Androgens
Clinical Research and Therapeutics

Mary Ann Liebert, Inc. publishers

ANDROGENS AND THE NERVOUS SYSTEM: REVIEW   Open Access

Synthesis and Actions of 5α-Reduced Metabolites of Testosterone in the Nervous System

Roberto Cosimo Melcangi,[1,*,i] Lucia Cioffi,[1] Silvia Diviccaro,[1] and Abdulmaged M. Traish[2,3,ii]

Abstract

Neuroactive testosterone metabolites synthesized by enzymes 5α-reductase and 3α/3β-hydroxysteroid oxidoreductase are critical physiological modulators of the central nervous system (CNS) function. Indeed, as discussed in this review, they exert a variety of effects on myelination, brain maturation, neurotransmission, reproductive behavior, and the stress responses. The expression and synthesis of these enzymes as well as the levels of testosterone derivatives exhibit specific localized regional and sex differences in the CNS and are influenced under various physiological and neuropathological conditions. In addition, neuroactive metabolites of testosterone exert a variety of neuroprotective effects in several neuropathological animal models. Therefore, neuroactive testosterone metabolites might represent an interesting potential therapeutic option to treat some neurological disorders.

Keywords: neuroactive steroids; neurodegenerative disorders; psychiatric disorders; androgen; sex difference

Introduction

Testosterone (T) and its various metabolites play an essential role in physiology and pathophysiology of the central nervous system (CNS). For example, it is well established that androgens play a seminal role in brain development and neurogenesis and in sexual differentiation.[1] Furthermore, androgens exerted a key role in CNS myelination and remyelination[2] and provide neuroprotection effects on the hypothalamus in conditions of metabolic syndrome.[3] Androgens are also thought to increase synaptic density in the gyrus dentatus[4] and improve regional brain perfusion[5] and enhances survival of new hippocampal neurons.[6] T also mediates rewarding properties in the nucleus accumbens[7] and its metabolite 3α-diol facilitates female sexual motivation when infused in the nucleus accumbens.[8] T metabolites were thought to serve as an anxiolytic and cognitive-enhancing modulator in the hippocampus[9] and T and its metabolites may modulate seizure susceptibility.[10]

T appears to regulate Alzheimer-like neuropathology in male 3xTg-AD mice[11] and most recently it was demonstrated that blockade of 5α-reductase

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano, Italy.
Departments of [2]Biochemistry and [3]Urology, Boston University School of Medicine, Boston, Massachusetts, USA.
[i]ORCID ID (https://orcid.org/0000-0003-0861-8967).
[ii]ORCID ID (https://orcid.org/0000-0003-3850-0329).

*Address correspondence to: Roberto Cosimo Melcangi, PhD, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Via Balzaretti 9, Milano 20133, Italy, Email: roberto.melcangi@unimi.it

(5α-R) impedes cognitive performance, modifies dendritic morphology, and upregulates Tau phosphorylation in the hippocampus of male 3xTg-AD mice.[12] In this review, we provide a discussion of the biology, biochemistry, and regional distribution of key enzymes involved in T metabolism in the nervous system, such as 5α-Rs and 3α/3β-hydroxysteroid oxidoreductases (3α/3β-HSORs) (Fig. 1) and discuss the critical role of such biochemical pathways and potential clinical implication on brain function in health and disease.

The Biology of 5α-R

5α-Rs and 3α/3β-HSORs are families of enzymes expressed in the CNS and in the periphery, and are responsible for transformation of androgens, progestins, glucocorticoids, and mineralocorticoids into neuroactive signaling steroid molecules.[13–28] 5α-Rs catalyze the formation of 5α derivatives of neuroactive steroids with second-order kinetics representing the rate-*limiting step*[19,26,29–31] (Fig. 1). One critical pathway in the CNS physiological function is the transformation of T or Δ^4 androstenedione into neuroactive androgen derivatives by 5α-Rs concomitant with 3α/3β-HSORs.[19,26,31–34] As is discussed in detail in this review, these neuroactive androgens metabolites modulate a host of physiological processes including myelination,[2,35] neurotransmission,[36–39] response to stress,[40–43] and serve as anti-inflammatory agents.[44–47] The expression of 5α-Rs and 3α/3β- HSORs exhibit sex-specific differences and regional differences within the CNS and are modified by various physiological modulators as well as pathological conditions.

Traish[25] reviewed the distribution of 5α-R types 1, 2, and 3 and reported on localization of these enzymes in various tissues including liver, prostate, epididymis, seminal vesicle, and spinal cord, as well as the white and gray matter. 5α-R type 2 is expressed in glial cells of the white matter and in oligodendrocytes, dorsal horn, and ventral horn in the gray matter and motoneuronal cells of the anterior horn. Type 1 activity predominates in other peripheral tissues. 5α-R type 1 activity is localized in many regions of the CNS encompassing mixed glia, type 1 astrocytes, oligodendrocytes, and neurons, and is localized in white matter of the spinal cord. Type 1 activity is detected in many other tissues such as adrenal glands, liver, kidney, skin, and scalp. 5α-R type 3 is distributed in many tissues including the hippocampus, cerebellum retina, heart, skin, kidney, prostate, liver, skeletal, muscle, myometrium, and pancreas.
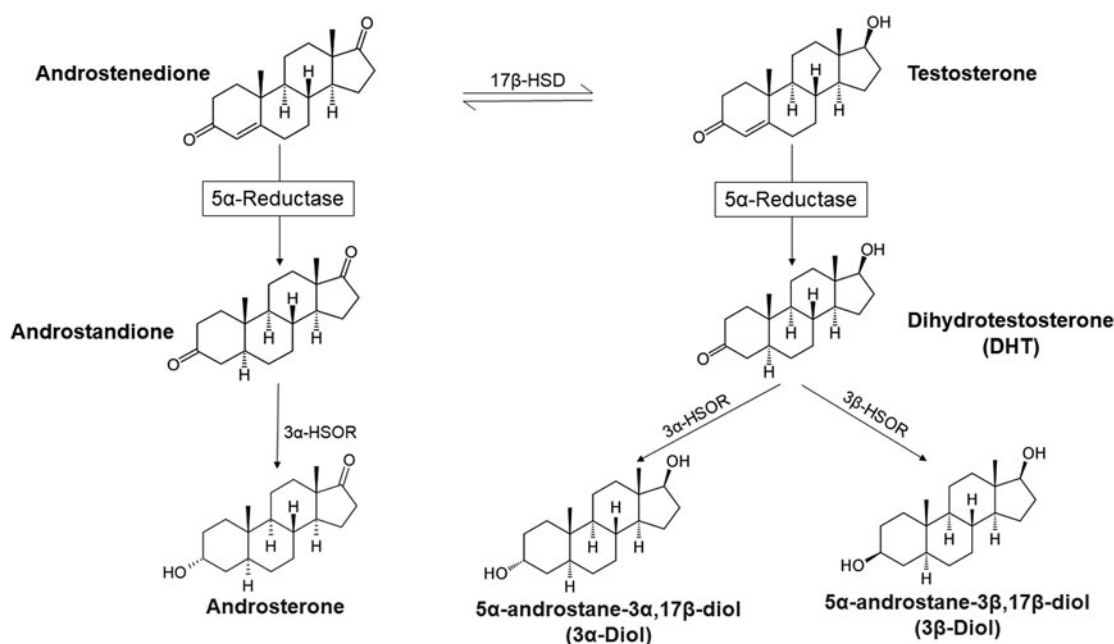

FIG. 1. Androgen metabolism by the enzymes 5α-reductase and 3α/3β-hydroxysteroid oxidoreductase.

Expression and regional distribution of 5α-Rs in the CNS

5α-Rs catalyze a key biochemical reaction in the regulation of development and physiology of male sexual differentiation and metabolism.[26,27,48] The family of 5α-Rs is currently thought to encompass five different enzymes, based on differences in the primary DNA sequences. The members of this family include three well-characterized 5α-R isotypes (types 1, 2, and 3; SRD5A1, SRD5A2, SRD5A3). In addition, two trans-2,3 enoyl-CoA reductases (TECR and TECR-like) have been identified and partially characterized.[18,27,28,49–51]

5α-R type 1 activity is found in high levels in various regions of the CNS, more specifically those rich in white matter, such as midbrain, corpus callosum, anterior commissure, optic chiasm, pons, and spinal cord.[14,18,52] Similarly, 5α-R type 1 activity is elevated in samples of purified myelin preparations isolated from rat brain[13,14,53] and is detected in oligodendrocytes, the myelin forming cells of the CNS, and in neurons.[54–56] Interestingly, 5α-R type 1 activity was also detected in the myelinating cells of the peripheral nervous system (PNS) (i.e., Schwann cells).[57–60] Further observations indicate the presence of 5α-R type 1 in microglia determined by reverse transcriptase-polymerase chain reaction[61] and in astrocytes by functional enzymatic activity.[54,55] Interestingly, as reported in cell cultures, type 2 astrocytes, which probably correspond to fibrous astrocytes *in vivo*, but not type 1 astrocytes, which probably correspond to protoplasmatic astrocytes, express 5α-R type 1, assessed by functional enzymatic activity.[54,55] However, the 5α-R activity detected in type 1 astrocytes is enhanced by the concomitant presence of neurons.[62] 5α-R type 1 mRNA has also been detected in cortical, hippocampal, and olfactory bulb glutamatergic neurons and in some output neurons of the amygdala and thalamus.[63]

5α-R type 1 enzyme genetic deficiency has not been reported to date and has not been related to any human disorder. In contrast, congenital 5α-R type 2 deficiencies are well characterized with adverse clinical consequences such as significant changes in sexual differentiation.[48,50,64–68] 5α-R type 3 deficiencies due to mutations have been described and were thought to be associated with visual disturbances and various mental retardation.[69–72] Recently, TECR mutations were also thought to be associated with mental retardation[73] even though the exact functions of these enzymes (as well as TECRL) remain poorly characterized, and information on its relevance in the process of steroidogenesis is currently not available.

5α-R type 2 is also widely detected in most key regions of the adult rat brain using immunofluorescent staining.[74] Based on detection by immunoreactivity, 5α-R type 2 is present in higher concentrations in the spinal cord (i.e., dorsal and ventral horn of the gray matter) and particularly in oligodendrocytes.[52] It is also present in motoneuronal cells of the anterior horn, with greater levels in the L1 to L3 tract of the lumbar spinal cord.[75] It is worth noting that not only differentiated CNS cells but also stem cells originating from the mouse striatum express 5α-R.[76] We should also emphasize that 5α-R type 3 expression and tissue distribution encompass the hippocampus and cerebellum of human brain.[72,77] 5α-R type 3 is thought to be more responsible in N-linked protein glycosylation through a mechanism that involves polyprenol reductase[69] and may not be critical in steroid metabolism.[78] SRD5A3, a gene encoding an enzyme responsible for dolichol synthesis, and when defective, leading to impaired N-glycan biosynthesis in the endoplasmic reticulum.[72] Only few reports suggesting that it is expressed in liver and prostate were reported. Nikolaou et al.[79] suggested that the role of 5α-R type 3 is not entirely clear. This isoform is found in a variety of human tissues, including liver, skin, kidney, skeletal muscle, pancreas, and testis, and it is the mostly highly expressed in human abdominal adipose. A number of studies have tried to elucidate the role of this enzyme in steroid hormone metabolism, but data have been conflicting.[79] A role for this enzyme in the nervous system remains unclear, at present.

Biochemistry of 5α-Rs

5α-Rs catalyze the chemical reduction of the double bond in the A ring at the 4,5 positions in C19 and C21 steroids. 5α-Rs transfer a hydride from nicotine amide adenine dinucleotide phosphate (NADPH) to the 5α position of the steroid nucleus to produce its 5α-reduced derivative.[29,80] The precursors for 5α-Rs include T, progesterone (PROG), deoxycorticosterone, corticosterone, cortisol, and aldosterone. The derivatives produced from these reactions result in formation of 5α-dihydro-derivatives such as 5α-dihydrotestosterone (5α-DHT), 5α-dihydroprogesterone (5α-DHP), 5α-dihydrodeoxycorticosterone, 5α-dihydrocorticosterone, 5α-dihydrocortisol, and 5α-dihydroxyaldosterone. More importantly, 5α-dihydrocortisol and 5α-dihydroxyaldosterone may serve as a potential active mineralocorticoid. The activity of 5α-Rs determines the rate limiting step in the synthesis of 3α, 5α steroid

derivatives.[26,27] Thus, 5α-Rs reactions generate products that serve as substrates for 3α-HSOR) and 3β-HSOR) enzymes.[31] 3α-HSOR transforms DHT to 3α-diol, DHP to tetrahydroprogesterone (THP; also known as allopregnanolone), and DHDOC to tetrahydrodeoxycorticosterone (THDOC). 3β-HSOR transforms DHT to 3β-diol and DHP to isoallopregnanolone. We have previously addressed the role of 5α-Rs and 3α/3β-HSOR in the metabolism of other steroids, like for instance PROG.[26,31] Therefore, in this review we focus our attention on T metabolites.

Molecular mechanisms of 5α-R catalysis and inhibition

5α-Rs are NADPH-dependent enzymes that catalyze the reduction of 3-keto, Δ^4, steroids. The binding of T and NADPH to the enzyme results in the formation of an enzyme–substrate complex that undergoes activation into the transition state complex (Fig. 2). The pyridine group from NADPH initiates a nucleophilic attack that results in isomerization of the three keto group into the enol group and reduction of the C-4 double bond. In a subsequent reaction, and acid–base catalysis step results in enol to keto formation and abstraction of the pair of the electrons, resulting in dissociation of NADP+ from the intermediate and dissociation of DHT from the complex and eventually the enzyme active site. As reported by Bull et al.[29] at standard conditions of pH 7.20 and 37°C, the catalytic constants for conversion of T to DHT are $K_m = 24.6 \pm 0.7$ nM for T; $K_m = \sim 1\,\mu$M for NADPH; $k_{cat} = 0.075 \pm 0.006$/S. From the kinetic constants reported by Bull et al.,[29] the second-order rate constant for reduction of T to DHT equivalent to $k_{cat}/K_m = (3.0 \pm 0.3) \times 10^6$ / (M S). These catalytic parameters point to an efficient enzymatic reaction with high affinity for the substrate (T). Finasteride: (17β–(N-*tert*-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one (MK-906) is a potent inhibitor of 5α-R types 1 and 2. Bull et al.[29] proposed a novel mechanism related to catalysis, in which finasteride
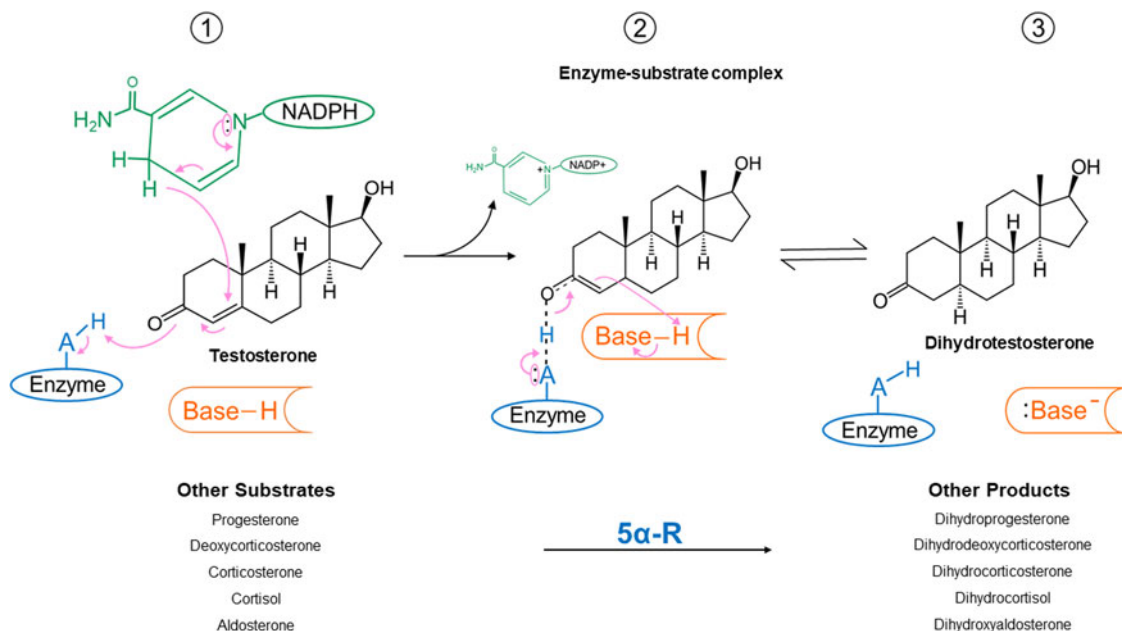

FIG. 2. Mechanism of action of 5α-R. The action of the enzyme 5α-R on testosterone and other substrates is divided into three main steps: the first step (1) shows a nucleophilic attack of pyridine group from NADPH to testosterone leading to the binding to substrate with enzyme. The second step (2) consists in isomerization of the three keto group into the enole group and an abstraction of the pair of the electrons resulting in dissociation of NADP+ from the intermediate; the third step (3) shows the dissociation of 5α-DHT from the enzyme–substrate complex and eventually the enzyme active site. 5α-DHT, 5α-dihydrotestosterone; 5α-R, 5α-reductase; NADPH, nicotine amide adenine dinucleotide phosphate.

acts through a mechanism-based inhibitor (suicide substrate).[81] The enzyme binds finasteride and reduces finasteride to dihydrofinasteride through NADPH as a cosubstrate and the NADP-dihydrofinasteride adduct remains very tightly bound to the enzyme and does not dissociate fast, keeping the enzyme locked up in an inactive state. This NADP-dihydrofinasteride adduct serves as a bisubstrate analog inhibitor, and ranks among the most potent noncovalently bound enzyme inhibitors known for this enzyme. The *mechanism-based inhibition* of 5α-R by finasteride proceeds through closely related enol(ate) intermediates, but then diverges significantly from the natural substrate because finasteride redirects the carbanion center to a position where it escapes the proton transfer that normally completes the reduction. The adduct phosphor-adenosine diphosphate-dihydro-finasteride remains bound to the enzyme and does not dissociate easily, resulting in a dead end (suicide substrate) and is lethal to enzyme function. Although the enzyme is bound to phosphor-adenosine diphosphate-dihydro-finasteride, it is not available to perform its anticipated cellular activity of transforming T to DHT. Over a longer period of time, the adduct is hydrolyzed either through enzyme catalyzed or nonenzymatic reaction releasing the dihydrofinasteride. However, because of these pharmacokinetic parameters, the finasteride adduct may remain attached to the enzyme for days, weeks, or perhaps months, thus rendering the enzyme incapable of its regular biological function. For this reason, finasteride is considered a suicide substrate for the enzyme. Bull et al.[29] suggested that the kinetic data demonstrate that the rate constants for the forward and backward reactions of the enzyme–inhibitor complex show that the (steady-state) dissociation constant of finasteride is $Ki^* = k_{off}/k_{on} \leq 3 \times 10^{-13}$ M.

It is important to emphasize that human 5α-R type 2 isozyme has apparent Ki values for finasteride and dutasteride in the nanomolar range (69 and 7 nM, respectively), suggesting that both inhibitors significantly reduce DHT biosynthesis.[82] The apparent Ki values for type of 5α-R type 1 isozyme for finasteride and dutasteride (360 and 6 nM, respectively), suggest that finasteride is not highly selective for 5α-R type 1 and may not significantly inhibit DHT.[82] This is not the case in the animal model studies in which finasteride and dutasteride inhibit both 5α-R types 1 and 2. Finally, it should be pointed out that 5α-R type 2 isozyme has apparent K values in the nM range for steroid substrates, whereas the type 1 isozyme has apparent K values in

the μM range. Rat type 2 enzyme is maximally active over a narrow acidic pH range, whereas the rat type 1 enzyme expressed in these cells has a broad neutral basic pH optimum.[83,84]

The Biology of 3α/3β-HSORs

3α- and 3β-HSORs are members of the aldo-keto reductase superfamily that includes aldehyde reductase, aldoreductase, and dihydrodiol dehydrogenase.[85–91] This group of enzymes catalyzes conversion between aldehydes or ketones and alcohols, using NAD(H) or NADPH(H) as cofactors. In humans, multiple cDNAs encode proteins related to 3α-HSOR,[92–96] and four isozymes of 3α-HSOR were identified.[90,96] In contrast, in rats only one type of enzyme was identified in both brain and liver.[97–100] The Δ^4-3-ketosteroids, which are reduced by 5α-R, are metabolized consecutively to 3α,5α-tetrahydrosteroids by 3α-HSOR.[90,101] The enzyme activity of 3α-HSOR is also widely distributed in the CNS (e.g., in rat cerebral cortex and the cerebellum, with lower expression in the latter structure)[102] and is especially high in the olfactory bulb.[103,104] Agis-Balboa et al.[63] reported that 5α-R type 1 and 3α-HSOR colocalize in cortical, hippocampal, and olfactory bulb glutamatergic principal neurons and in some output neurons of the amygdala and thalamus of mice. Neither 5α-R type 1 nor 3α-HSOR mRNAs are expressed in S100β- or glial fibrillary acidic protein-positive glial cells. 5α-R type 1 and 3α-HSOR were not detected in cortical and hippocampal GABAergic interneurons. However, 5α-R type 1 and 3α-HSOR are significantly expressed in principal GABAergic output neurons, such as striatal medium spiny, reticular thalamic nucleus, and cerebellar Purkinje neurons. A similar distribution and cellular location of neurosteroidogenic enzymes were observed in rat brain. These findings demonstrate that THP and THDOC, which may be synthesized in principal output neurons, modulate gamma-aminobutyric acid (GABA) function at GABA-A receptors, through an autocrine mechanism or through a paracrine mechanism or by allosteric interaction with GABA-A receptor intracellular sites through lateral membrane diffusion.

In the spinal cord, 3α-HSOR presence, as detected by immunostaining, is mainly distributed in both white and gray matters, even if the greatest density is found in sensory regions of the dorsal horn.[52] The 3α-HSOR activity is little in neurons and high in astrocytes,

especially in type 1 cells[54,55] and oligodendrocytes,[52,105] although this activity has not been observed in myelin membranes of the CNS.[13,14]

Physiological Effects of Testosterone Metabolites

The metabolites of T as a result of the action of the enzymatic complex 5α-R and 3α- or 3β-HSOR exert critical physiological action in the CNS, since such metabolites serve as chemical messengers (ligands) for a host of neuronal and glial receptors that are not known to be directly modulated by T. Indeed, although the 5α-reduced metabolite of T (i.e., DHT) is still able to bind to the androgen receptor (AR), showing higher affinity for this receptor than its precursor T,[106] the further 3α,5α-reduced metabolites interact with different receptor molecules. Indeed, 3α-diol and 3β-diol are known to act as agonists of GABA-A receptors, as well as ERβ.[107–112]

Activation of AR by DHT initiates a cascade of molecular mechanisms that regulate neuronal, glial, and synaptic differentiation and in the coordination of the appearance of typical male features in specific brain regions.[113,114] Interestingly, DHT regulates synaptic density and transmission in male, but not in female rodent hippocampal cultures. In this experimental model, finasteride, blocking DHT synthesis, diminishes dendritic spine maturation and long-term potentiation.[115] In addition, this T metabolite affects plasticity of CA1 hippocampal pyramidal neurons in adolescent male rats,[116] it is necessary for inducing long-term depression of synaptic transmission,[117] and plays a role in the regulation of spine plasticity in circuits related with motivated behaviors.[118] DHT mediates also the age-dependent change in GnRH neuron firing in adult prenatally androgenized female mice[119] and facilitates sexual behavior in gonadectomized female rats primed with estradiol.[120] Finally, activation of AR-dependent signaling pathway promotes neurogenesis,[121] increasing the neural progenitor cells in the developing mouse cortex.[122]

3α-diol, by the interaction with GABA-A receptors, regulates behavior.[123,124] 3β-diol seems to modulate the activity exerted by 3α-diol on the stress response mediated by HPA axis.[107] In particular, 3β-diol is effective in attenuating corticosterone and ACTH increases in response to restraint stress.[125,126] These effects are inhibited by administration of tamoxifen (i.e., an estrogen receptor [ER] antagonist) suggesting an involvement of ERβ.[125–127]

5α-reduced derivatives of T exert physiological effects in the PNS. In mature male animals, gonadectomy significantly reduces gene expression of myelin proteins such as P0 and PMP22 in the sciatic nerve, whereas subsequent treatment with 5α-DHT or 3α-diol restores P0 mRNA to precastration levels.[128,129] Only 3α-diol treatment is effective on PMP22 levels.[128] These results demonstrate that 5α-DHT and 3α-diol increase P0 and PMP22 mRNA levels, respectively, in cultures of rat Schwann cells, in agreement with previous observations.[129,130] These results seem to suggest that P0 and PMP22 are regulated by the action of AR and GABA-A receptor, respectively. Indeed, inhibition of AR action by flutamide (i.e., an antagonist of AR) or with bicuculline (i.e., an antagonist of GABA-A receptor) reduces the synthesis of these two myelin proteins.[128,129] The regulation of P0 expression by classical steroid receptors is further inferred from findings that putative androgen responsive elements have been shown in the promoter region of P0 gene.[129]

Levels of Testosterone Metabolites Under Physiological Conditions

Data based on analyses of metabolites of T using liquid chromatography-tandem mass spectrometry demonstrated that the levels of reduced T metabolites are significantly different in the nervous system, plasma, and cerebrospinal fluid (CSF), especially, in the CNS versus PNS and in male versus female rat.[131]

The sex differences in the levels of 5α-reduced T metabolites may be related to sex dimorphism of the steroidogenic enzymes responsible for their synthesis. Indeed, as reported in the brain of green anole lizards, the gene expression of 5α-R type 2 is higher in females than in males.[132] Sex differences in the brain expression of 5α-R have also been reported in rats. Thus, the gene expression of this enzyme is significantly higher in the cerebellum of males, whereas that of 3α-HSOR is significantly higher in the cerebellum of proestrus females. It is important to highlight, that this sex dimorphism is brain area specific; indeed, does not occur in the cerebral cortex.[102]

Interestingly, the levels of 5α-reduced metabolites of T in the nervous system are influenced by their circulating levels. Thus, gonadectomy affects the levels of these molecules in the CNS and the PNS.[133] As reported in rodents, the changes induced by gonadectomy are different depending on the nervous regions and sex considered, as well as by the duration of gonadal hormone deprivation.[133] For instance, 3α-diol levels present in the cerebellum, cerebral cortex, and spinal cord are decreased, by long-term gonadectomy, only in male animals, whereas in the sciatic nerve,

they occurred only in female animals.[133] On the contrary, short-term gonadectomy does not show any effects in the cerebellum, cerebral cortex, and sciatic nerve of both sexes, but it induces an increase of 3α-diol levels in spinal cord of female animals.[133] A sex-specific effect of long-term gonadectomy on 3α-HSOR expression has also been recently reported.[102] Similar alterations in the levels of T metabolites have been recently reported in quail brain of control and gonadectomized animals.[134] In summary, these findings demonstrated that the levels of 5α-reduced metabolites of T detected in the nervous system, even if modulated by the sex steroid hormone milieu, point to a specific pattern depending on sex and brain region.

Levels of Testosterone Metabolites Under Pathological Conditions

The levels of T-reduced metabolites in the nervous system are also altered by psychiatric and neurodegenerative diseases (Fig. 3). In addition, many neurodegenerative and psychiatric disorders exhibit a sex difference with regard to incidence and/or manifestations of the pathology. Interestingly, in some of these pathologies, a sex difference is also reported in the magnitude of 5α-reduced metabolites of T in the nervous system.

For instance, 3α-diol levels are decreased in association with increased depression and anxiety symptoms in the plasma of anorexic and overweight/obese women.[135] As shown in prefrontal cortex Brodmann's area of depressed patients, 5α-R type 1 enzyme is also downregulated.[136] Changes in expression of 5α-R type 1 as well as of types 2 and 3 were reported in the prefrontal cortex of adolescent male rats after ethanol treatment.[137] Polymorphism in 5α-R type 1 and 3α-HSOR has also been related to elevated risk of alcohol dependence.[138]

Changes in T metabolism have also been reported in autism. At postnatal day 5, a decrease of DHT levels was reported in male, but not in female cerebellum of the reeler mouse (an experimental model of autism).[139] Interestingly, a significant association between single nucleotide polymorphisms rs523349 (Leu89Val) located on the gene encoding 5α-R type 2 and autism

Pathology	DHT	3α-diol	Experimental models
Autism	↓	–	Cerebellum of male reeler mouse model
TBI	↓	–	Male rats
ALS	↓	–	CSF of male/female rats
PD	↑	–	Male rats
MS	↓	↓	Spinal cord of male rats affected by EAE
Diabetes mellitus	↓	↓	Spinal cord, cerebral cortex and cerebellum of male rats after long-term diabetes
Peripheral neuropathies	↓	–	Sciatic nerve of male rats after short-term diabetes
	↓	↓	Sciatic nerve of male rats after long-term diabetes
	–	↓	Male rats affected by CMT disease

FIG. 3. Levels of testosterone metabolites in experimental models of neuropathological conditions. The arrows indicate the increase or decrease of testosterone metabolites, DHT, and 3α-diol. ALS, amyotrophic lateral sclerosis; CMT, Charcot-Marie-Tooth; CSF, cerebrospinal fluid; DHT, dihydrotestosterone; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; PD, Parkinson's disease; TBI, traumatic brain injury.

has also been reported.[140] Moreover, knockout mice for 5α-R type 2 enzyme exhibited reduced dominance-related behaviors, as well as deficits of novelty-seeking and risk-taking responses.[141]

In experimental model of traumatic brain injury (TBI), levels of DHT are significantly altered in the brain.[142–144] In particular, in agreement with the reported sex-dimorphic incidence and outcome of TBI,[145] sex differences in T derivative levels were also observed in animal models. Indeed, a decrease of DHT levels was observed after TBI in the male, but not in the female brain.[143]

Changes in T metabolism were also observed in neurodegenerative disorders. For instance, DHT levels are decreased in the CSF of male and female patients affected by amyotrophic lateral sclerosis.[146] In Parkinson's disease (PD) patients, the expression of the 5α-R type 1 enzyme is downregulated in the substantia nigra, whereas that of 3α-HSOR type 3 is upregulated in the caudate nucleus.[147] The levels of the 5α-reduced metabolites of T are modified in PD rodent models. For instance, injection with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; i.e., an experimental model of PD) increases the levels of DHT in the brain of male mice.[148]

Observations performed in patients affected by multiple sclerosis (MS) show altered levels of T metabolites. In the CSF of relapsing-remitting MS male adult patients, a decrease in the levels of DHT associated with an increase in those of 3α-diol was reported.[149] In agreement, in the hippocampus and cerebral cortex of the mouse cuprizone demyelination model, a decrease of the 5α-R expression was reported.[150] In addition, observations performed in experimental autoimmune encephalomyelitis (EAE) rat MS model showed alterations of T-reduced metabolite levels in CNS.[151,152] Indeed, a decrease in the levels of DHT and 3α-diol was reported in the spinal cord at the chronic phase of the disease.[152]

In agreement with the higher incidence of MS in females[153–155] and the existence of sex differences in MS symptomatology (i.e., course of the disease, age of onset, and pathological alterations),[156] different levels of T-reduced metabolites have been detected in male and female EAE animals.

Both at the acute phase (i.e., 14 days postimmunization)[151] and chronic phase of the disease (i.e., 40 days postimmunization),[152] DHT and 3α-diol levels are decreased, in the spinal cord of male, but not of female animals. In the cerebellum, only a significant decrease of 3α-diol levels was reported in males.[151,152] In the male cerebral cortex, there is a decrease in the levels of DHT and 3α-diol at the acute phase of the disease,[151] however, at the chronic phase, only the levels of DHT remain decreased.[152] The levels of 5α-reduced metabolites of T were unaffected in these nervous areas of female animals.[151,152]

Diabetic encephalopathy (i.e., the damage in the CNS induced by diabetes mellitus) also induces changes in the levels of T-reduced metabolites.[157–162] As reported in an experimental model (i.e., rats raised diabetic by streptozotocin injection), the specific alterations in the levels of these T metabolites depend on the duration of pathology. For instance, the levels of 3α-diol are decreased in the spinal cord of male rats after long- but not after short-term diabetes.[159,160] In agreement with sex dimorphic features of diabetic encephalopathy,[163] changes in the nervous levels of T-reduced metabolites depending on the sex were also reported. For instance, in diabetic animals the levels of DHT and 3α-diol are decreased in the cerebral cortex, cerebellum, and spinal cord of males but not of females.[159] Diabetes mellitus also induces damage in the PNS (i.e., diabetic peripheral neuropathy). Also, in this case the specific alterations in the levels of 3α-diol depend on the duration of pathology. Indeed, the levels of this T metabolite are decreased in the sciatic nerve of male rats after long- but not after short-term diabetes.[159,164] Sex-specific changes in the levels of T metabolites also occur in diabetic peripheral neuropathy as well as other peripheral neuropathies. For instance, in the sciatic nerve of male but not in female rats, DHT levels are decreased after short-term diabetes.[164] After longer time of diabetes, a decrease not only of DHT but also of 3α-diol levels is observed in the male rat sciatic nerve.[159] In another model of peripheral neuropathy, such as the crush injury of the sciatic nerve, a decrease in the expression of enzyme 5α-R has been observed in the distal portion of the injured nerve in male rats.[165] In addition, in an animal model Charcot-Marie-Tooth disease type 1A (i.e., an inherited peripheral neuropathy), the levels of 3α-diol are decreased in the peripheral nerves of male but not of female animals.[166] Altogether these observations clearly show that CNS and PNS damage is also associated with altered nervous levels of 5α-reduced metabolites of T. On this basis, the neuroprotective actions of these molecules have been explored in several experimental models of psychiatric and neurodegenerative disorders.

Neuroprotective Effects of Testosterone Metabolites

Some of the 5α-reduced metabolites of T have been reported to exert effects in mood and anxiety

disorders.[167] In particular, sex-specific features are an important aspect of these actions. For instance, 3β-diol normalized the HPA axis responses to interleukin-1β only in adult male prenatally stressed rats.[168]

T-reduced metabolites also exert protective effects in a variety of *in vitro* and *in vivo* models of neurodegeneration (Fig. 4). Indeed, DHT is neuroprotective in experimental model of PD,[169,170] in EAE models,[171–174] as well as in an experimental model of TBI.[175] This T metabolite is also able to protect hippocampal neurons from damages, like for instance kainic acid injection,[176] apoptosis,[177,178] and serum deprivation.[179] In addition, it is also a protective agent for motoneurons[180] and also after spinal cord injury.[181] On stroke, both protective and deleterious effects have been reported.[182]

Also the further metabolite of DHT, 3α-diol, exerts neuroprotective effects. In SH-SY5Y neuronal cells and in primary cortical neurons, this metabolite, by both GABA-A receptor-dependent and independent mechanisms, inhibits the phosphorylation of extracellular signal-regulated kinase induced by amyloid β peptide 1–42[183] as well as modulates mitogen-activated protein kinase phosphatase 3/dual specificity phosphatase 6.[184] Some of the protective effects exerted by the metabolites of T show sex-specific features. In cultured hippocampal neurons, DHT shows protective effects for apoptosis induced by glutamate in males, but not in females.[185]

5α-reduced metabolites of T are also protective in the PNS. For example, it has been reported that DHT induces a faster regeneration and functional recovery of injured nerves.[180,186–189] In the streptozotocin (STZ) experimental diabetic rat model, this metabolite stimulates the activity of Na$^+$, K$^+$-ATPase in the sciatic nerve and counteracts the impairment of nerve conduction
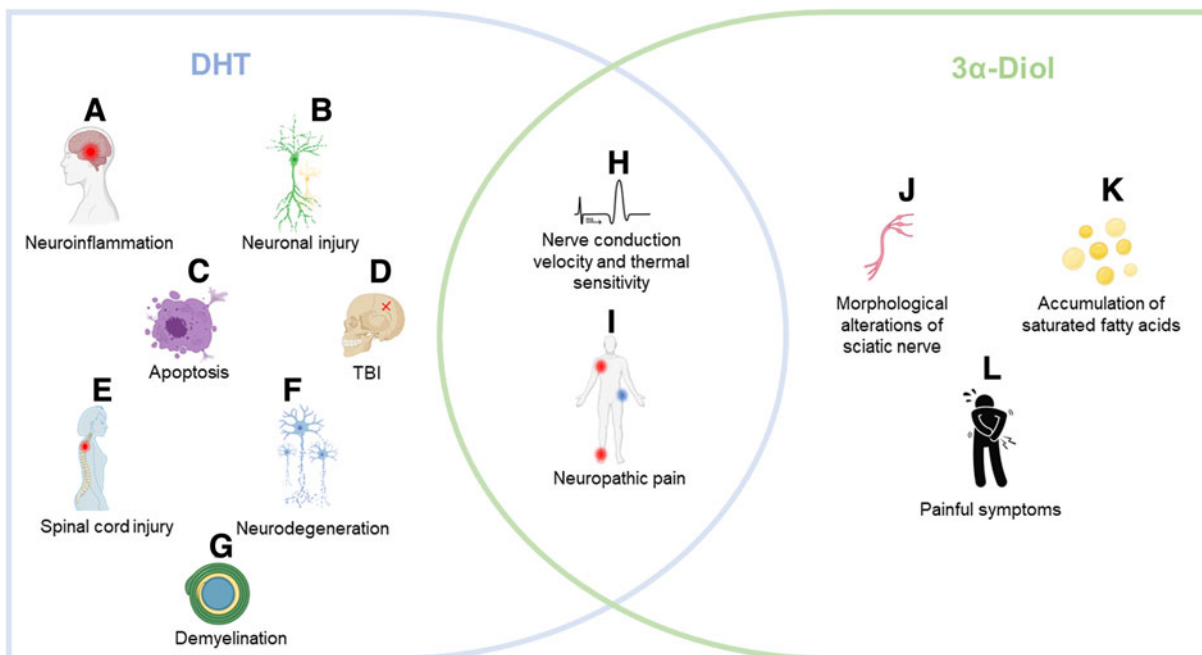

FIG. 4. Neuroprotective effects of testosterone metabolites. The main protective effects of DHT and 3α-diol are shown. 5α-DHT exerts neuroprotective effect on **(A)** neuroinflammation in EAE, **(B)** damage of hippocampal neurons, **(C)** apoptosis induced by glutamate in cultured hippocampal neurons, **(D)** TBI, **(E)** spinal cord injury, **(F)** PD, **(G)** acute demyelination induced in cerebellar organotypic cultures. 5α-DHT or 3α-diol treatment improves **(H)** nerve conduction velocity and thermal sensitivity, in experimental diabetic rat model, as well as reduces **(I)** neuropathic pain. Moreover, 3α-diol reduces **(J)** morphological alterations in the sciatic nerve and **(K)** the accumulation of saturated fatty acids in the myelin of sciatic nerve of experimental diabetic rat model. This molecule is also able to exert beneficial effects on **(L)** painful symptoms in paclitaxel-induced peripheral neuropathy.

velocity, thermal sensitivity, and skin innervation density.[190] Also 3α-diol is effective; indeed, it reduces morphological alterations in the sciatic nerve[191] and improves nerve conduction velocity, thermal sensitivity, and skin innervation density observed in STZ experimental diabetic rat model.[190]

Pain is an important component of the peripheral neuropathy. Interestingly, 5α-R and 3α-HSOR are expressed in pain information processing centers of the CNS, such as the dorsal root ganglia and the dorsal horn of the spinal cord.[192,193] In agreement, T-reduced metabolites decrease neuropathic pain associated with diabetes mellitus. In particular, DHT and 3α-diol treatments are effective on alterations of mechanical nociceptive threshold and tactile allodynia induced by diabetes, respectively.[160] 3α-diol is also able to exert beneficial effects on painful symptom- occurring in paclitaxel-induced peripheral neuropathy.[194]

Myelin compartment is another target of the neuroprotective effects of T-reduced metabolites. Indeed, in cerebellar organotypic cultures, DHT protects against acute demyelination,[195] and in the STZ experimental model, DHT stimulates the expression of P0 in the sciatic nerve.[190] In addition, 3α-diol reduces the accumulation of saturated fatty acids in the myelin of sciatic nerve reported in STZ experimental diabetic rat model.[191] Neuroinflammation, a common aspect among neurodegenerative and psychiatric diseases,[196–201] may be regulated by T metabolites. In the EAE model, DHT reduces proinflammatory IFN-γ expression and gliosis in the spinal cord[174] and increases the expression of anti-inflammatory IL-10 by autoantigen-specific T lymphocytes.[173] In addition, this T metabolite inhibits microglia inflammatory responses by suppressing tool-like receptor 4/nuclear factor-kappa B signaling.[45,46] Altogether these observations indicate that the treatment with T-reduced metabolites exert a variety of protective actions in the nervous system.

An alternative to the systemic treatment with these molecules, it is to enhance their endogenous synthesis. One option could be represented by the pharmacological activation of steroidogenesis with ligands of translocator protein of 18kDa (TSPO) (i.e., a molecule involved in the transfer of cholesterol into mitochondria).[202] Indeed, some TSPO ligands stimulating steroidogenesis and, therefore, the synthesis of T-reduced metabolites,[203–205] exert neuroprotective actions in animal models. Examples of that are provided by the etifoxine[206] and XBD173[207] in EAE mice, midazolam[208] and YL-IPA08 in rat models of post-traumatic stress disorder (PTSD),[209] chronic stress-

related depression[210] and anxiety disorders,[211] PK11195, in a rat *ex vivo* glaucoma model,[212] and Ro5–4864 and AC-5216 in diabetic rats.[213,214]

Activation of liver X receptors (LXRs), it has also been demonstrated to increase the levels of T-reduced metabolites in the nervous system as well as to exert neuroprotective effects in animal models of cerebral ischemia,[215] MS, AD, and PD.[216,217] In particular, the treatment with an LXR ligand, such as GW3965, increases the levels of 3α-diol in the spinal cord, cerebral cortex, and sciatic nerve of STZ experimental model.[213,218] Interestingly, at least in the sciatic nerve, the protective effect of LXR ligands is associated with an increase in the expression of 5α-R, among other steroidogenic molecules.[218] The modulation of 5α-R activity has been proposed as a therapeutic treatment for disorders of the nervous system. In observational studies, dutasteride in adult males was shown to be associated with reduced self-reported sedative effects of a moderate dose of alcohol in a laboratory setting and reduced alcohol self-administration for 1–2 weeks after a single 4-mg dose in the natural environment.[219] Moreover, a clinical study performed in women with premenstrual dysphoric disorder showed that this 5α-R inhibitor reduces irritability, sadness, anxiety, food cravings, and bloating.[220] We should point out that limited studies in the animal model, as well as in human, have investigated the effects of dutasteride in the CNS. Nevertheless, it was postulated that dutasteride elicits a neuroprotective activity, in the animal model of PD. Dutasteride but not finasteride protected dopamine (DA) neurons against MPTP-induced toxicity. MPTP mice treated with dutasteride (5 mg/kg and 12.5 mg/kg) showed higher striatal DA and metabolites (i.e., 3,4-dihydroxyphenylacetic acid and homovanillic acid) with a decrease of metabolites/DA ratios compared with saline-treated MPTP mice.[221,222] Dutasteride has been proposed for therapy of human glioblastoma.[219,223] In addition, another 5α-R inhibitor, such as the finasteride, is protective in ischemic brain injury induced in aged rats.[221] Finally, in adolescent male rats, this 5α-R inhibitor inhibits dopaminergic system and, therefore, has been proposed for neuropsychiatric disorders associated with hyperactivity of this neurotransmitter system.[222]

In contrast, it is critical to emphasize that endocrine and neuropsychiatric side effects have been reported in a subset of male patients treated with 5α-R inhibitors for androgenetic alopecia (i.e., erectile and ejaculatory dysfunctions, loss of libido, depression, anxiety, suicidal thoughts, and sleep problems). It is significant that such effects may persist despite treatment

discontinuation,[26,224–227] leading to new clinical disorder referred to as "postfinasteride syndrome." Interestingly, changes in the methylation pattern of the gene encoding for 5α-R type 2 and in the levels of the T-reduced metabolites were detected in the CSF of patients with this newly defined syndrome[228,229] associated with alterations in the gut microbiota population[230] as well as major depressive disorders, erectile dysfunction, and peripheral neuropathy.[227,228]

Conclusions

Significant literature indicating that 5α-Rs and 3α/3β-HSORs are widely expressed and distributed in various regions of the nervous system suggests a critical function of these enzymes in the effects exerted by testosterone and its metabolites. Indeed, 5α-Rs catalyze a key *rate-limiting step* in formation of 5α-reduced neuroactive steroids and, therefore, is critical in maintaining the physiological function of the nervous system. Indeed, T and its metabolites are implicated in processes of neurogenesis, myelination and remyelination, neuroprotection, and attenuation of neuroinflammation. Furthermore, these metabolites play an important role in the reduction of stress responses and modulation of behavior. Significant changes in the concentrations of testosterone and its various metabolites have been noted in the nervous system under various pathological conditions, such as neurodegenerative and psychiatric disorders. Altogether, observations reported in this review suggest that a better understanding of the biochemical actions of testosterone and its metabolites in the nervous system will have important clinical implications in understanding of and in treatment of neurological disorders.

Author Contributions

R.C.M. and A.M.T. carried out data collection and analyses. L.C. and S.D. prepared the figures. R.C.M., L.C., S.D., and A.M.T. were involved in writing, review, and editing the article.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

No funding was received for this article.

References

1. Negri-Cesi P, Colciago A, Celotti F, Motta M. Sexual differentiation of the brain: Role of testosterone and its active metabolites. J Endocrinol Invest. 2004;27(6 Suppl):120–127.
2. Ghoumari AM, Abi Ghanem C, Asbelaoui N, Schumacher M, Hussain R. Roles of progesterone, testosterone and their nuclear receptors in central nervous system myelination and remyelination. Int J Mol Sci. 2020;21(9):3163.
3. Sarchielli E, Comeglio P, Filippi S, et al. Neuroprotective effects of testosterone in the hypothalamus of an animal model of metabolic syndrome. Int J Mol Sci. 2021;22(4):1589.
4. Fattoretti P, Malatesta M, Mariotti R, Zancanaro C. Testosterone administration increases synaptic density in the gyrus dentatus of old mice independently of physical exercise. Exp Gerontol. 2019;125:110664.
5. Azad N, Pitale S, Barnes WE, Friedman N. Testosterone treatment enhances regional brain perfusion in hypogonadal men. J Clin Endocrinol Metab. 2003;88(7):3064–3068.
6. Spritzer MD, Galea LA. Testosterone and dihydrotestosterone, but not estradiol, enhance survival of new hippocampal neurons in adult male rats. Dev Neurobiol. 2007;67(10):1321–1333.
7. Frye CA, Rhodes ME, Rosellini R, Svare B. The nucleus accumbens as a site of action for rewarding properties of testosterone and its 5alpha-reduced metabolites. Pharmacol Biochem Behav. 2002;74(1):119–127.
8. Sanchez Montoya EL, Hernandez L, Barreto-Estrada JL, Ortiz JG, Jorge JC. The testosterone metabolite 3alpha-diol enhances female rat sexual motivation when infused in the nucleus accumbens shell. J Sex Med. 2010;7(11):3598–3609.
9. Edinger KL, Frye CA. Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. Behav Neurosci. 2004;118(6):1352–1364.
10. Reddy DS. Testosterone modulation of seizure susceptibility is mediated by neurosteroids 3alpha-androstanediol and 17beta-estradiol. Neuroscience. 2004;129(1):195–207.
11. Rosario ER, Carroll J, Pike CJ. Testosterone regulation of Alzheimer-like neuropathology in male 3xTg-AD mice involves both estrogen and androgen pathways. Brain Res. 2010;1359:281–290.
12. Mendell AL, Creighton SD, Wilson HA, et al. Inhibition of 5alpha reductase impairs cognitive performance, alters dendritic morphology and increases Tau phosphorylation in the hippocampus of male 3xTg-AD mice. Neuroscience. 2020;429:185–202.
13. Melcangi RC, Celotti F, Ballabio M, Poletti A, Castano P, Martini L. Testosterone 5 alpha-reductase activity in the rat brain is highly concentrated in white matter structures and in purified myelin sheaths of axons. J Steroid Biochem. 1988;31(2):173–179.
14. Melcangi RC, Celotti F, Ballabio M, et al. Ontogenetic development of the 5 alpha-reductase in the rat brain: Cerebral cortex, hypothalamus, purified myelin and isolated oligodendrocytes. Brain Res Dev Brain Res. 1988;44(2):181–188.
15. Melcangi RC, Poletti A, Cavarretta I, et al. The 5alpha-reductase in the central nervous system: Expression and modes of control. J Steroid Biochem Mol Biol. 1998;65(1–6):295–299.
16. Celotti F, Melcangi RC, Negri-Cesi P, Ballabio M, Martini L. Differential distribution of the 5-alpha-reductase in the central nervous system of the rat and the mouse: Are the white matter structures of the brain target tissue for testosterone action? J Steroid Biochem. 1987;26(1):125–129.
17. Celotti F, Melcangi RC, Negri-Cesi P, Poletti A. Testosterone metabolism in brain cells and membranes. J Steroid Biochem Mol Biol. 1991;40(4–6):673–678.
18. Celotti F, Melcangi RC, Martini L. The 5 alpha-reductase in the brain: Molecular aspects and relation to brain function. Front Neuroendocrinol. 1992;13(2):163–215.
19. Martini L, Melcangi RC. Androgen metabolism in the brain. J Steroid Biochem Mol Biol. 1991;39(5B):819–828.
20. 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(4):2171–2178.
21. 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(5–6):246–251.
22. Poletti A, Martini L. Androgen-activating enzymes in the central nervous system. J Steroid Biochem Mol Biol. 1999;69(1–6):117–122.
23. Tsuruo Y. Topography and function of androgen-metabolizing enzymes in the central nervous system. Anat Sci Int. 2005;80(1):1–11.
24. Lephart ED. Brain 5alpha-reductase: Cellular, enzymatic, and molecular perspectives and implications for biological function. Mol Cell Neurosci. 1993;4(6):473–484.
25. Traish AM. 5alpha-reductases in human physiology: An unfolding story. Endocr Pract. 2012;18(6):965–975.
26. Traish AM, Melcangi RC, Bortolato M, Garcia-Segura LM, Zitzmann M. Adverse effects of 5alpha-reductase inhibitors: What do we know, don't know, and need to know? Rev Endocr Metab Disord. 2015;16:177–198.

27. Paba S, Frau R, Godar SC, Devoto P, Marrosu F, Bortolato M. Steroid 5alpha-reductase as a novel therapeutic target for schizophrenia and other neuropsychiatric disorders. Curr Pharm Des. 2011;17(2):151–167.

28. 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(3):489–497.

29. Bull HG, Garcia-Calvo M, Andersson S, et al. Mechanism-based inhibition of human steroid 5α-reductase by finasteride: Enzyme-catalyzed formation of NADP—dihydrofinasteride, a potent bisubstrate analog inhibitor. J Am Chem Soc. 1996;118(10):2359–2365.

30. Ellsworth KP, Azzolina BA, Cimis G, Bull HG, Harris GS. Cloning, expression and characterization of rhesus macaque types 1 and 2 5alpha-reductase: Evidence for mechanism-based inhibition by finasteride. J Steroid Biochem Mol Biol. 1998;66(5–6):271–279.

31. Giatti S, Diviccaro S, Falvo E, Garcia-Segura LM, Melcangi RC. Physiopathological role of the enzymatic complex 5alpha-reductase and 3alpha/beta-hydroxysteroid oxidoreductase in the generation of progesterone and testosterone neuroactive metabolites. Front Neuroendocrinol. 2020;57:100836.

32. Martini L, Melcangi RC, Maggi R. Androgen and progesterone metabolism in the central and peripheral nervous system. J Steroid Biochem Mol Biol. 1993;47(1–6):195–205.

33. Stoffel-Wagner B, Beyenburg S, Watzka M, et al. Expression of 5alpha-reductase and 3alpha-hydroxisteroid oxidoreductase in the hippocampus of patients with chronic temporal lobe epilepsy. Epilepsia. 2000;41(2):140–147.

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

35. Baulieu EE, Robel P. Neurosteroids: A new brain function? J Steroid Biochem Mol Biol. 1990;37(3):395–403.

36. Mellon SH. Neurosteroid regulation of central nervous system development. Pharmacol Ther. 2007;116(1):107–124.

37. Benarroch EE. Neurosteroids: Endogenous modulators of neuronal excitability and plasticity. Neurology. 2007;68(12):945–947.

38. Belelli D, Lambert JJ. Neurosteroids: Endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci. 2005;6(7):565–575.

39. Carver CM, Reddy DS. Neurosteroid structure-activity relationships for functional activation of extrasynaptic deltaGABA(A) receptors. J Pharmacol Exp Ther. 2016;357(1):188–204.

40. Mellon SH. Neurosteroids: Biochemistry, modes of action, and clinical relevance. J Clin Endocrinol Metab. 1994;78(5):1003–1008.

41. Barbaccia ML, Serra M, Purdy RH, Biggio G. Stress and neuroactive steroids. Int Rev Neurobiol. 2001;46:243–272.

42. Dubrovsky B. [Neurobiology of depression and stress syndromes. Focus on active neurosteroids and neurosteroids]. Vertex. 2004;15(57):183–195.

43. Girdler SS, Klatzkin R. Neurosteroids in the context of stress: Implications for depressive disorders. Pharmacol Ther. 2007;116(1):125–139.

44. Troubat R, Barone P, Leman S, et al. Neuroinflammation and depression: A review. Eur J Neurosci. 2021;53(1):151–171.

45. Yang L, Tong Y, Chen PF, Miao S, Zhou RY. Neuroprotection of dihydrotestosterone via suppression of the toll-like receptor 4/nuclear factor-kappa B signaling pathway in high glucose-induced BV-2 microglia inflammatory responses. Neuroreport. 2020;31(2):139–147.

46. Yang L, Zhou R, Tong Y, et al. Neuroprotection by dihydrotestosterone in LPS-induced neuroinflammation. Neurobiol Dis. 2020;140:104814.

47. Yilmaz C, Karali K, Fodelianaki G, et al. Neurosteroids as regulators of neuroinflammation. Front Neuroendocrinol. 2019;55:100788.

48. Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE. Steroid 5alpha-reductase deficiency in man: An inherited form of male pseudohermaphroditism. Science. 1974;186(4170):1213–1215.

49. 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(27):19548–19554.

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

51. Stiles AR, Russell DW. SRD5A3: A surprising role in glycosylation. Cell. 2010;142(2):196–198.

52. Patte-Mensah C, Penning TM, Mensah-Nyagan AG. Anatomical and cellular localization of neuroactive 5 alpha/3 alpha-reduced steroid-synthesizing enzymes in the spinal cord. J Comp Neurol. 2004;477(3):286–299.

53. Melcangi RC, Celotti F, Ballabio M, Carnaghi R, Poletti A, Martini L. Effect of postnatal starvation on the 5 alpha-reductase activity of the brain and of the isolated myelin membranes. Exp Clin Endocrinol. 1989;94(3):253–261.

54. 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(3):1252–1259.

55. Melcangi RC, Celotti F, Martini L. Progesterone 5-alpha-reduction in neuronal and in different types of glial cell cultures: Type 1 and 2 astrocytes and oligodendrocytes. Brain Res. 1994;639(2):202–206.

56. Melcangi RC, Celotti F, Ballabio M, et al. 5 alpha-reductase activity in isolated and cultured neuronal and glial cells of the rat. Brain Res. 1990;516(2):229–236.

57. Melcangi RC, Celotti F, Ballabio M, Poletti A, Martini L. Testosterone metabolism in peripheral nerves: Presence of the 5 alpha-reductase-3 alpha-hydroxysteroid-dehydrogenase enzymatic system in the sciatic nerve of adult and aged rats. J Steroid Biochem. 1990;35(1):145–148.

58. Yokoi H, Tsuruo Y, Ishimura K. Steroid 5alpha-reductase type 1 immunolocalized in the rat peripheral nervous system and paraganglia. Histochem J. 1998;30(10):731–739.

59. Melcangi RC, Magnaghi V, Galbiati M, Martini L. Formation and effects of neuroactive steroids in the central and peripheral nervous system. Int Rev Neurobiol. 2001;46:145–176.

60. Schaeffer V, Meyer L, Patte-Mensah C, Mensah-Nyagan AG. Progress in dorsal root ganglion neurosteroidogenic activity: Basic evidence and pathophysiological correlation. Prog Neurobiol. 2010;92(1):33–41.

61. Gottfried-Blackmore A, Sierra A, Jellinck PH, McEwen BS, Bulloch K. Brain microglia express steroid-converting enzymes in the mouse. J Steroid Biochem Mol Biol. 2008;109(1–2):96–107.

62. Melcangi RC, Ballabio M, Magnaghi V, Celotti F. Metabolism of steroids in pure cultures of neurons and glial cells: Role of intracellular signalling. J Steroid Biochem Mol Biol. 1995;53(1–6):331–336.

63. Agis-Balboa RC, Pinna G, Zhubi A, et al. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proc Natl Acad Sci U S A. 2006;103(39):14602–14607.

64. Imperato-McGinley J. 5alpha-reductase-2 deficiency and complete androgen insensitivity: Lessons from nature. Adv Exp Med Biol. 2002;511:121–131; discussion 131–134.

65. Imperato-McGinley J, Zhu YS. Androgens and male physiology the syndrome of 5alpha-reductase-2 deficiency. Mol Cell Endocrinol. 2002;198(1–2):51–59.

66. Sasaki G, Nakagawa K, Hashiguchi A, Hasegawa T, Ogata T, Murai M. Giant seminoma in a patient with 5 alpha-reductase type 2 deficiency. J Urol. 2003;169(3):1080–1081.

67. Sasaki G, Ogata T, Ishii T, et al. Micropenis and the 5alpha-reductase-2 (SRD5A2) gene: Mutation and V89L polymorphism analysis in 81 Japanese patients. J Clin Endocrinol Metab. 2003;88(7):3431–3436.

68. Katz MD, Kligman I, Cai LQ, et al. Paternity by intrauterine insemination with sperm from a man with 5alpha-reductase-2 deficiency. N Engl J Med. 1997;336(14):994–997.

69. Cantagrel V, Lefeber DJ, Ng BG, et al. SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder. Cell. 2010;142(2):203–217.

70. Kasapkara CS, Tumer L, Ezgu FS, et al. SRD5A3-CDG: A patient with a novel mutation. Eur J Paediatr Neurol. 2012;16(5):554–556.

71. Grundahl JE, Guan Z, Rust S, et al. Life with too much polyprenol: Polyprenol reductase deficiency. Mol Genet Metab. 2012;105(4):642–651.

72. Morava E, Wevers RA, Cantagrel V, et al. A novel cerebello-ocular syndrome with abnormal glycosylation due to abnormalities in dolichol metabolism. Brain. 2010;133(11):3210–3220.

73. Caliskan M, Chong JX, Uricchio L, et al. Exome sequencing reveals a novel mutation for autosomal recessive non-syndromic mental retardation in the TECR gene on chromosome 19p13. Hum Mol Genet. 2011;20(7):1285–1289.

74. Castelli MP, Casti A, Casu A, et al. Regional distribution of 5alpha-reductase type 2 in the adult rat brain: An immunohistochemical analysis. Psychoneuroendocrinology. 2013;38(2):281–293.

75. Pozzi P, Bendotti C, Simeoni S, et al. Androgen 5-alpha-reductase type 2 is highly expressed and active in rat spinal cord motor neurones. J Neuroendocrinol. 2003;15(9):882–887.

76. Melcangi RC, Froelichsthal P, Martini L, Vescovi AL. Steroid metabolizing enzymes in pluripotential progenitor central nervous system cells: Effect of differentiation and maturation. Neuroscience. 1996;72(2):467–475.

77. Godoy A, Kawinski E, Li Y, et al. 5alpha-reductase type 3 expression in human benign and malignant tissues: A comparative analysis during prostate cancer progression. Prostate. 2011;71(10):1033–1046.

78. Uemura M, Tamura K, Chung S, et al. Novel 5 alpha-steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. Cancer Sci. 2008;99(1):81–86.

79. Nikolaou N, Hodson L, Tomlinson JW. The role of 5-reduction in physiology and metabolic disease: Evidence from cellular, pre-clinical and human studies. J Steroid Biochem Mol Biol. 2021;207:105808.

80. 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(3):1496–1502.

81. Morrison JF, Walsh CT. The behavior and significance of slow-binding enzyme inhibitors. Adv Enzymol Relat Areas Mol Biol. 1988;61:201–301.

82. Schmidt LJ, Tindall DJ. Steroid 5 alpha-reductase inhibitors targeting BPH and prostate cancer. J Steroid Biochem Mol Biol. 2011;125(1–2):32–38.

83. 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(5–6):273–279.

84. Thigpen AE, Cala KM, Russell DW. Characterization of Chinese hamster ovary cell lines expressing human steroid 5 alpha-reductase isozymes. J Biol Chem. 1993;268(23):17404–17412.

85. Jez JM, Bennett MJ, Schlegel BP, Lewis M, Penning TM. Comparative anatomy of the aldo-keto reductase superfamily. Biochem J. 1997;326 (Pt 3):625–636.

86. Jez JM, Flynn TG, Penning TM. A new nomenclature for the aldo-keto reductase superfamily. Biochem Pharmacol. 1997;54(6):639–647.

87. Jez JM, Penning TM. The aldo-keto reductase (AKR) superfamily: An update. Chem Biol Interact. 2001;130–132(1–3):499–525.

88. Penning TM, Pawlowski JE, Schlegel BP, et al. Mammalian 3 alpha-hydroxysteroid dehydrogenases. Steroids. 1996;61(9):508–523.

89. Penning TM. Molecular determinants of steroid recognition and catalysis in aldo-keto reductases. Lessons from 3alpha-hydroxysteroid dehydrogenase. J Steroid Biochem Mol Biol. 1999;69(1–6):211–225.

90. Penning TM, Jin Y, Heredia VV, Lewis M. Structure-function relationships in 3alpha-hydroxysteroid dehydrogenases: A comparison of the rat and human isoforms. J Steroid Biochem Mol Biol. 2003;85(2–5):247–255.

91. Degtiar WG, Kushlinsky NE. 3alpha-Hydroxysteroid dehydrogenase in animal and human tissues. Biochemistry (Mosc). 2001;66(3):256–266.

92. Qin KN, New MI, Cheng KC. Molecular cloning of multiple cDNAs encoding human enzymes structurally related to 3 alpha-hydroxysteroid dehydrogenase. J Steroid Biochem Mol Biol. 1993;46(6):673–679.

93. Khanna M, Qin KN, Cheng KC. Distribution of 3 alpha-hydroxysteroid dehydrogenase in rat brain and molecular cloning of multiple cDNAs encoding structurally related proteins in humans. J Steroid Biochem Mol Biol. 1995;53(1–6):41–46.

94. Khanna M, Qin KN, Wang RW, Cheng KC. Substrate specificity, gene structure, and tissue-specific distribution of multiple human 3 alpha-hydroxysteroid dehydrogenases. J Biol Chem. 1995;270(34):20162–20168.

95. Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev. 1997;18(3):281–305.

96. Penning TM, Burczynski ME, Jez JM, et al. Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: Functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochem J. 2000;351(Pt 1):67–77.

97. Pawlowski JE, Huizinga M, Penning TM. Cloning and sequencing of the cDNA for rat liver 3 alpha-hydroxysteroid/dihydrodiol dehydrogenase. J Biol Chem. 1991;266(14):8820–8825.

98. Stolz A, Rahimi-Kiani M, Ameis D, Chan E, Ronk M, Shively JE. Molecular structure of rat hepatic 3 alpha-hydroxysteroid dehydrogenase. A member of the oxidoreductase gene family. J Biol Chem. 1991;266(23):15253–15257.

99. Hoog SS, Pawlowski JE, Alzari PM, Penning TM, Lewis M. Three-dimensional structure of rat liver 3 alpha-hydroxysteroid/dihydrodiol dehydrogenase: A member of the aldo-keto reductase superfamily. Proc Natl Acad Sci U S A. 1994;91(7):2517–2521.

100. Bennett MJ, Schlegel BP, Jez JM, Penning TM, Lewis M. Structure of 3 alpha-hydroxysteroid/dihydrodiol dehydrogenase complexed with NADP+. Biochemistry. 1996;35(33):10702–10711.

101. Jin Y, Penning TM. Steroid 5alpha-reductases and 3alpha-hydroxysteroid dehydrogenases: Key enzymes in androgen metabolism. Best Pract Res Clin Endocrinol Metab. 2001;15(1):79–94.

102. Giatti S, Diviccaro S, Garcia-Segura LM, Melcangi RC. Sex differences in the brain expression of steroidogenic molecules under basal conditions and after gonadectomy. J Neuroendocrinol. 2019;31(6):e12736.

103. Krieger NR, Scott RG. 3 alpha-Hydroxysteroid oxidoreductase in rat brain. J Neurochem. 1984;42(3):887–890.

104. Li X, Bertics PJ, Karavolas HJ. Regional distribution of cytosolic and particulate 5alpha-dihydroprogesterone 3alpha-hydroxysteroid oxido-reductases in female rat brain. J Steroid Biochem Mol Biol. 1997;60(5–6): 311–318.

105. Gago N, Akwa Y, Sananes N, et al. Progesterone and the oligodendroglial lineage: Stage-dependent biosynthesis and metabolism. Glia. 2001; 36(3):295–308.

106. Wilson EM, French FS. Binding properties of androgen receptors. Evidence for identical receptors in rat testis, epididymis, and prostate. J Biol Chem. 1976;251(18):5620–5629.

107. Handa RJ, Pak TR, Kudwa AE, Lund TD, Hinds L. An alternate pathway for androgen regulation of brain function: Activation of estrogen receptor beta by the metabolite of dihydrotestosterone, 5alpha-androstane-3beta,17beta-diol. Horm Behav. 2008;53(5):741–752.

108. Melcangi RC, Garcia-Segura LM, Mensah-Nyagan AG. Neuroactive steroids: State of the art and new perspectives. Cell Mol Life Sci. 2008;65(5): 777–797.

109. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science. 1986;232(4753):1004–1007.

110. Paul SM, Purdy RH. Neuroactive steroids. FASEB J. 1992;6(6):2311–2322.

111. Puia G, Santi MR, Vicini S, et al. Neurosteroids act on recombinant human GABAA receptors. Neuron. 1990;4(5):759–765.

112. Lambert JJ, Belelli D, Hill-Venning C, Peters JA. Neurosteroids and GABAA receptor function. Trends Pharmacol Sci. 1995;16(9):295–303.

113. Cooke GM, Price CA, Oko RJ. Effects of in utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on serum androgens and steroidogenic enzyme activities in the male rat reproductive tract. J Steroid Biochem Mol Biol. 1998;67(4):347–354.

114. Morris JA, Jordan CL, Breedlove SM. Sexual differentiation of the vertebrate nervous system. Nat Neurosci. 2004;7(10):1034–1039.

115. Brandt N, Vierk R, Fester L, et al. Sex-specific difference of hippocampal synaptic plasticity in response to sex neurosteroids. Cereb Cortex. 2020; 30(4):2627–2641.

116. Islam MN, Sakimoto Y, Jahan MR, et al. Androgen affects the dynamics of intrinsic plasticity of pyramidal neurons in the CA1 hippocampal subfield in adolescent male rats. Neuroscience. 2020;440:15–29.

117. Tozzi A, Bellingacci L, Pettorossi VE. Rapid estrogenic and androgenic neurosteroids effects in the induction of long-term synaptic changes: Implication for early memory formation. Front Neurosci. 2020;14: 572511.

118. Huijgens PT, Snoeren EMS, Meisel RL, Mermelstein PG. Effects of gonadectomy and dihydrotestosterone on neuronal plasticity in motivation and reward related brain regions in the male rat. J Neuroendocrinol. 2021;33(1):e12918.

119. Dulka EA, Burger LL, Moenter SM. Ovarian androgens maintain high GnRH neuron firing rate in adult prenatally-androgenized female mice. Endocrinology. 2020;161(1):bqz038.

120. Maseroli E, Santangelo A, Lara-Fontes B, et al. The non-aromatizable androgen dihydrotestosterone (DHT) facilitates sexual behavior in ovariectomized female rats primed with estradiol. Psychoneuroendocrinology. 2020;115:104606.

121. Spritzer MD, Roy EA. Testosterone and adult neurogenesis. Biomolecules. 2020;10(2):225.

122. La Rosa P, Bartoli G, Farioli Vecchioli S, Cesari E, Pagliarini V, Sette C. Androgen receptor signaling promotes the neural progenitor cell pool in the developing cortex. J Neurochem. 2021;157(4):1153–1166.

123. Frye CA. Some rewarding effects of androgens may be mediated by actions of its 5alpha-reduced metabolite 3alpha-androstanediol. Pharmacol Biochem Behav. 2007;86(2):354–367.

124. Rosellini RA, Svare BB, Rhodes ME, Frye CA. The testosterone metabolite and neurosteroid 3alpha-androstanediol may mediate the effects of testosterone on conditioned place preference. Brain Res Brain Res Rev. 2001;37(1–3):162–171.

125. Lund TD, Munson DJ, Haldy ME, Handa RJ. Dihydrotestosterone may inhibit hypothalamo-pituitary-adrenal activity by acting through estrogen receptor in the male mouse. Neurosci Lett. 2004;365(1):43–47.

126. Lund TD, Munson DJ, Haldy ME, Handa RJ. Androgen inhibits, while oestrogen enhances, restraint-induced activation of neuropeptide neurones in the paraventricular nucleus of the hypothalamus. J Neuroendocrinol. 2004;16(3):272–278.

127. Lund TD, Hinds LR, Handa RJ. The androgen 5alpha-dihydrotestosterone and its metabolite 5alpha-androstan-3beta, 17beta-diol inhibit the hypothalamo-pituitary-adrenal response to stress by acting through estrogen receptor beta-expressing neurons in the hypothalamus. J Neurosci. 2006;26(5):1448–1456.

128. Magnaghi V, Ballabio M, Gonzalez LC, Leonelli E, Motta M, Melcangi RC. The synthesis of glycoprotein Po and peripheral myelin protein 22 in sciatic nerve of male rats is modulated by testosterone metabolites. Brain Res Mol Brain Res. 2004;126(1):67–73.

129. Magnaghi V, Cavarretta I, Zucchi I, et al. Po gene expression is modulated by androgens in the sciatic nerve of adult male rats. Brain Res Mol Brain Res. 1999;70(1):36–44.

130. Melcangi RC, Magnaghi V, Galbiati M, Ghelarducci B, Sebastiani L, Martini L. The action of steroid hormones on peripheral myelin proteins: A possible new tool for the rebuilding of myelin? J Neurocytol. 2000;29(5–6):327–339.

131. Caruso D, Pesaresi M, Abbiati F, et al. Comparison of plasma and cerebrospinal fluid levels of neuroactive steroids with their brain, spinal cord and peripheral nerve levels in male and female rats. Psychoneuroendocrinology. 2013;38(10):2278–2290.

132. Cohen RE, Wade J. Distribution of two isozymes of 5alpha-reductase in the brains of adult male and female green anole lizards. Brain Behav Evol. 2010;76(3–4):279–288.

133. Caruso D, Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Melcangi RC. Effects of short- and long-term gonadectomy on neuroactive steroid levels in the central and peripheral nervous system of male and female rats. J Neuroendocrinol. 2010;22:1137–1147.

134. Liere P, Cornil CA, de Bournonville MP, et al. Steroid profiles in quail brain and serum: Sex and regional differences and effects of castration with steroid replacement. J Neuroendocrinol. 2019;31(2):e12681.

135. Dichtel LE, Lawson EA, Schorr M, et al. Neuroactive steroids and affective symptoms in women across the weight spectrum. Neuropsychopharmacology. 2018;43(6):1436–1444.

136. Agis-Balboa RC, Guidotti A, Pinna G. 5alpha-reductase type I expression is downregulated in the prefrontal cortex/Brodmann's area 9 (BA9) of depressed patients. Psychopharmacology (Berl). 2014;231(17):3569–3580.

137. Sanchez P, Castro B, Torres JM, Ortega E. Effects of different ethanol-administration regimes on mRNA and protein levels of steroid 5alpha-reductase isozymes in prefrontal cortex of adolescent male rats. Psychopharmacology (Berl). 2014;231(17):3273–3280.

138. Milivojevic V, Kranzler HR, Gelernter J, Burian L, Covault J. Variation in genes encoding the neuroactive steroid synthetic enzymes 5alpha-reductase type 1 and 3alpha-reductase type 2 is associated with alcohol dependence. Alcohol Clin Exp Res. 2011;35(5):946–952.

139. Biamonte F, Assenza G, Marino R, et al. Interactions between neuroactive steroids and reelin haploinsufficiency in Purkinje cell survival. Neurobiol Dis. 2009;36(1):103–115.

140. Zettergren A, Jonsson L, Johansson D, et al. Associations between polymorphisms in sex steroid related genes and autistic-like traits. Psychoneuroendocrinology. 2013;38(11):2575–2584.

141. Mosher LJ, Godar SC, Morissette M, et al. Steroid 5alpha-reductase 2 deficiency leads to reduced dominance-related and impulse-control behaviors. Psychoneuroendocrinology. 2018;91:95–104.

142. Lopez-Rodriguez AB, Acaz-Fonseca E, Giatti S, et al. Correlation of brain levels of progesterone and dehydroepiandrosterone with neurological recovery after traumatic brain injury in female mice. Psychoneuroendocrinology. 2015;56:1–11.

143. Lopez-Rodriguez AB, Acaz-Fonseca E, Spezzano R, et al. Profiling neuroactive steroid levels after traumatic brain injury in male mice. Endocrinology. 2016;157(10):3983–3993.

144. Meffre D, Pianos A, Liere P, et al. Steroid profiling in brain and plasma of male and pseudopregnant female rats after traumatic brain injury: Analysis by gas chromatography/mass spectrometry. Endocrinology. 2007;148(5):2505–2517.

145. Mollayeva T, Mollayeva S, Colantonio A. Traumatic brain injury: Sex, gender and intersecting vulnerabilities. Nat Rev Neurol. 2018;14(12):711–722.

146. Sawal N, Kaur J, Kaur K, Gombar S. Dihydrotestosterone in amyotrophic lateral sclerosis-the missing link? Brain Behav. 2020;10(11):e01645.

147. Luchetti S, Bossers K, Frajese GV, Swaab DF. Neurosteroid biosynthetic pathway changes in substantia nigra and caudate nucleus in Parkinson's disease. Brain Pathol. 2010;20(5):945–951.

148. Bourque M, Morissette M, Al Sweidi S, Caruso D, Melcangi RC, Di Paolo T. Neuroprotective effect of progesterone in MPTP-treated male mice. Neuroendocrinology. 2016;103(3–4):300–314.

149. Caruso D, Melis M, Fenu G, et al. Neuroactive steroid levels in plasma and cerebrospinal fluid of male multiple sclerosis patients. J Neurochem. 2014;130(4):591–597.

150. Leicaj ML, Pasquini LA, Lima A, et al. Changes in neurosteroidogenesis during demyelination and remyelination in cuprizone-treated mice. J Neuroendocrinol. 2018;30(11):e12649.

151. Giatti S, D'Intino G, Maschi O, et al. Acute experimental autoimmune encephalomyelitis induces sex dimorphic changes in neuroactive steroid levels. Neurochem Int. 2010;56:118–127.

152. Caruso D, D'Intino G, Giatti S, et al. Sex-dimorphic changes in neuroactive steroid levels after chronic experimental autoimmune encephalomyelitis. J Neurochem. 2010;114(3):921–932.

153. Dooley MA, Hogan SL. Environmental epidemiology and risk factors for autoimmune disease. Curr Opin Rheumatol. 2003;15(2):99–103.

154. Gleicher N, Barad DH. Gender as risk factor for autoimmune diseases. J Autoimmun. 2007;28(1):1–6.

155. Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. Clin Immunol Immunopathol. 1997;84(3):223–243.

156. Golden LC, Voskuhl R. The importance of studying sex differences in disease: The example of multiple sclerosis. J Neurosci Res. 2017;95(1–2):633–643.

157. Giatti S, Mastrangelo R, D'Antonio M, et al. Neuroactive steroids and diabetic complications in the nervous system. Front Neuroendocrinol. 2018;48:58–69.

158. Pesaresi M, Giatti S, Calabrese D, Maschi O, Caruso D, Melcangi RC. Dihydroprogesterone increases the gene expression of myelin basic protein in spinal cord of diabetic rats. J Mol Neurosci. 2010;42(2):135–139.

159. Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Caruso D, Melcangi RC. Sex differences in neuroactive steroid levels in the nervous system of diabetic and non-diabetic rats. Horm Behav. 2010;57(1):46–55.

160. Calabrese D, Giatti S, Romano S, et al. Diabetic neuropathic pain: A role for testosterone metabolites. J Endocrinol. 2014;221(1):1–13.

161. Romano S, Mitro N, Diviccaro S, et al. Short-term effects of diabetes on neurosteroidogenesis in the rat hippocampus. J Steroid Biochem Mol Biol. 2017;167:135–143.

162. Romano S, Mitro N, Giatti S, et al. Diabetes induces mitochondrial dysfunction and alters cholesterol homeostasis and neurosteroidogenesis in the rat cerebral cortex. J Steroid Biochem Mol Biol. 2018;178:108–116.

163. Giatti S, Diviccaro S, Melcangi RC. Neuroactive steroids and sex-dimorphic nervous damage induced by diabetes mellitus. Cell Mol Neurobiol. 2019;39(4):493–502.

164. Pesaresi M, Giatti S, Spezzano R, et al. Axonal transport in a peripheral diabetic neuropathy model: Sex-dimorphic features. Biol Sex Differ. 2018;9(1):6.

165. Roglio I, Bianchi R, Gotti S, et al. Neuroprotective effects of dihydroprogesterone and progesterone in an experimental model of nerve crush injury. Neuroscience. 2008;155(3):673–685.

166. Caruso D, Scurati S, Roglio I, Nobbio L, Schenone A, Melcangi RC. Neuroactive Steroid Levels in a transgenic rat model of CMT1A Neuropathy. J Mol Neurosci. 2008;34(3):249–253.

167. Domonkos E, Hodosy J, Ostatnikova D, Celec P. On the role of testosterone in anxiety-like behavior across life in experimental rodents. Front Endocrinol (Lausanne). 2018;9:441.

168. Brunton PJ, Donadio MV, Yao ST, et al. 5alpha-Reduced neurosteroids sex-dependently reverse central prenatal programming of neuroendocrine stress responses in rats. J Neurosci. 2015;35(2):666–677.

169. Khasnavis S, Ghosh A, Roy A, Pahan K. Castration induces Parkinson disease pathologies in young male mice via inducible nitric-oxide synthase. J Biol Chem. 2013;288(29):20843–20855.

170. Bourque M, Dluzen DE, Di Paolo T. Neuroprotective actions of sex steroids in Parkinson's disease. Front Neuroendocrinol. 2009;30(2):142–157.

171. Bebo BF, Jr., Schuster JC, Vandenbark AA, Offner H. Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. J Immunol. 1999;162(1):35–40.

172. Palaszynski KM, Loo KK, Ashouri JF, Liu HB, Voskuhl RR. Androgens are protective in experimental autoimmune encephalomyelitis: Implications for multiple sclerosis. J Neuroimmunol. 2004;146(1–2):144–152.

173. Dalal M, Kim S, Voskuhl RR. Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. J Immunol. 1997;159(1):3–6.

174. Giatti S, Rigolio R, Romano S, et al. Dihydrotestosterone as a protective agent in chronic experimental autoimmune encephalomyelitis. Neuroendocrinology. 2015;101:296–308.

175. Barreto G, Veiga S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: Role of its metabolites, oestradiol and dihydrotestosterone. Eur J Neurosci. 2007;25(10):3039–3046.

176. Ramsden M, Shin TM, Pike CJ. Androgens modulate neuronal vulnerability to kainate lesion. Neuroscience. 2003;122(3):573–578.

177. Nguyen TV, Jayaraman A, Quaglino A, Pike CJ. Androgens selectively protect against apoptosis in hippocampal neurones. J Neuroendocrinol. 2010;22(9):1013–1022.

178. Zhang Y, Champagne N, Beitel LK, Goodyer CG, Trifiro M, LeBlanc A. Estrogen and androgen protection of human neurons against intracellular amyloid beta1–beta42 toxicity through heat shock protein 70. J Neurosci. 2004;24(23):5315–5321.

179. Hammond J, Le Q, Goodyer C, Gelfand M, Trifiro M, LeBlanc A. Testosterone-mediated neuroprotection through the androgen receptor in human primary neurons. J Neurochem. 2001;77(5):1319–1326.

180. Huppenbauer CB, Tanzer L, DonCarlos LL, Jones KJ. Gonadal steroid attenuation of developing hamster facial motoneuron loss by axotomy: Equal efficacy of testosterone, dihydrotestosterone, and 17-beta estradiol. J Neurosci. 2005;25(16):4004–4013.

181. Sengelaub DR, Han Q, Liu NK, et al. Protective effects of estradiol and dihydrotestosterone following spinal cord injury. J Neurotrauma. 2018;35(6):825–841.

182. Quillinan N, Deng G, Grewal H, Herson PS. Androgens and stroke: Good, bad or indifferent? Exp Neurol. 2014;259:10–15.

183. Mendell AL, Chung BYT, Creighton CE, Kalisch BE, Bailey CDC, MacLusky NJ. Neurosteroid metabolites of testosterone and progesterone differentially inhibit ERK phosphorylation induced by amyloid beta in SH-SY5Y cells and primary cortical neurons. Brain Res. 2018;1686:83–93.

184. Mendell AL, MacLusky NJ. The testosterone metabolite 3alpha-androstanediol inhibits oxidative stress-induced ERK phosphorylation and neurotoxicity in SH-SY5Y cells through an MKP3/DUSP6-dependent mechanism. Neurosci Lett. 2019;696:60–66.

185. Zup SL, Edwards NS, McCarthy MM. Sex- and age-dependent effects of androgens on glutamate-induced cell death and intracellular calcium regulation in the developing hippocampus. Neuroscience. 2014;281C:77–87.

186. Vita G, Dattola R, Girlanda P, Oteri G, Lo Presti F, Messina C. Effects of steroid hormones on muscle reinnervation after nerve crush in rabbit. Exp Neurol. 1983;80(2):279–287.

187. Yu WH. Effect of testosterone on the regeneration of the hypoglossal nerve in rats. Exp Neurol. 1982;77(1):129–141.

188. Tanzer L, Jones KJ. Neurotherapeutic action of testosterone on hamster facial nerve regeneration: Temporal window of effects. Horm Behav. 2004;45(5):339–344.

189. Jones KJ, Brown TJ, Damaser M. Neuroprotective effects of gonadal steroids on regenerating peripheral motoneurons. Brain Res Brain Res Rev. 2001;37(1–3):372–382.

190. Roglio I, Bianchi R, Giatti S, et al. Testosterone derivatives are neuroprotective agents in experimental diabetic neuropathy. Cell Mol Life Sci. 2007;64(9):1158–1168.

191. Mitro N, Cermenati G, Brioschi E, et al. Neuroactive steroid treatment modulates myelin lipid profile in diabetic peripheral neuropathy. J Steroid Biochem Mol Biol. 2014;143:115–121.

192. Patte-Mensah C, Meyer L, Schaeffer V, Mensah-Nyagan AG. Selective regulation of 3 alpha-hydroxysteroid oxido-reductase expression in dorsal root ganglion neurons: A possible mechanism to cope with peripheral nerve injury-induced chronic pain. Pain. 2010;150(3):522–534.

193. Meyer L, Taleb O, Patte-Mensah C, Mensah-Nyagan AG. Neurosteroids and neuropathic pain management: Basic evidence and therapeutic perspectives. Front Neuroendocrinol. 2019;55:100795.

194. Meyer L, Patte-Mensah C, Taleb O, Mensah-Nyagan AG. Neurosteroid 3alpha-androstanediol efficiently counteracts paclitaxel-induced peripheral neuropathy and painful symptoms. PLoS One. 2013;8(11):e80915.

195. Hussain R, Ghoumari AM, Bielecki B, et al. The neural androgen receptor: A therapeutic target for myelin repair in chronic demyelination. Brain. 2013;136(Pt 1):132–146.

196. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell. 2010;140(6):918–934.

197. Tansey MG, Goldberg MS. Neuroinflammation in Parkinson's disease: Its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis. 2010;37(3):510–518.

198. Tansey MG. Inflammation in neuropsychiatric disease. Neurobiol Dis. 2010;37(3):491–492.

199. Wee Yong V. Inflammation in neurological disorders: A help or a hindrance? Neuroscientist. 2010;16(4):408–420.

200. Wuwongse S, Chang RC, Law AC. The putative neurodegenerative links between depression and Alzheimer's disease. Prog Neurobiol. 2010;91(4):362–375.

201. Meyer U, Schwarz MJ, Muller N. Inflammatory processes in schizophrenia: A promising neuroimmunological target for the treatment of negative/cognitive symptoms and beyond. Pharmacol Ther. 2011;132(1):96–110.

202. Papadopoulos V, Amri H, Li H, Boujrad N, Vidic B, Garnier M. Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. J Biol Chem. 1997;272(51):32129–32135.

203. Da Pozzo E, Giacomelli C, Costa B, et al. TSPO PIGA ligands promote neurosteroidogenesis and human astrocyte well-being. Int J Mol Sci. 2016;17(7).

204. Papadopoulos V. On the role of the translocator protein (18-kDa) TSPO in steroid hormone biosynthesis. Endocrinology. 2014;155(1):15–20.

205. Papadopoulos V, Fan J, Zirkin B. Translocator protein (18 kDa): An update on its function in steroidogenesis. J Neuroendocrinol. 2018;30(2):10.1111/jne.12500.

206. Daugherty DJ, Selvaraj V, Chechneva OV, Liu XB, Pleasure DE, Deng W. A TSPO ligand is protective in a mouse model of multiple sclerosis. EMBO Mol Med. 2013;5(6):891–903.

207. Leva G, Klein C, Benyounes J, et al. The translocator protein ligand XBD173 improves clinical symptoms and neuropathological markers in the SJL/J mouse model of multiple sclerosis. Biochim Biophys Acta Mol Basis Dis. 2017;1863(12):3016–3027.

208. Miao YL, Guo WZ, Shi WZ, et al. Midazolam ameliorates the behavior deficits of a rat posttraumatic stress disorder model through dual 18 kDa translocator protein and central benzodiazepine receptor and neurosteroidogenesis. PLoS One. 2014;9(7):e101450.

209. Shang C, Guo Y, Yao JQ, et al. Rapid anti-PTSD-like activity of the TSPO agonist YL-IPA08: Emphasis on brain GABA, neurosteroids and HPA axis function. Behav Brain Res. 2019:112320.

210. Zhang LM, Wang YL, Liu YQ, et al. Antidepressant-like effects of YL-IPA08, a potent ligand for the translocator protein (18 kDa) in chronically stressed rats. Neuropharmacology. 2017;113(Pt A):567–575.

211. Yao JQ, Liu C, Jin ZL, et al. Serotonergic transmission is required for the anxiolytic-like behavioral effects of YL-IPA08, a selective ligand targeting TSPO. Neuropharmacology. 2020;178:108230.

212. Ishikawa M, Yoshitomi T, Covey DF, Zorumski CF, Izumi Y. TSPO activation modulates the effects of high pressure in a rat ex vivo glaucoma model. Neuropharmacology. 2016;111:142–159.

213. Mitro N, Cermenati G, Giatti S, et al. LXR and TSPO as new therapeutic targets to increase the levels of neuroactive steroids in the central nervous system of diabetic animals. Neurochem Int. 2012;60(6):616–621.

214. Giatti S, Pesaresi M, Cavaletti G, et al. Neuroprotective effects of a ligand of translocator protein-18kDa (Ro5-4864) in experimental diabetic neuropathy. Neuroscience. 2009;164:520–529.

215. Cheng O, Ostrowski RP, Liu W, Zhang JH. Activation of liver X receptor reduces global ischemic brain injury by reduction of nuclear factor-kappaB. Neuroscience. 2010;166(4):1101–1109.

216. Paterniti I, Campolo M, Siracusa R, et al. Liver X receptors activation, through TO901317 binding, reduces neuroinflammation in Parkinson's disease. PLoS One. 2017;12(4):e0174470.

217. Xu P, Li D, Tang X, et al. LXR agonists: New potential therapeutic drug for neurodegenerative diseases. Mol Neurobiol. 2013;48(3):715–728.

218. Cermenati G, Giatti S, Cavaletti G, et al. Activation of the liver X receptor increases neuroactive steroid levels and protects from diabetes-induced peripheral neuropathy. J Neurosci. 2010;30(36):11896–11901.

219. Rodriguez-Lozano DC, Velazquez-Vazquez DE, Del Moral-Morales A, Camacho-Arroyo I. Dihydrotestosterone induces proliferation, migration, and invasion of human glioblastoma cell lines. Onco Targets Ther. 2020;13:8813–8823.

220. Martinez PE, Rubinow DR, Nieman LK, et al. 5alpha-reductase inhibition prevents the luteal phase increase in plasma allopregnanolone levels and mitigates symptoms in women with premenstrual dysphoric disorder. Neuropsychopharmacology. 2016;41(4):1093–1102.

221. Tanaka M, Ogaeri T, Samsonov M, Sokabe M. The 5alpha-reductase inhibitor finasteride exerts neuroprotection against ischemic brain injury in aged male rats. Transl Stroke Res. 2019;10(1):67–77.

222. Li L, Kang YX, Ji XM, et al. Finasteride inhibited brain dopaminergic system and open-field behaviors in adolescent male rats. CNS Neurosci Ther. 2018;24(2):115–125.

223. Orozco M, Valdez RA, Ramos L, Cabeza M, Segovia J, Romano MC. Dutasteride combined with androgen receptor antagonists inhibit glioblastoma U87 cell metabolism, proliferation, and invasion capacity: Androgen regulation. Steroids. 2020;164:108733.

224. Irwig MS. Depressive symptoms and suicidal thoughts among former users of finasteride with persistent sexual side effects. J Clin Psychiatry. 2012;73(9):1220–1223.

225. Irwig MS. Persistent sexual side effects of finasteride: Could they be permanent? J Sex Med. 2012;9(11):2927–2932.

226. Giatti S, Diviccaro S, Panzica G, Melcangi RC. Post-finasteride syndrome and post-SSRI sexual dysfunction: Two sides of the same coin? Endocrine. 2018;2(61):180–193.

227. Diviccaro S, Melcangi RC, Giatti S. Post-finasteride syndrome: An emerging clinical problem. Neurobiol Stress. 2020:100209.

228. Melcangi RC, Santi D, Spezzano R, et al. Neuroactive steroid levels and psychiatric and andrological features in post-finasteride patients. J Steroid Biochem Mol Biol. 2017;171:229–235.

229. Melcangi RC, Casarini L, Marino M, et al. Altered methylation pattern of the SRD5A2 gene in the cerebrospinal fluid of post-finasteride patients: A pilot study. Endocr Connect. 2019;8(8):1118–1125.

230. Borgo F, Macandog AD, Diviccaro S, et al. Alterations of gut microbiota composition in post-finasteride patients: A pilot study. J Endocrinol Invest. 2021;44(6):1263–1273.

Abbreviations Used

3α-HSOR	= 3α-hydroxysteroid oxidoreductase
3β-HSOR	= 3β-hydroxysteroid oxidoreductase
3α/3β-HSORs	= 3α/3β-hydroxysteroid oxidoreductases
5α-DHT	= 5α-dihydrotestosterone
5α-R	= 5α-reductase
ALS	= amyotrophic lateral sclerosis
AR	= androgen receptor
CMT	= Charcot-Marie-Tooth
CNS	= central nervous system
CSF	= cerebrospinal fluid
DA	= dopamine
EAE	= experimental autoimmune encephalomyelitis
ER	= estrogen receptor
GABA	= gamma-aminnobutyric acid
LXRs	= liver X receptors
MPTP	= 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	= multiple sclerosis
NADPH	= nicotine amide adenine dinucleotide phosphate
PD	= Parkinson's disease
PNS	= peripheral nervous system
PROG	= progesterone
STZ	= streptozotocin
TBI	= traumatic brain injury
THDOC	= tetrahydrodeoxycorticosterone
THP	= tetrahydroprogesterone
TSPO	= translocator protein of 18 kDa