GE. Response of rat ventral prostate to a new and novel 5 alpha-reductase inhibitor. Endocrinology, 1981; 109: 830-6.

[124] Brandt M, Levy MA. 3 Beta-hydroxy-delta 5-steroid dehydrogenase/3-keto-delta 5-steroid isomerase from bovine adrenals: mechanism of inhibition by 3-oxo-4-aza steroids and kinetic mechanism of the dehydrogenase. Biochemistry, 1989; 28: 140-8.

[125] Rasmusson GH, Reynolds GF, Utne T, Jobson RB, Primka RL, Berman C, Brooks JR. Azasteroids as inhibitors of rat prostatic 5 alpha-reductase. J Med Chem, 1984; 27: 1690-701.

[126] Rasmusson GH, Reynolds GF, Steinberg NG, Walton E, Patel GF, Liang T, Cascieri MA, Cheung AH, Brooks JR, Berman C. Azasteroids: structure-activity relationships for inhibition of 5 alpha-reductase and of androgen receptor binding. J Med Chem, 1986; 29: 2298-315.

[127] Bull HG, Garcia-Calvo M, Andersson S, Baginsky WF, Chan HK, Ellsworth DE, Miller RR, Stearns RA, Bakshi RK, Rasmusson GH, Tolman RL, Myers RW, Kozarich JW, Harris GS. Mechanism-Based Inhibition of Human Steroid 5α-Reductase by Finasteride: Enzyme-Catalyzed Formation of NADP−Dihydrofinasteride, a Potent Bisubstrate Analog Inhibitor. Journal of the American Chemical Society, 1996; 118: 2359-2365.

[128] Bramson HN, Hermann D, Batchelor KW, Lee FW, James MK, Frye SV. Unique preclinical characteristics of GG745, a potent dual inhibitor of 5AR. J Pharmacol Exp Ther, 1997; 282: 1496-502.

[129] Frye SV, Bramson HN, Hermann DJ, Lee FW, Sinhababu AK, Tian G. Discovery and development of GG745, a potent inhibitor of both isozymes of 5 alpha-reductase. Pharm Biotechnol, 1998; 11: 393-422.

[130] Roehrborn CG, Boyle P, Nickel JC, Hoefner K, Andriole G. Efficacy and safety of a dual inhibitor of 5-alpha-reductase types 1 and 2 (dutasteride) in men with benign prostatic hyperplasia. Urology, 2002; 60: 434-41.

[131] Debruyne F, Barkin J, van Erps P, Reis M, Tammela TL, Roehrborn C. Efficacy and safety of long-term treatment with the dual 5 alpha-reductase inhibitor dutasteride in men with symptomatic benign prostatic hyperplasia. Eur Urol, 2004; 46: 488-94; discussion 495.

[132] Bakshi RK, Patel GF, Rasmusson GH, Baginsky WF, Cimis G, Ellsworth K, Chang B, Bull H, Tolman RL, Harris GS. 4,7 beta-Dimethyl-4-azacholestan-3-one (MK-386) and related 4-azasteroids as selective inhibitors of human type 1 5 alpha-reductase. J Med Chem, 1994; 37: 3871-4.

[133] Ellsworth K, Azzolina B, Baginsky W, Bull H, Chang B, Cimis G, Mitra S, Toney J, Bakshi RK, Rasmusson GR, Tolman RL, Harris GS. MK386: a potent, selective inhibitor of the human type 1 5alpha-reductase. J Steroid Biochem Mol Biol, 1996; 58: 377-84.

[134] Nakayama O, Hirosumi J, Chida N, Takahashi S, Sawada K, Kojo H, Notsu Y. FR146687, a novel steroid 5 alpha-reductase inhibitor: in vitro and in vivo effects on prostates. Prostate, 1997; 31: 241-9.

[135] Sawada K, Okada S, Golden P, Kayakiri N, Sawada Y, Hashimoto M, Tanaka H. 4-(1-benzoylindol-3-yl)butyric acids and FK143: novel nonsteroidal inhibitors of steroid 5 alpha-reductase (II). Chem Pharm Bull (Tokyo), 1999; 47: 481-91.

[136] Aggarwal S, Thareja S, Verma A, Bhardwaj TR, Kumar M. An overview on 5alpha-reductase inhibitors. Steroids, 2010; 75: 109-53

[137] Liang T, Liao S. Inhibition of steroid 5 alpha-reductase by specific aliphatic unsaturated fatty acids. Biochem J, 1992; 285 (Pt 2): 557-62.

[138] Iehle C, Delos S, Guirou O, Tate R, Raynaud JP, Martin PM. Human prostatic steroid 5 alpha-reductase isoforms--a comparative study of selective inhibitors. J Steroid Biochem Mol Biol, 1995; 54: 273-9.

[139] Nickel JC, Fradet Y, Boake RC, Pommerville PJ, Perreault JP, Afridi SK, Elhilali MM. Efficacy and safety of finasteride therapy for benign prostatic hyperplasia: results of a 2-year randomized controlled trial (the PROSPECT study). PROscar Safety Plus Efficacy Canadian Two year Study. CMAJ, 1996; 155: 1251-9.

[140] Castello R, Tosi F, Perrone F, Negri C, Muggeo M, Moghetti P. Outcome of long-term treatment with the 5 alpha-reductase inhibitor finasteride in idiopathic hirsutism: clinical and hormonal effects during a 1-year course of therapy and 1-year follow-up. Fertil Steril, 1996; 66: 734-40.

[141] Rittmaster RS. 5alpha-reductase inhibitors in benign prostatic hyperplasia and prostate cancer risk reduction. Best Pract Res Clin Endocrinol Metab, 2008; 22: 389-402.

[142] Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA, Jr. The influence of finasteride on the development of prostate cancer. N Engl J Med, 2003; 349: 215-24.

[143] Klotz L, Saad F. PCPT, MTOPS and the use of 5ARIs: a Canadian consensus regarding implications for clinical practice. Can Urol Assoc J, 2007; 1: 17-21.

[144] Gravas S, Oelke M. Current status of 5alpha-reductase inhibitors in the management of lower urinary tract symptoms and BPH. World J Urol, 2010; 28: 9-15.

[145] Xu Y, Dalrymple SL, Becker RE, Denmeade SR, Isaacs JT. Pharmacologic basis for the enhanced efficacy of dutasteride against prostatic cancers. Clin Cancer Res, 2006; 12: 4072-9.

[146] Andriole G, Bostwick D, Brawley O, Gomella L, Marberger M, Tindall D, Breed S, Somerville M, Rittmaster R. Chemoprevention of prostate cancer in men at high risk:

rationale and design of the reduction by dutasteride of prostate cancer events (REDUCE) trial. J Urol, 2004; 172: 1314-7.

[147] Bergfeld WF. Androgenetic alopecia: an autosomal dominant disorder. Am J Med, 1995; 98: 95S-98S.

[148] Price VH, Roberts JL, Hordinsky M, Olsen EA, Savin R, Bergfeld W, Fiedler V, Lucky A, Whiting DA, Pappas F, Culbertson J, Kotey P, Meehan A, Waldstreicher J. Lack of efficacy of finasteride in postmenopausal women with androgenetic alopecia. J Am Acad Dermatol, 2000; 43: 768-76.

[149] 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: 578-89.

[150] Serafini P, Lobo RA. The effects of spironolactone on adrenal steroidogenesis in hirsute women. Fertil Steril, 1985; 44: 595-9.

[151] Moghetti P, Castello R, Magnani CM, Tosi F, Negri C, Armanini D, Bellotti G, Muggeo M. Clinical and hormonal effects of the 5 alpha-reductase inhibitor finasteride in idiopathic hirsutism. J Clin Endocrinol Metab, 1994; 79: 1115-21.

[152] Moghetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F, Caputo M, Muggeo M, Castello R. Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. J Clin Endocrinol Metab, 2000; 85: 89-94.

[153] Stoner E. The clinical development of a 5 alpha-reductase inhibitor, finasteride. J Steroid Biochem Mol Biol, 1990; 37: 375-8.

[154] De Schepper PJ, Imperato-McGinley J, Van Hecken A, De Lepeleire I, Buntinx A, Carlin J, Gressi MH, Stoner E. Hormonal effects, tolerability, and preliminary kinetics in men of MK-906, a 5 alpha-reductase inhibitor. Steroids, 1991; 56: 469-71.

[155] McConnell JD, Bruskewitz R, Walsh P, Andriole G, Lieber M, Holtgrewe HL, Albertsen P, Roehrborn CG, Nickel JC, Wang DZ, Taylor AM, Waldstreicher J. The effect of finasteride on the risk of acute urinary retention and the need for surgical treatment among men with benign prostatic hyperplasia. Finasteride Long-Term Efficacy and Safety Study Group. N Engl J Med, 1998; 338: 557-63.

[156] McConnell JD, Roehrborn CG, Bautista OM, Andriole GL, Jr., Dixon CM, Kusek JW, Lepor H, McVary KT, Nyberg LM, Jr., Clarke HS, Crawford ED, Diokno A, Foley JP, Foster HE, Jacobs SC, Kaplan SA, Kreder KJ, Lieber MM, Lucia MS, Miller GJ, Menon M, Milam DF, Ramsdell JW, Schenkman NS, Slawin KM, Smith JA. The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. N Engl J Med, 2003; 349: 2387-98.

[157] Lowe FC, McConnell JD, Hudson PB, Romas NA, Boake R, Lieber M, Elhilali M, Geller J, Imperto-McGinely J, Andriole GL, Bruskewitz RC, Walsh PC, Bartsch G, Nacey JN, Shah S, Pappas F, Ko A, Cook T, Stoner E, Waldstreicher J. Long-term 6-year experience with finasteride in patients with benign prostatic hyperplasia. Urology, 2003; 61: 791-6.

[158] Makridakis N, Reichardt JK. Pharmacogenetic analysis of human steroid 5 alpha reductase type II: comparison of finasteride and dutasteride. J Mol Endocrinol, 2005; 34: 617-23.

[159] Ramot Y, Czarnowicki T, Zlotogorski A. Finasteride induced Gynecomastia: Case report and Review of the Literature. Int J Trichology, 2009; 1: 27-9.

[160] Wilton L, Pearce G, Edet E, Freemantle S, Stephens MD, Mann RD. The safety of finasteride used in benign prostatic hypertrophy: a non-interventional observational cohort study in 14,772 patients. Br J Urol, 1996; 78: 379-84.

[161] Green L, Wysowski DK, Fourcroy JL. Gynecomastia and breast cancer during finasteride therapy. N Engl J Med, 1996; 335: 823.

[162] Ferrando J, Grimalt R, Alsina M, Bulla F, Manasievska E. Unilateral gynecomastia induced by treatment with 1 mg of oral finasteride. Arch Dermatol, 2002; 138: 543-4.

[163] Moreno-Fernandez A, Mira Laguarda JM, Ruiz-Hornillos FJ, Rubio Sotes M. Urticarial rush due to finasteride. Allergy, 2010; 65: 405-6.

[164] Kappas A, Bradlow HL, Bickers DR, Alvares AP. Induction of a deficiency of steroid delta 4-5 alpha-reductase activity in liver by a porphyrinogenic drug. J Clin Invest, 1977; 59: 159-64.

[165] Andriole GL, Kirby R. Safety and tolerability of the dual 5alpha-reductase inhibitor dutasteride in the treatment of benign prostatic hyperplasia. Eur Urol, 2003; 44: 82-8.

[166] Schulman C, Pommerville P, Hofner K, Wachs B. Long-term therapy with the dual 5alpha-reductase inhibitor dutasteride is well tolerated in men with symptomatic benign prostatic hyperplasia. BJU Int, 2006; 97: 73-9; discussion 79-80.

[167] Hudak SJ, Hernandez J, Thompson IM. Role of 5 alpha-reductase inhibitors in the management of prostate cancer. Clin Interv Aging, 2006; 1: 425-31.

[168] Mostallino MC, Sanna E, Concas A, Biggio G, Follesa P. Plasticity and function of extrasynaptic GABA(A) receptors during pregnancy and after delivery. Psychoneuroendocrinology, 2009; 34 Suppl 1: S74-83.

[169] de Sousa FL, Lazzari V, de Azevedo MS, de Almeida S, Sanvitto GL, Lucion AB, Giovenardi M. Progesterone and maternal aggressive behavior in rats. Behav Brain Res, 2010; 212: 84-9.

[170] Molina-Hernandez M, Tellez-Alcantara NP, Olivera-Lopez JI, Jaramillo MT. Olanzapine plus 17-beta estradiol produce antidepressant-like actions in rats forced to swim. Pharmacol Biochem Behav, 2009; 93: 491-7.

[171] Crawley JN, Glowa JR, Majewska MD, Paul SM. Anxiolytic activity of an endogenous adrenal steroid. Brain Res, 1986; 398: 382-5.

[172] Zimmerberg B, Brunelli SA, Hofer MA. Reduction of rat pup ultrasonic vocalizations by the neuroactive steroid allopregnanolone. Pharmacol Biochem Behav, 1994; 47: 735-8.

[173] Zimmerberg B, McDonald BC. Prenatal alcohol exposure influences the effects of neuroactive steroids on separation-induced ultrasonic vocalizations in rat pups. Pharmacol Biochem Behav, 1996; 55: 541-7.

[174] Pibiri F, Nelson M, Guidotti A, Costa E, Pinna G. Decreased corticolimbic allopregnanolone expression during social isolation enhances contextual fear: A model relevant for posttraumatic stress disorder. Proc Natl Acad Sci U S A, 2008; 105: 5567-72.

[175] Agis-Balboa RC, Pinna G, Pibiri F, Kadriu B, Costa E, Guidotti A. Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. Proc Natl Acad Sci U S A, 2007; 104: 18736-41.

[176] Agis-Balboa RC, Pibiri F, Nelson M, Pinna G. Enhanced fear responses in mice treated with anabolic androgenic steroids. Neuroreport, 2009; 20: 617-21.

[177] Pinna G. In a mouse model relevant for post-traumatic stress disorder, selective brain steroidogenic stimulants (SBSS) improve behavioral deficits by normalizing allopregnanolone biosynthesis. Behav Pharmacol, 2010; 21: 438-50.

[178] Frye CA, Walf AA. Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Horm Behav, 2002; 41: 306-15.

[179] Mann PE. Finasteride delays the onset of maternal behavior in primigravid rats. Physiol Behav, 2006; 88: 333-8.

[180] Walf AA, Sumida K, Frye CA. Inhibiting 5alpha-reductase in the amygdala attenuates antianxiety and antidepressive behavior of naturally receptive and hormone-primed ovariectomized rats. Psychopharmacology (Berl), 2006; 186: 302-11.

[181] Martin-Garcia E, Darbra S, Pallares M. Neonatal finasteride induces anxiogenic-like profile and deteriorates passive avoidance in adulthood after intrahippocampal neurosteroid administration. Neuroscience, 2008; 154: 1497-505.

[182] Reddy DS. Pharmacology of endogenous neuroactive steroids. Crit Rev Neurobiol, 2003; 15: 197-234.

[183] Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. J Neurosci, 2002; 22: 3795-805.

[184] Frye CA, Koonce CJ, Edinger KL, Osborne DM, Walf AA. Androgens with activity at estrogen receptor beta have anxiolytic and cognitive-enhancing effects in male rats and mice. Horm Behav, 2008; 54: 726-34.

[185] Frye CA, Edinger KL, Lephart ED, Walf AA. 3alpha-androstanediol, but not testosterone, attenuates age-related decrements in cognitive, anxiety, and depressive behavior of male rats. Front Aging Neurosci, 2010; 2: 15.

[186] Azzolina B, Ellsworth K, Andersson S, Geissler W, Bull HG, Harris GS. Inhibition of rat alpha-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. J Steroid Biochem Mol Biol, 1997; 61: 55-64.

[187] Girman CJ, Kolman C, Liss CL, Bolognese JA, Binkowitz BS, Stoner E. Effects of finasteride on health-related quality of life in men with symptomatic benign prostatic hyperplasia. Finasteride Study Group. Prostate, 1996; 29: 83-90.

[188] Altomare G, Capella GL. Depression circumstantially related to the administration of finasteride for androgenetic alopecia. J Dermatol, 2002; 29: 665-9.

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

[190] Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, Guidotti A. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci U S A, 1998; 95: 3239-44.

[191] van Broekhoven F, Verkes RJ. Neurosteroids in depression: a review. Psychopharmacology (Berl), 2003; 165: 97-110.

[192] Strohle A, Romeo E, Hermann B, Pasini A, Spalletta G, di Michele F, Holsboer F, Rupprecht R. Concentrations of 3 alpha-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. Biol Psychiatry, 1999; 45: 274-7.

[193] Griffin LD, Mellon SH. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. Proc Natl Acad Sci U S A, 1999; 96: 13512-7.

[194] Romeo E, Strohle A, Spalletta G, di Michele F, Hermann B, Holsboer F, Pasini A, Rupprecht R. Effects of antidepressant treatment on neuroactive steroids in major depression. Am J Psychiatry, 1998; 155: 910-3.

[195] Uzunova V, Sampson L, Uzunov DP. Relevance of endogenous 3alpha-reduced neurosteroids to depression and antidepressant action. Psychopharmacology (Berl), 2006; 186: 351-61.

[196] Eser D, Romeo E, Baghai TC, di Michele F, Schule C, Pasini A, Zwanzger P, Padberg F, Rupprecht R. Neuroactive steroids as modulators of depression and anxiety. Neuroscience, 2006; 138: 1041-8.

[197] Rupprecht R. Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. Psychoneuroendocrinology, 2003; 28: 139-68.

[198] Baghai TC, di Michele F, Schule C, Eser D, Zwanzger P, Pasini A, Romeo E, Rupprecht R. Plasma concentrations of neuroactive steroids before and after electroconvulsive therapy in major depression. Neuropsychopharmacology, 2005; 30: 1181-6.

[199] Padberg F, di Michele F, Zwanzger P, Romeo E, Bernardi G, Schule C, Baghai TC, Ella R, Pasini A, Rupprecht R. Plasma concentrations of neuroactive steroids before and after repetitive transcranial magnetic stimulation (rTMS) in major depression. Neuropsychopharmacology, 2002; 27: 874-8.

[200] Eser D, Schule C, Baghai TC, Romeo E, Rupprecht R. Neuroactive steroids in depression and anxiety disorders: clinical studies. Neuroendocrinology, 2006; 84: 244-54.

[201] Hafner H. Gender differences in schizophrenia. Psychoneuroendocrinology, 2003; 28 Suppl 2: 17-54.

[202] Halari R, Kumari V, Mehrotra R, Wheeler M, Hines M, Sharma T. The relationship of sex hormones and cortisol with cognitive functioning in Schizophrenia. J Psychopharmacol, 2004; 18: 366-74.

[203] Elias A, Kumar A. Testosterone for schizophrenia. Cochrane Database Syst Rev, 2007: CD006197.

[204] Andreasen NC, Swayze VW, 2nd, Flaum M, Yates WR, Arndt S, McChesney C. Ventricular enlargement in schizophrenia evaluated with computed tomographic scanning. Effects of gender, age, and stage of illness. Arch Gen Psychiatry, 1990; 47: 1008-15.

[205] Tamminga CA. Gender and schizophrenia. J Clin Psychiatry, 1997; 58 Suppl 15: 33-7.

[206] Gur RE, Turetsky BI, Cowell PE, Finkelman C, Maany V, Grossman RI, Arnold SE, Bilker WB, Gur RC. Temporolimbic volume reductions in schizophrenia. Arch Gen Psychiatry, 2000; 57: 769-75.

[207] Goldstein JM, Cohen LS, Horton NJ, Lee H, Andersen S, Tohen M, Crawford A, Tollefson G. Sex differences in clinical response to olanzapine compared with haloperidol. Psychiatry Res, 2002; 110: 27-37.

[208] Seeman MV. Gender differences in the prescribing of antipsychotic drugs. Am J Psychiatry, 2004; 161: 1324-33.

[209] Kendell RE, Chalmers JC, Platz C. Epidemiology of puerperal psychoses. Br J Psychiatry, 1987; 150: 662-73.

[210] Goyal RO, Sagar R, Ammini AC, Khurana ML, Alias AG. Negative correlation between negative symptoms of schizophrenia and testosterone levels. Ann N Y Acad Sci, 2004; 1032: 291-4.

[211] Ko YH, Jung SW, Joe SH, Lee CH, Jung HG, Jung IK, Kim SH, Lee MS. Association between serum testosterone levels and the severity of negative symptoms in male patients with chronic schizophrenia. Psychoneuroendocrinology, 2007; 32: 385-91.

[212] Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull. 1987;13(2):261-76.

[213] Ko YH, Lew YM, Jung SW, Joe SH, Lee CH, Jung HG, Lee MS. Short-term testosterone augmentation in male schizophrenics: a randomized, double-blind, placebo-controlled trial. J Clin Psychopharmacol, 2008; 28: 375-83.

[214] Di Michele F, Caltagirone C, Bonaviri G, Romeo E, Spalletta G. Plasma. Dehydroepiandrosterone levels are strongly increased in schizophrenia. J Psychiatr Res, 2005; 39: 267-73.

[215] Ritsner MS, Strous RD. Neurocognitive deficits in schizophrenia are associated with alterations in blood levels of neurosteroids: a multiple regression analysis of findings from a double-blind, randomized, placebo-controlled, crossover trial with DHEA. J Psychiatr Res, 2010; 44: 75-80.

[216] Silver H, Knoll G, Isakov V, Goodman C, Finkelstein Y. Blood DHEAS concentrations correlate with cognitive function in chronic schizophrenia patients: a pilot study. J Psychiatr Res, 2005; 39: 569-75.

[217] Ritsner M, Gibel A, Maayan R, Ratner Y, Ram E, Biadsy H, Modai I, Weizman A. Cortisol/dehydroepiandrosterone ratio and responses to antipsychotic treatment in schizophrenia. Neuropsychopharmacology, 2005; 30: 1913-22.

[218] Nachshoni T, Ebert T, Abramovitch Y, Assael-Amir M, Kotler M, Maayan R, Weizman A, Strous RD. Improvement of extrapyramidal symptoms following dehydroepiandrosterone (DHEA) administration in antipsychotic treated schizophrenia patients: a randomized, double-blind placebo controlled trial. Schizophr Res, 2005; 79: 251-6.

[219] Strous RD, Stryjer R, Maayan R, Gal G, Viglin D, Katz E, Eisner D, Weizman A. Analysis of clinical symptomatology, extrapyramidal symptoms and neurocognitive dysfunction following dehydroepiandrosterone (DHEA) administration in olanzapine treated schizophrenia patients: a randomized, double-blind placebo controlled trial. Psychoneuroendocrinology, 2007; 32: 96-105.

[220] Akwa Y, Ladurelle N, Covey DF, Baulieu EE. The synthetic enantiomer of pregnenolone sulfate is very active on memory in rats and mice, even more so than its physiological neurosteroid counterpart: distinct mechanisms? Proc Natl Acad Sci U S A, 2001; 98: 14033-7.

[221] Darnaudery M, Pallares M, Piazza PV, Le Moal M, Mayo W. The neurosteroid pregnenolone sulfate infused into the medial septum nucleus increases hippocampal acetylcholine and spatial memory in rats. Brain Res, 2002; 951: 237-42.

[222] Flood JF, Morley JE, Roberts E. Pregnenolone sulfate enhances post-training memory processes when injected in very low doses into limbic system structures: the amygdala is by far the most sensitive. Proc Natl Acad Sci U S A, 1995; 92: 10806-10.

[223] Ladurelle N, Eychenne B, Denton D, Blair-West J, Schumacher M, Robel P, Baulieu E. Prolonged intracerebroventricular infusion of neurosteroids affects cognitive performances in the mouse. Brain Res, 2000; 858: 371-9.

[224] Mayo W, Dellu F, Robel P, Cherkaoui J, Le Moal M, Baulieu EE, Simon H. Infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processes in the rat. Brain Res, 1993; 607: 324-8.

[225] Pallares M, Darnaudery M, Day J, Le Moal M, Mayo W. The neurosteroid pregnenolone sulfate infused into the nucleus basalis increases both acetylcholine release in the frontal cortex or amygdala and spatial memory. Neuroscience, 1998; 87: 551-8.

[226] Vallee M, Mayo W, Darnaudery M, Corpechot C, Young J, Koehl M, Le Moal M, Baulieu EE, Robel P, Simon H. Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. Proc Natl Acad Sci U S A, 1997; 94: 14865-70.

[227] Vallee M, Mayo W, Koob GF, Le Moal M. Neurosteroids in learning and memory processes. Int Rev Neurobiol, 2001; 46: 273-320.

[228] Ritsner MS. Pregnenolone, dehydroepiandrosterone, and schizophrenia: alterations and clinical trials. CNS Neurosci Ther, 2010; 16: 32-44.

[229] Marx CE, Stevens RD, Shampine LJ, Uzunova V, Trost WT, Butterfield MI, Massing MW, Hamer RM, Morrow AL, Lieberman JA. Neuroactive steroids are altered in schizophrenia and bipolar disorder: relevance to pathophysiology and therapeutics. Neuropsychopharmacology, 2006; 31: 1249-63.

[230] Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Front Neuroendocrinol, 2009; 30: 65-91.

[231] van Winkel R, Stefanis NC, Myin-Germeys I. Psychosocial stress and psychosis. A review of the neurobiological mechanisms and the evidence for gene-stress interaction. Schizophr Bull, 2008; 34: 1095-105.

[232] Barbaccia ML, Roscetti G, Trabucchi M, Purdy RH, Mostallino MC, Concas A, Biggio G. The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations. Br J Pharmacol, 1997; 120: 1582-8.

[233] Monnet FP, Maurice T. The sigma1 protein as a target for the non-genomic effects of neuro(active)steroids: molecular, physiological, and behavioral aspects. J Pharmacol Sci, 2006; 100: 93-118.

[234] Sedlacek M, Korinek M, Petrovic M, Cais O, Adamusova E, Chodounska H, Vyklicky L, Jr. Neurosteroid modulation of ionotropic glutamate receptors and excitatory synaptic transmission. Physiol Res, 2008; 57 Suppl 3: S49-57.

[235] Stevens JR. Schizophrenia: reproductive hormones and the brain. Am J Psychiatry, 2002; 159: 713-9.

[236] Grigoriadis S, Seeman MV. The role of estrogen in schizophrenia: implications for schizophrenia practice guidelines for women. Can J Psychiatry, 2002; 47: 437-42.

[237] Rao ML, Kolsch H. Effects of estrogen on brain development and neuroprotection--implications for negative symptoms in schizophrenia. Psychoneuroendocrinology, 2003; 28 Suppl 2: 83-96.

[238] Bortolato M, Frau R, Orru M, Bourov Y, Marrosu F, Mereu G, Devoto P, Gessa GL. Antipsychotic-like properties of 5-alpha-reductase inhibitors. Neuropsychopharmacology, 2008; 33: 3146-56.

[239] Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl), 2001; 156: 234-58.

[240] Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl), 2001; 156: 117-54.

[241] Perry W, Minassian A, Feifel D, Braff DL. Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. Biol Psychiatry, 2001; 50: 418-24.

[242] Castellanos FX, Giedd JN, Hamburger SD, Marsh WL, Rapoport JL. Brain morphometry in Tourette's syndrome: the influence of comorbid attention-deficit/hyperactivity disorder. Neurology, 1996; 47: 1581-3.

[243] Swerdlow NR, Karban B, Ploum Y, Sharp R, Geyer MA, Eastvold A. Tactile prepuff inhibition of startle in children with Tourette's syndrome: in search of an "fMRI-friendly" startle paradigm. Biol Psychiatry, 2001; 50: 578-85.

[244] Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. Endocr Rev, 2002; 23: 175-200.

[245] Rhodes ME, Frye CA. Inhibiting progesterone metabolism in the hippocampus of rats in behavioral estrus decreases anxiolytic behaviors and enhances exploratory and antinociceptive behaviors. Cogn Affect Behav Neurosci, 2001; 1: 287-96.

[246] Molina-Hernandez M, Tellez-Alcantara NP, Garcia JP, Lopez JI, Jaramillo MT. Antidepressant-like actions of intra-accumbens infusions of allopregnanolone in ovariectomized Wistar rats. Pharmacol Biochem Behav, 2005; 80: 401-9.

[247] Engin E, Treit D. The anxiolytic-like effects of allopregnanolone vary as a function of intracerebral microinfusion site: the amygdala, medial prefrontal cortex, or hippocampus. Behav Pharmacol, 2007; 18: 461-70.

[248] Koethe D, Bortolato M, Piomelli D, Leweke FM. Improvement of general symptoms in a chronic psychotic patient treated with finasteride: case report. Pharmacopsychiatry, 2008; 41: 115-6.

[249] Bortolato M, Cannas A, Solla P, Puligheddu M, Muroni A, Marrosu F. Treatment of benign essential blepharospasm with finasteride: a case report. Clin Neuropharmacol, 2010; 33: 207-8.

[250] Mauriello JA, Jr., Dhillon S, Leone T, Pakeman B, Mostafavi R, Yepez MC. Treatment selections of 239 patients with blepharospasm and Meige syndrome over 11 years. Br J Ophthalmol, 1996; 80: 1073-6.

[251] Napolitano A, Bonuccelli U, Rossi B. Different effects of levodopa and apomorphine on blink reflex recovery cycle in essential blepharospasm. Eur Neurol, 1997; 38: 119-22.

[252] Bhatia MS, Gupta R. Essential blepharospasm responding to haloperidol. J Neuropsychiatry Clin Neurosci, 2006; 18: 554-6.

[253] Weiner WJ, Nausieda PA, Glantz RH. Meige syndrome (blepharospasm-oromandibular dystonia) after long-term neuroleptic therapy. Neurology, 1981; 31: 1555-6.

[254] Fahn S. Systemic therapy of dystonia. Can J Neurol Sci, 1987; 14: 528-32.

[255] Ananth J, Edelmuth E, Dargan B. Meige's syndrome associated with neuroleptic treatment. Am J Psychiatry, 1988; 145: 513-5.

[256] Maisto SA, Connors GJ, Tucker JA, McCollam JB, Adesso VJ. Validation of the Sensation Scale, a measure of subjective physiological responses to alcohol. Behav Res Ther, 1980; 18: 37-43.

[257] Giovannoni G, O'Sullivan JD, Turner K, Manson AJ, Lees AJ. Hedonistic homeostatic dysregulation in patients with Parkinson's disease on dopamine replacement therapies. J Neurol Neurosurg Psychiatry, 2000; 68: 423-8.

[258] Voon V, Hassan K, Zurowski M, de Souza M, Thomsen T, Fox S, Lang AE, Miyasaki J. Prevalence of repetitive and reward-seeking behaviors in Parkinson disease. Neurology, 2006; 67: 1254-7.

[259] Reuter J, Raedler T, Rose M, Hand I, Glascher J, Buchel C. Pathological gambling is linked to reduced activation of the mesolimbic reward system. Nat Neurosci, 2005; 8: 147-8.

[260] Evans AH, Katzenschlager R, Paviour D, O'Sullivan JD, Appel S, Lawrence AD, Lees AJ. Punding in Parkinson's disease: its relation to the dopamine dysregulation syndrome. Mov Disord, 2004; 19: 397-405.

[261] American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DMS-IV-TR). Washington (DC): American Psychiatric Association, 2000.

[262] Robertson MM: Tourette syndrome, associated conditions and the complexities of treatment. Brain 2000; 123:425–462.

[263] Sandor P: Pharmacological management of tics in patients with TS. Journal of Psychosomatic research 55 (2003) 41-48

[264] Leckman JF, Peterson BS. The pathogenesis of Tourette's syndrome: epigenetic factors active in early CNS development. Biol Psychiatry, 1993; 34: 425-7.

[265] Lombroso PJ, Scahill L. Tourette syndrome and obsessive-compulsive disorder. Brain Dev. 2008 Apr;30(4):231-7.

[266] Leckman JF, Bloch MH, Scahill L, King RA. Tourette syndrome: the self under siege. J Child Neurol. 2006 Aug;21(8):642-9.

[267] Jankovic J. Phenomenology and classification of tics, in Neurologic Clinics. Edited by Jankovic J. Philadelphia, WH Saunders Company, 1997, pp 267-275.

[268] Robertson MM, Stern JS. Gilles de la Tourette syndrome: symptomatic treatment based on evidence. Eur Child Adolesc Psychiatry. 2000;9 Suppl 1:I60-75.

[269] Albin RL, Mink JW. Recent advances in Tourette syndrome researchTrends Neurosci. 2006 Mar;29(3):175-82.

[270] Coffey BJ, Biederman J, Spencer T, Geller DA, Faraone SV, Bellordre CA: Informativeness of structured diagnostic interviews in the identification of Tourette's disorder in referred youth. J Nerv Ment Dis 188:583–588, 2000.

[271] Scahill L, Erenberg G, Berlin CM Jr, Budman C, Coffey BJ, Jankovic J, Kiessling L, King RA, Kurlan R, Lang A, Mink J, Murphy T, Zinner S, Walkup J; Tourette Syndrome Association Medical Advisory Board: Practice Committee. Contemporary assessment and pharmacotherapy of Tourette syndrome. NeuroRx. 2006 Apr;3(2):192-206.

[272] Shapiro AK, Shapiro E, Wayne HL: Treatment of Gilles de la Tourette's syndrome with haloperidol: Review of 34 cases. Arch Gen Psychiatry 28:702–723, 1973.

[273] Pringsheim T, Marras C. Pimozide for tics in Tourette's syndrome. Cochrane Database Syst Rev. 2009 Apr 15;(2):CD006996.

[274] Green WH: Child and Adolescent Clinical Psychopharmacology. Philadelphia: Lippincott Williams & Wilkins, 2001.

[275] Swerdlow NR, Sutherland AN. Preclinical models relevant to Tourette syndrome. Adv Neurol, 2006; 99: 69-88.

[276] Carucci S., Paba S., Di Martino A., Zuddas A. Neuropsychiatric disorders of the basal ganglia: the neurobiology of Tourette Syndrome, obsessive-compulsive disorder, and attention deficit hyperactivity disorder. In. D. Riva and C. Njiokiktjien. Mariani Foundation Pediatric Neurology Series: Localization of brain lesions and developmental functions. 2010.John Libbey Eurotext, chapter 4, 1-17.

[277] Peterson BS, Leckman JF, Scahill L, Naftolin F, Keefe D, Charest NJ, Cohen DJ. Steroid hormones and CNS sexual dimorphisms modulate symptom expression in Tourette's syndrome. Psychoneuroendocrinology. 1992 Nov;17(6):553-63.

[278] Bryden MP, McManus IC, Bulman-Fleming MB. Evaluating the empirical support for the Geschwind-Behan-Galaburda model of cerebral lateralization. Brain Cogn. 1994 Nov;26(2):103-67.

[279] Velísková J, Moshé SL. Sexual dimorphism and developmental regulation of substantia nigra function. Ann Neurol. 2001 Nov; 50(5):596-601.

[280] Alexander GM, Peterson BS. (2004). Testing the prenatal hormone hypothesis of tic-related disorders: gender identity and gender role behavior. Dev Psychopathol. 16(2):407-20.

[281] Pauls DL, Pakstis AJ, Kurlan R, Kidd KK, Leckman JF, Cohen DJ, Kidd JR, Como P, Sparkes R. Segregation and linkage analyses of Tourette's syndrome and related disorders. J Am Acad Child Adolesc Psychiatry, 1990; 29: 195-203.

[282] Leckman JF, Scahill L. Possible exacerbation of tics by androgenic steroids. N Engl J Med, 1990; 322: 1674.

[283] Budman SH, Demby A, Redondo JP, Hannan M, Feldstein M, Ring J, Springer T. Comparative outcome in time-limited individual and group psychotherapy. Int J Group Psychother, 1988; 38: 63-86.

[284] Comings DE, Comings BG. A controlled study of Tourette syndrome. IV. Obsessions, compulsions, and schizoid behaviors. Am J Hum Genet, 1987; 41: 782-803.

[285] Peterson BS, Leckman JF, Scahill L, Naftolin F, Keefe D, Charest NJ, King RA, Hardin MT, Cohen DJ. Steroid hormones and Tourette's syndrome: early experience with antiandrogen therapy. J Clin Psychopharmacol, 1994; 14: 131-5.

[286] Peterson BS, Zhang H, Anderson GM, Leckman JF. A double-blind, placebo-controlled, crossover trial of an antiandrogen in the treatment of Tourette's syndrome. J Clin Psychopharmacol, 1998; 18: 324-31.

[287] Izmir M, Dursun SM. Cyproterone acetate treatment of Tourette's syndrome. Can J Psychiatry, 1999; 44: 710-1.

[288] Migliari R, Muscas G, Murru M, Verdacchi T, De Benedetto G, De Angelis M. Antiandrogens: a summary review of pharmacodynamic properties and tolerability in prostate cancer therapy. Arch Ital Urol Androl, 1999; 71: 293-302.

[289] Sandor P. Pharmacological management of tics in patients with TS. J Psychosom Res, 2003; 55: 41-8.

[290] Swerdlow NR, Sutherland AN. Preclinical models relevant to Tourette syndrome. Adv Neurol, 2006; 99: 69-88.

[291] Cohen AJ, Leckman JF. Sensory phenomena associated with Gilles de la Tourette's syndrome. J Clin Psychiatry, 1992; 53: 319-23.

[292] Miguel EC, Baer L, Coffey BJ, Rauch SL, Savage CR, O'Sullivan RL, Phillips K, Moretti C, Leckman JF, Jenike MA. Phenomenological differences appearing with repetitive behaviours in obsessive-compulsive disorder and Gilles de la Tourette's syndrome. Br J Psychiatry, 1997; 170: 140-5.

[293] Miguel EC, do Rosario-Campos MC, Prado HS, do Valle R, Rauch SL, Coffey BJ, Baer L, Savage CR, O'Sullivan RL, Jenike MA, Leckman JF. Sensory phenomena in obsessive-compulsive disorder and Tourette's disorder. J Clin Psychiatry, 2000; 61: 150-6; quiz 157.

[294] Steinberg T, Shmuel Baruch S, Harush A, Dar R, Woods D, Piacentini J, Apter A. Tic disorders and the premonitory urge. J Neural Transm. 2010 Feb;117(2):277-84.

[295] Bortolato M, Muroni A, Marrosu F. Treatment of Tourette's syndrome with finasteride. Am J Psychiatry, 2007; 164: 1914-5.

[296] Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, Cohen DJ. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. J Am Acad Child Adolesc Psychiatry, 1989; 28: 566-73.

[297] Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, et al. (1989) The Yale-Brown Obsessive Compulsive Scale. I. Development, use, and reliability. Arch Gen Psychiatry 46: 1006–1011.

[298] Sandyk R. A case of Tourette's syndrome with midbrain involvement. Int J Neurosci, 1988; 43: 171-5.

[299] Sandyk R, Bamford CR, Binkiewicz A, Finley PR. Gonadotropin deficiency in Tourette's syndrome: a preliminary communication. Int J Neurosci, 1988; 42: 121-5.

[300] Paba S, Frau R, Godar SC, Devoto P, Marrosu F, Bortolato M. (2011) Steroid 5α-reductase as a novel therapeutic target for schizophrenia and other neuropsychiatric disorders. Curr Pharmaceutical Design, Special Issue: "Novel Approaches for Potential Therapeutic Drugs in Schizophrenia". In press.

[301] Maurice T. Neurosteroids and sigma1 receptors, biochemical and behavioral relevance. Pharmacopsychiatry. 2004 Nov;37 Suppl 3:S171-82.

[302] Cohen DJ, Leckman JF. Tourette's syndrome. JAMA. 1991 Apr 3;265(13):1738

[303] Wilde MI, Goa KL. Finasteride: an update of its use in the management of symptomatic benign prostatic hyperplasia. Drugs. 1999 Apr; 57(4):557-81.

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

[305] Smith AB, Carson CC. Finasteride in the treatment of patients with benign prostatic hyperplasia: a review. Ther Clin Risk Manag. 2009 Jun;5(3):535-45.

[306] Stout SM, Stumpf JL. Finasteride treatment of hair loss in women. Ann Pharmacother. 2010 Jun;44(6):1090-7

[307] Townsend KA, Marlowe KF. Relative safety and efficacy of finasteride for treatment of hirsutism. Ann Pharmacother. 2004 Jun;38(6):1070-3.