Overview of the Molecular Steps in Steroidogenesis of the GABAergic Neurosteroids Allopregnanolone and Pregnanolone

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Abstract
Allopregnanolone and pregnanolone—neurosteroids synthesized from progesterone in the brain, adrenal gland, ovary and testis—have been implicated in a range of neuropsychiatric conditions including seizure disorders, post-traumatic stress disorder, major depression, post-partum depression, pre-menstrual dysphoric disorder, chronic pain, Parkinson’s disease, Alzheimer’s disease, neurotrauma, and stroke. Allopregnanolone and pregnanolone equipotently facilitate the effects of gamma-amino-butyric acid (GABA) at GABA A receptors, and when sulfated, antagonize N-methyl-D-aspartate receptors. They play myriad roles in neurophysiological homeostasis and adaptation to stress while exerting anxiolytic, antidepressant, anti-nociceptive, anticonvulsant, anti-inflammatory, sleep promoting, memory stabilizing, neuroprotective, pro-myelinating, and neurogenic effects. Given that these neurosteroids are synthesized de novo on demand, this review details the molecular steps involved in the biochemical conversion of cholesterol to allopregnanolone and pregnanolone within steroidogenic cells. Although much is known about the early steps in neurosteroidogenesis, less is known about transcriptional, translational, and post-translational processes in allopregnanolone- and pregnanolone-specific synthesis. Further research to elucidate these mechanisms as well as to optimize the timing and dose of interventions aimed at altering the synthesis or levels of these neurosteroids is much needed. This should include the development of novel therapeutics for the many neuropsychiatric conditions to which dysregulation of these neurosteroids contributes.

Keywords
allopregnanolone, steroidogenesis, biosynthesis, transcriptional regulation, post-traumatic stress disorder

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Introduction
Allopregnanolone (3α-hydroxy-5α-pregn-20-one) and pregnanolone (3α-hydroxy-5β-pregn-20-one) are neurosteroid metabolites of progesterone that equipotently and positively modulate the action of gamma-aminobutyric acid (GABA) at GABA A synaptic and extrasynaptic receptors. At nanomolar doses, these stereoisomers increase the effects of GABA on GABA A receptor-mediated chloride ion influx ~7 to 10 times, thus markedly enhancing the inhibitory impact of GABA on neuronal firing.1–6 In contrast, when sulfated, allopregnanolone and pregnanolone potently antagonize N-methyl-D-aspartate (NMDA) receptors. In addition, allopregnanolone and pregnanolone play a role in regulating GABA A receptor number and subunit composition, further shaping the dynamic balance between inhibitory and excitatory neuronal signaling.1

The effects of allopregnanolone and pregnanolone on GABA neurotransmission are thought to be responsible for their potent antidepressant, anxiolytic, anti-conflict, anticonvulsant, anti-nociceptive, anesthetic, sleep-promoting, and memory modulating effects. In addition to these pharmacological effects, these neurosteroids can

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suppress inflammation, reduce apoptosis, and promote neurogenesis and myelination.\textsuperscript{7–11} At a higher translational level, such neuroprotective effects manifest as reduced infarct volume and cerebral edema after cerebral ischemia, delays in neuronal death after traumatic brain injury or in the context of Niemann-Pick type C disease,\textsuperscript{2,12} and lastly as interruption and reversal of neurodegenerative processes associated with Parkinson’s and Alzheimer’s disease.\textsuperscript{13,14}

However, preclinical and clinical research suggests that high as well as low levels of these neurosteroids may be associated with phenotypically similar neuropsychiatric disorders.\textsuperscript{15–17} Even apparently normal fluctuations in these neurosteroids that induce changes in GABA\textsubscript{A} receptor subunit composition can be associated with dysphoria and depressive symptoms during the post-partum period or luteal phase of the menstrual cycle,\textsuperscript{18–21} while subnormal increases of their synthesis in response to stress occur across the menstrual cycle in women with post-traumatic stress disorder (PTSD),\textsuperscript{15} which shares many clinical symptoms with premenstrual dysphoric disorder (PMDD). PTSD is also associated with substantially increased risk for PMDD.\textsuperscript{9}

Given the manifold neurophysiological roles of allopregnanolone and pregnanolone, as well as their dysregulation in a range of disorders for which treatments warrant improvement, a review of the complex molecular pathways by which these GABAergic neurosteroids are synthesized and released may highlight opportunities for the development of novel precision medicine-based interventions for these disorders. This review and series of graphics thus were developed from the extant literature characterizing molecular regulatory factors and key enzymes that interact in steroidogenic cells from a variety of tissues, including the adrenal gland, brain, ovary, and testis, to produce allopregnanolone and pregnanolone.

**Overview of Steroid Biosynthesis Leading to Allopregnanolone and Pregnanolone**

Due to their lipophilic nature, steroids such as allopregnanolone and pregnanolone cannot be stored in steroidogenic cells for later release; thus, nearly all circulating steroid hormones are synthesized de novo and diffuse out of the cell. This accounts for the delayed peaking of blood and brain steroid levels in response to tissue-specific stimulation of neurosteroidogenesis by adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), or NMDA receptor activation\textsuperscript{22,23} as cells must activate and increase their steroidogenic capacity on demand. Therefore, understanding the mechanisms by which allopregnanolone is synthesized is necessary to understand when and how allopregnanolone and pregnanolone function. The schematic in Figure 1 summarizes the events of allopregnanolone synthesis, which are

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic depicting the steps in the steroidogenesis of allopregnanolone and pregnanolone starting with uptake or synthesis of cholesterol and ending with allopregnanolone and pregnanolone diffusion out of the cell. Dashed lines represent movement, whereas solid lines represent a catalytic reaction. OMM: outer mitochondrial membrane; IMM: inner mitochondrial membrane; IMS: intermembrane space; S\textsubscript{x}-DHP: S\textsubscript{x}-dihydroprogesterone; S\textsubscript{\beta}-DHP: S\textsubscript{\beta}-dihydroprogesterone.}
\end{figure}
amino acids capable of binding lipids. Among the 15 described below) and contain a domain of 210 conserved steroidogenic acute regulatory protein (StAR, lipid transfer (START) domain proteins are related to Steroidogenic acute regulatory protein (StAR)-related proteins in the human START family, StarD4 and StarD5 are cytosolic proteins that bind cholesterol with high specificity and affinity in steroidogenic cells. StarD4 has been co-localized with Acyl-CoA cholesterol acyltransferase (ACAT), which is responsible for catalyzing the reversible conversion of free cholesterol into cholesterol ester, suggesting a role for these closely related START domain proteins in transporting cholesterol throughout the cytosol, including to the outer mitochondrial membrane (OMM). In addition, activation of steroidogenic cells induces changes in mitochondrial morphology that enable a greater number of close contacts between mitochondria and the endoplasmic reticulum. The resulting domains of the endoplasmic reticulum, called mitochondria-associated membrane (MAM), are necessary for cholesterol transfer to steroidogenic mitochondria.

Cholesterol Synthesis and Import

All steroids are synthesized from a common cholesterol precursor. Human steroidogenic cells source their cholesterol from two processes: uptake of cholesterol from low density lipoproteins (LDL) via receptor-mediated endocytosis or de novo cholesterol synthesis. It is thought that the majority of the cholesterol used in steroid biosynthesis originates from LDLs. Steroidogenic cells activated by trophic hormones upregulate the presence of LDL receptors on their cellular surface in order to endocytose a greater volume of LDLs, which are rich in cholesteryl ester. These LDLs are broken down in lysosomes to release the cholesteryl esters, which are further processed by lysosomal acid lipase (LAL) into cholesterol for use in steroidogenesis. Free cholesterol is then transferred from lysosomes via the soluble glycoprotein Niemann Pick type C 2 (NPC2) to the transmembrane protein Niemann Pick type C 1 (NPC1), which inserts the cholesterol into the lysosomal membrane for eventual delivery throughout the cytoplasm.

Alternatively, cells may synthesize cholesterol de novo from three acetyl CoA molecules. This reaction, catalyzed by 3-hydroxy-3-methylglutaryl CoA (HMGCoA) reductase, represents the committed, rate-limiting step for cholesterol biosynthesis; activated steroidogenic cells upregulate this enzyme. Although LDL endocytosis is conventionally thought to be the main source of cholesterol for steroidogenesis, patients with low LDL levels—such as those with congenital abetalipoproteinemia—have normal basal cortisol production and normal, or mildly impaired cortisol secretion in response to ACTH. This suggests that de novo cholesterol synthesis may be adequate to provide the precursors for steroidogenesis under normal conditions; it is not clear whether such individuals maintain adequate cortisol production during extreme or chronic stress, conditions under which compensation for impaired steroid production at the single cell level may be compensated by hyperplasia of the steroidogenic tissue.

Intracellular Transport of Cholesterol to Mitochondria

Steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain proteins are related to the steroidogenic acute regulatory protein (StAR, described below) and contain a domain of 210 conserved amino acids capable of binding lipids. Among the 15 proteins in the human START family, StarD4 and StarD5 are cytosolic proteins that bind cholesterol with high specificity and affinity in steroidogenic cells.
into the cytosol.\textsuperscript{28,49,51} TSPO is enriched at contact sites
between the OMM and IMM.\textsuperscript{28} Although TSPO is ini-
tially inserted into the OMM as a monomer, it is often
found in polymeric form, in complex with many other
proteins.\textsuperscript{28,49,52}

One hypothesis suggests that the aforementioned pro-
tein complexes involved in cholesterol transport collaborate:
StarD4/5 delivers cholesterol to the OMM where StAR
is located.\textsuperscript{53} Subsequently, StAR binds this cholesterol
and was thought to transfer it to TSPO.\textsuperscript{28} As TSPO is
localized to OMM-IMM contact sites, TSPO could move
cholesterol from the OMM to the IMM, a hypothesis
established by studies in vitro as well as a single in vivo
embryonic lethal knockout mouse study.\textsuperscript{28,52,54} However,
more recent studies with TSPO knockout mice demonstrat-
ed that loss of TSPO did not affect steroidogenesis or
gross aspect of development or behavior in compari-
son to wild-type mice, suggesting that TSPO does not
play an essential role in this process.\textsuperscript{55–57} Further-
ther research has demonstrated that a reduction in TSPO
does not affect gonadal steroidogenesis but significantly
impairs adrenal steroidogenesis.\textsuperscript{58} Given its intracellular
localization to the OMM, high expression in steroido-
genic tissue, and conflicting results from multiple research
groups, further research will need to be done to elucidate
the purpose of TSPO.

Nevertheless, once inserted into the IMM by a now
uncertain mechanism, the pools of cholesterol mobilized
for steroidogenesis remain segregated from that for struc-
tural cholesterol\textsuperscript{28,59,60} and undergo the first committed
step for steroidogenesis described below.\textsuperscript{28,60}

**P450 Side Chain Cleavage**

P450 side chain cleavage (P450scc; also known as
CYP11A1), located on the IMM, catalyzes the first com-
mitted step of steroidogenesis. P450scc transforms chole-
sterol into pregnenolone via a series of three distinct
chemical reactions eventuating in loss of the hydrophobic
six-carbon side chain of cholesterol (hence the name of
chemical reactions eventuating in loss of the hydrophobic
esterol into pregnenolone via a series of three distinct
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CYP11A1), located on the IMM, catalyzes the first com-
mitted step of steroidogenesis described below.\textsuperscript{28,51}

The first reaction involves 22-hydroxylation of cholesterol bound to P450scc.\textsuperscript{24} Due to the time
required for the sequence of electron transport events,
the binding of cholesterol, and the 22-hydroxylation of
cholesterol, this first P450scc reaction constitutes the rate-
limited step in steroidogenesis in which approximately
20 molecules of cholesterol are processed by each
P450scc molecule per second.\textsuperscript{24,61} In the second step,
the product of the first reaction undergoes 20-hydroxy-
lization.\textsuperscript{54} This is followed by the third step during which the
C20-22 bond is cleaved to produce isocaproaldehyde and
pregnenolone (Figure 1).\textsuperscript{24}

**3β Hydroxysteroid Dehydrogenase Type I and Type 2**

In the context of allopregnanolone and pregnanolone
synthesis, 3β hydroxysteroid dehydrogenase (3β-HSD)
is responsible for transforming pregnenolone into proges-
terone.\textsuperscript{62} 3β-HSD exists in two isoforms (3β-HSD1 and
3β-HSD2), which share 93% homology and a similar
quaternary structure except for key residues that confer
different ligand specificities.\textsuperscript{63} The 3β-HSD2 isoform is
thought to be the most relevant for steroid biosynthesis,
as it is expressed in the adrenals, ovaries, and testes—all
traditionally steroidogenic tissues.\textsuperscript{63} However, the 3β-
HSD1 isoform is found in non-steroidogenic tissues,\textsuperscript{63}
and therefore may play a role in steroidogenesis in the
central nervous system (CNS). This is important as 3β-
HSD1 has a significantly higher affinity for common sub-
strates, coenzymes, and inhibitors than 3β-HSD2\textsuperscript{24}
and thus may have the ability to convert substantially lower
concentrations of pregnenolone to progesterone as
might be seen in the CNS. Both isoforms likely have a
similar mechanism of action given the high similarity in
structure; 3β-HSD2 is discussed here, as it is more
widely studied and has a well-delineated mechanism of
action.

3β-HSD2 is localized to the IMM in close association
with P450scc but performs its catalytic function in the
intermembrane space because it requires a low pH envi-
ronment to function.\textsuperscript{63,65,66} 3β-HSD2 first performs a
dehydrogenation reaction that converts NAD\textsuperscript{+} (nicotina-
mide adenine dinucleotide) to NADH.\textsuperscript{62} NADH induces
a conformational change in 3β-HSD2, thus creating a
catalytic site for the isomerization reaction that converts
pregnenolone to progesterone.\textsuperscript{62,67} The catalytic part
of the protein can exist in three forms: a highly folded
form at physiological pH, a partially folded form (molten glob-
ule with a high degree of secondary structure but no
defined tertiary structure) at about pH 3.5, or a com-
pletely unfolded form at about pH 4.5, or a com-
pletely unfolded form at about pH 3.5.\textsuperscript{63,66} 3β-HSD2 is
most active when partially unfolded, a state stabilized by
chaperone proteins that prevent 3β-HSD2 aggregation
(in the case of denaturation) and facilitate recycling of
the protein back to the less-active highly folded state
during cell quiescence.\textsuperscript{63,66,68} On both the OMM and
IMM, 3β-HSD is tightly associated with P450scc
and mitochondrial translocase proteins (Tom22, Tim50, and
Tim23) that seem to be necessary for 3β-HSD2 func-
tion.\textsuperscript{63,65} After synthesis, progesterone crosses the
OMM into the cytosol via passive diffusion.\textsuperscript{28}
5α-Reductase Type 1 and Type 2 (5α-R2)

5α-Reductase (5α-R) exists in three isoforms. Type 1 (SRD5A1 or 5α-R1) is the most abundant isoform in brain, but is also found in peripheral tissues, including the adrenal gland. In the brain, 5α-R1 has been co-localized with 3α-hydroxysteroid dehydrogenase type 3 (also known as AKR1C2) (3α-HSD3) (see below) in: (a) glutamatergic principal output neurons of the hippocampus, olfactory bulb, amygdala, thalamus and cortex, including pyramidal neurons of the prefrontal cortex (PFC) that project to the amygdala as well as (b) GABAergic principal output neurons such as cerebellar Purkinje cells, striatal medium spiny neurons, and reticular thalamic neurons. 5α-R2 plays a prominent role in the male reproductive system, but also has been identified in brain, including the PFC (specifically pyramidal cells), the pituitary gland (specifically prolactin producing cells), hypothalamus, and hippocampus. 5α-R2 is also expressed at high levels in spinal motor neurons and in the glomerulosa layer of the adrenal gland, where it is likely involved in the synthesis of tetrahydrodeoxycorticosterone (Figure 1), another potent GABAergic neuroactive steroid released into the circulation in response to stress. 5α-R3 (SRD5A3) appears to be primarily responsible for mediating the N-glycosylation of proteins and does not seem to be active in steroidogenesis.

Differing physiological characteristics of 5α-R1 and 5α-R2 allow these enzymes to play distinct but overlapping roles in steroid regulation. Both are involved in catalyzing the reduction of testosterone into its more potent metabolite 5α-dihydrotestosterone (5α-DHT) and progesterone into 5α-dihydropregosterone (5α-DHP), the immediate precursor for allopregnanolone. 5α-R1 has micromolar affinity for its steroid substrates (progesterone > testosterone > androstenedione > glucocorticoids) and is localized to the endoplasmic reticulum. There it plays an important neuroprotective role by catalyzing the large quantities of steroids produced under conditions of stress and certain reproductive phases in females, such as the luteal phase of the menstrual cycle and pregnancy. 5α-R1 activity increases markedly as testosterone levels decrease—as occurs during intense stress in men. In female rodents, testosterone has no effect on 5α-R1 expression in the PFC, whereas 5α-DHT markedly increases 5α-R1 levels. In contrast, 5α-R2 has nanomolar affinity for its substrates and thus may be critical in maintaining adequate resting allopregnanolone levels. In male rodents, 5α-R2 levels fall markedly when testosterone levels decrease (as occurs during stress) and normalize when testosterone levels return to normal. In contrast, a preliminary unconfirmed report suggests that 5α-R2 expression in hippocampus may increase during acute stress. Testosterone and 5α-DHT have lesser effects on 5α-R2 levels in females. Of note, potential effects of estradiol on 5α-R levels have not been reported.

The mechanisms by which 5α-R1 and 5α-R2 reduce their steroid substrates have not been well elucidated. The hydrophobic nature of 5α-R1 makes it difficult to solubilize in a form that retains metabolic activity; thus, crystallography studies have not been possible. 5α-R1 also lacks homology with other NADPH/steroid-binding enzymes. Studies have, however, determined that the N-terminal part of this protein binds to steroid substrates, whereas the C-terminal portion containing a glycine-rich region binds NADPH. 5α-R1 works best at pH 5.0 to 8.0 and has a half-life of approximately 20 to 30 h. Less is known about the chemical conditions under which 5α-R2 activity is optimized.

5β-Reductase

5β-Reductase (5β-R; also known as AKR1D1) is a monomeric cytoplasmic protein that plays a role in steroid metabolism as well as in cholic acid and chenodeoxycholic acid synthesis. It shares homology with other AKR family members with a similar mechanism of action (e.g., 3α-HSD, described below). Northern blot studies on human tissues demonstrated abundant 5β-R in the liver in two mRNA isoforms, smaller amounts in the testes in two isoforms, and trace amounts in all other tissues including the brain in a single isoform. This likely reflects a significant role for 5β-R in the inactivation of steroid hormones, given that two-thirds of these hormones, by mass, undergo transformation by 5β-R before excretion.

Among other reactions, 5β-R catalyzes the reduction of progesterone to 5β-dihydropregosterone (5β-DHP) or 5β-pregnane-3-20-dione, the immediate precursor for pregnanolone and a potent activator of pregna-X receptor (PXR, discussed later). 5β-R requires NADPH, which induces a conformational change, and allows for binding of substrate. Although the binding and release of NADPH and NADP+ are generally the slowest steps in 5β-R-mediated reactions, 5β-R efficiency also depends on the substrate involved. Human 5β-R demonstrates high efficiency for the reduction of progesterone, androstenedione, 17α-hydroxyprogesterone, and testosterone; reduced efficiency for reduction of aldosterone and corticosterone; and poor efficiency for reduction of cortisol.

3α-Hydroxysteroid Dehydrogenase Type 3

3α-HSD exists in four different isoforms (types 1, 2, 3, and 20a). Type 3 (associated with the AKR1C2 gene) is a cytosolic protein found in steroidogenic tissues and the central nervous system. Although 3α-HSD3 has been primarily studied with regard to its role in
converting 5α-DHT to 5α-androstane-3α,17β-diol (3α-diol), a very weak androgen and moderately potent GABAergic neuroactive steroid, it is also responsible for the conversion of 5α-DHP and 5β-DHP to the potent GABAergic neurosteroid stereoisomers, allopregnanolone and pregnanolone, respectively.\textsuperscript{91}

Among the 3α-HSD family proteins, 3α-HSD3 has the lowest catalytic efficiency, but the highest specificity for reducing 3-ketosteroids.\textsuperscript{91,92} 3α-HSD3 exists as a monomeric α/β barrel with large loops on one side that confer high substrate binding specificity due to their unique orientation and a conserved catalytic tetrad that mediates the reduction reaction.\textsuperscript{91,92} 3α-HSD3 binds NADPH before binding its steroid substrates; it then catalyzes the reduction of the substrate before releasing the steroid product.\textsuperscript{91} The rate-limiting step in the 3α-HSD3 conversion of substrate into product is the dissociation of NADP+ from the enzyme.\textsuperscript{91,93}

### Diffusion and Receptor Engagement of Allopregnanolone and Pregnanolone

Once allopregnanolone and pregnanolone have been synthesized, they can bind to various receptors to mediate their effects. If synthesized in the CNS, they may travel in an unbound state short distances intraneuronally or through the interstitial fluid to act in an autocrine or paracrine manner.\textsuperscript{73} A vascular transport protein has not been identified for the endocrine action of allopregnanolone, although there are a few likely candidates. Transcortin, also known as corticosteroid-binding globulin, is synthesized by the liver and plays a major role in transporting many adrenal steroid products throughout the body.\textsuperscript{94,95} Albumin, the major serum protein, also carries a significant proportion of steroid hormones and has been shown to bind progesterone.\textsuperscript{96} A less likely binding carrier is sex hormone-binding globulin, as it has high binding affinity only for 17β-hydroxysteroid hormones.\textsuperscript{97}

The binding of allopregnanolone to the GABA\textsubscript{A} receptor hypersensitizes or prolongs the opening time of the chloride ion channel in the presence of GABA.\textsuperscript{1,2} At a high enough concentration, allopregnanolone can also act as a direct agonist at this receptor.\textsuperscript{98} Studies have identified allopregnanolone binding sites in the transmembrane domain of the GABA\textsubscript{A} receptor and suggest that allopregnanolone may access the receptor from an intracellular approach.\textsuperscript{1,73,98} Allopregnanolone also binds to membrane progesterone receptors—putative G-protein-coupled receptors that activate intracellular second messenger signaling systems—to mediate neuroprotective actions similar to those mediated by progesterone binding.\textsuperscript{2,99} Finally, the nuclear PXR represents the only non-plasma membrane receptor associated with allopregnanolone function, as allopregnanolone does not bind to the nuclear progesterone receptor.\textsuperscript{2,100,101} Other potential signaling partners for allopregnanolone have yet to be identified.

### Common Pathways for Activating Steroidogenic Enzymes

#### Steroidogenic Stimulation by Trophic Hormones

The release of trophic hormones—such as ACTH and LH from the pituitary gland—is the most prominent and studied stimulus for the activation of steroidogenic cells in the periphery. The downstream messaging subsequent to ACTH or LH receptor binding is discussed here as a potential common pathway upon which other activating signals may converge (Figure 2).

The steroidogenic effect of ACTH or LH can be parsed into an acute response that occurs within seconds and a long-term response that occurs on the order of minutes to days.\textsuperscript{102} Acutely, protein kinase A (PKA) phosphorylates StAR at Ser195, which doubles its enzymatic activity in transporting cholesterol from the OMM to the IMM.\textsuperscript{58,35} TSPO is also a PKA substrate; phosphorylation of TSPO changes its ligand binding affinity and subsequently increases its activity.\textsuperscript{48,112} Overall, this acute response seems to drive the influx of cholesterol precursor into the mitochondria—whether by the action of StAR or TSPO—to saturate catalytic sites available for the first committed step in steroidogenesis mediated by P450scc. The long-term response to ACTH/LH activation occurs via PKA or protein kinase C (PKC) phosphorylation of several transcription factors that regulate steroidogenic enzymes, thereby increasing the intracellular steroidogenic machinery capacity.

Allopregnanolone and pregnanolone are just two of many end products generated as steroidogenic cells are activated to higher rates of steroid production. Although regulation of P450scc and 3β-HSD2 has been fairly well characterized due to their role in all steroid synthesis pathways, the regulatory mechanisms of 5α-R, 5β-R, and 3α-HSD3 are less well known. Recent research has shown that steroidogenic factor 1 (SF-1) regulates 5α-R and 3α-HSD differently: male murine SF-1 knockout models demonstrate decreased 5α-R mRNA levels and, surprisingly, increased 3α-HSD mRNA levels.\textsuperscript{113} A few studies have demonstrated that male rat glial cells respond to increased cyclic adenosine monophosphate (cAMP) levels by enhancing de novo increases in 5α-R mRNA levels and activity.\textsuperscript{114,115} Similar experiments in male rodents show that 3α-HSD does not increase in response to increases in cAMP.\textsuperscript{114} However, these experiments were not conducted in other steroidogenic tissues or in females. Moreover, the activity of these enzymes is determined not only by their abundance in the cell but...
also by the changing redox state of the cell and the related availability of NADPH, a cofactor required for the reductive activities of these enzymes.83,91 The regulatory mechanisms behind dedicated allopregnanolone production and the means by which production might change in response to stress (or the many means used by individuals to cope with stress) are likely to be highly complex and require further investigation in both sexes. For example, binge alcohol consumption over a month followed by abrupt alcohol withdrawal in male rodents decreased levels of both 5α-R1 and 3α-HSD in brain.116—perhaps by increasing the activity of NADPH oxidase and reducing availability of NADPH.

**Figure 2.** Schematic depicting the activation of a steroidogenic cell with ACTH or LH. In the adrenal gland, ACTH binds to the GPCR melanocortin 2 receptor (MC2R), whereas in the gonads, LH binds to the GPCR LH/choriogonadotropin receptor (LHCGR).103 Upon ligand binding to MC2R or LHCGR, these receptors mediate a signaling cascade through their associated G-protein. The Go subunit of the cell membrane-bound G-protein binds guanosine triphosphate (GTP) and dissociates from the G-protein in order to activate adenylyl cyclase which converts ATP to 3',5'-cyclic AMP (cAMP). The increase in cAMP levels activates protein kinase A (PKA). Meanwhile, the Gbg subunit of the membrane-bound G-protein activates phospholipase C β (PLCβ), which cleaves diacylglycerols (DAG) from the plasma membrane. The increase in DAG levels activates PKC. Acute changes secondary to this activation include the phosphorylation of StAR and TSPO, which allows for a higher influx of cholesterol across the mitochondrial membranes allowing for increased steroidogenesis. PKA phosphorylates and activates CAMP responsive element binding protein (CREB), which binds to CAMP response elements (CREs) to promote transcription of several downstream genes involved in neurosteroidogenesis.102 CREs have been identified in the promoters of HMG-CoA reductase, NPC1, StAR, and P450sc.c.49,102,104,105 PKC also has been shown to activate two transcription factors: steroidogenic factor 1 (SF-1) and activating protein 1 (AP-1).102 SF-1 binding domains have been found in the promoters of NPC1, StAR, P450sc.c, and 3β-HSD2.102,106,107 AP-1 binding domains have been found in the promoters of TSPO, P450sc.c and 3β-HSD2.49,62,102 The Nuclear Receptor 4A (NR4A) family of transcription are involved in inflammation and have been shown to upregulate StAR and 3β-HSD2.108-111 Long-term changes include activation of transcription factors which upregulate the transcription of genes associated with allopregnanolone synthesis. OMM: outer mitochondrial membrane; IMM: inner mitochondrial membrane; IMS: intermembrane space; HMG-CoA: 3-hydroxy-3-methylglutaryl CoA; NPC1: Niemann Pick type C1.

**Role of Allopregnanolone in Psychophysiological and Cellular Stress Responses**

Exposing male rodents to intense physical stress, prolonged social isolation, or drugs that reduce the activity of enzymes in the allopregnanolone synthesis pathway reduces serum and brain allopregnanolone levels in association with increases in anxiety and aggression, enhancement of contextual fear conditioning, decreases in the rate of fear extinction, and deficits in extinction retention.117-121 In contrast, compounds that induce neurosteroidogenesis or otherwise increase brain allopregnanolone levels reduce these negative behavioral
outcomes. Effects of stress on allopregnanolone synthesis have been less well studied in female rodents. For example, while isolation stress appeared to reduce brain allopregnanolone levels only in ovariectomized, testosterone-replaced female rodents, potential effects of the diestrus cycle in the normal females were not taken into account, possibly obscuring the effects of isolation. Other stressor types have not been investigated in female rodents. In humans, cross-sectional investigations have demonstrated an inverse relationship between the sum of allopregnanolone and pregnanolone level in cerebrospinal fluid (CSF) and PTSD severity as well as sex differences in the enzyme sites at which allopregnanolone synthesis appears to be blocked in PTSD. It is unclear whether deficits in allopregnanolone synthesis precede trauma exposure or result from trauma exposure or both. Longitudinal studies in humans exposed to severe stressors such as intense military training exercises or deployment would help to answer these important questions.

As illustrated in Figure 3, several enzymes involved in allopregnanolone biosynthesis, as well as allopregnanolone itself, also moderate the impact of cellular stressors such as xenobiotics or reactive oxygen species (ROS) requiring clearance. For example, TSPO expression increases in response to pro-pathology stressors and likely protects against apoptosis while preserving cellular mitochondrial metabolic and signaling capabilities in the face of increasing cellular ROS. 3α-HSD3 plays a role in phase II detoxification of polycyclic aromatic hydrocarbons (PAHs), particulate pollutants released into the air by the burning of fossil fuels. Allopregnanolone is one of the many ligands for PXR, a nuclear receptor that binds promiscuously to both endogenous ligands and xenobiotic ligands of a hydrophobic nature. When bound to a ligand, PXR binds regulatory elements to the promoter of a variety of genes responsible for the metabolism and clearance of xenobiotics. A well-studied and important effect of PXR is on the CYP3A family of enzymes responsible for the biotransformation of more than half of all prescription medications. Additionally, PXR drives the expression of phase II detoxification proteins such as glutathione-S-transferase and xenobiotic transporting polypeptide 2 (OATP2) and multidrug resistance-associated protein 2 (MRP2), which are responsible for excreting xenobiotic metabolites into the biliary system. Changes in PXR activity—such as those associated with elevations in allopregnanolone induced by stress, exercise, or the luteal phase of the menstrual cycle—thus may significantly alter drug metabolism and complicate drug dosing. PXR also upregulates TSPO, potentially creating positive feedback for allopregnanolone synthesis—which may be necessary to preserve adequate allopregnanolone levels in the face of increased PXR-mediated allopregnanolone metabolism. It is not known whether 3α-HSD3 is a target for PXR-induced upregulation, despite its role as a phase II detoxification protein.

**Clinical Implications**

**Phenotypes Associated With Defects Along the Allopregnanolone Synthesis Pathway**

Various defects can arise in the steroidogenic pathway synthesizing allopregnanolone, many of which are common to steroidogenesis in general. Some defects are related to specific severe medical disorders that may overshadow comorbid psychiatric conditions, while others so far have been linked primarily to psychiatric disorders despite increasing evidence that they contribute to comorbid medical and substance abuse disorders. Thus, further research will be important to characterize the full impact of these defects.

At the start of the steroid synthetic process, cells may have deficiencies that interfere with obtaining, synthesizing, or storing cholesterol precursor. Defects in LAL result in a range of phenotypes. Wolman disease is one rare genetic disorder affecting LAL function, may lead to multi-organ failure and death in early childhood. Cholesterol ester storage disease is a milder, later-onset form of Wolman disease. Even so, Wolman disease has less effect on steroidogenesis than congenital adrenal hyperplasia (CAH), likely due to the ability of steroidogenic cells to obtain free cholesterol from other sources. Niemann-Pick disease type C results from the mutation of NPC1, resulting in an inability to shuttle digested LDL products out of lysosomes. Lysosomal glycosphingolipids and cholesterol accumulate to produce a variable phenotype that generally includes progressive neurodegeneration. The impact on allopregnanolone synthesis by statins—which inhibit HMG-CoA reductase and thus endogenous cholesterol production—has not been thoroughly assessed. It is possible that patients with pre-treatment synthesis pathway vulnerabilities are more prone to the negative side effects of these drugs. Cortisol responses to ACTH were reduced in a small sample of individuals with familial hypercholesterolemia before and after statin treatment for two months. In a larger and longer study, dehydroepiandrosterone sulfate levels in children and adolescents with familial hypercholesterolemia were similar to those of their siblings before treatment with a statin, but substantially lower after 10 years of treatment. In a large randomized, placebo-controlled study of otherwise healthy middle-aged men with hypercholesterolemia, testosterone levels decreased, while depression, somatization, and self-reported aggression increased after two years on simvastatin.
Investigation of the effects of statins on resting and stress-induced increases in allopregnanolone and other neuroactive steroids are therefore warranted.

Defects can occur in the trafficking of cholesterol from the OMM to the IMM. Although controversy exists over whether loss of TSPO results in a significant clinical phenotype, loss of StAR results in lipoid CAH, a condition characterized by a nearly complete absence of all circulating steroid hormones, salt-wasting due to loss of aldosterone, and feminized external genitalia in patients with an XY genotype. Patients with defects in 3β-HSD are also diagnosed with CAH as 3β-HSD is required for synthesis of nearly all steroidogenic end-products. Less severe forms of 3β-HSD deficiency may present as hyperandrogenism in women, as progesterone precursors are routed into the androgen pathways. Due to the accumulation of dehydroepiandrosterone precursors, they may also present with psychiatric disorders typified by anxiety, depression, aggression, and increased risk for smoking dependence and other substance abuse disorders. Severe mutations in 5α-R2 result in male pseudohermaphroditism wherein patients with an XY genotype are unable to convert testosterone to 5α-DHT, resulting in an

Figure 3. Schematic depicting the response of steroidogenic cells to stressors such as polycyclic aromatic hydrocarbons and ROS. TSPO responds to stress-related increases in ROS by working with voltage-dependent anion-selective channel protein 1 (VDAC1) to increase the level of ROS in the mitochondria, thereby amplifying ROS signaling in the cell, driving the upregulation of cyclooxygenases, nitric oxide synthases, lipoxygenases, and NAPDH oxidase. It also has been shown to play a role in blocking ubiquitination of the mitochondria by other factors, thereby blocking mitophagy. Among the regulatory elements that drive expression of the TSPO gene is an ROS response element in the promoter that binds specificity protein 1 (SP1), which accumulates in the nucleus upon activation by ROS. Additionally, a PXR binding site in the TSPO gene promoter plays a part in the allopregnanolone stress response. On the other hand, 3α-HSD3 plays a role in detoxification of polycyclic aromatic hydrocarbons. PAH first undergoes a phase I detoxification reaction by the P450 family enzymes (but not P450scx, which strictly plays a role in steroidogenesis; this family is also known as the CYP enzymes), to form a dihydrodiol. Then 3α-HSD3 converts the dihydrodiol to an ortho-quinone, which is further conjugated to glutathione by a quinone reductase, thus producing a nontoxic metabolite for excretion from the body. Both the dihydrodiol and the ortho-quinone intermediates are capable of producing ROS and are considered genotoxic. However, the reaction pathway through 3α-HSD3 is the major route for eliminating PAHs, making the synthesis of these intermediates necessary. 3α-HSD3 is regulated by the nuclear factor E2-related factor 2 (Nrf2), which dissociates from its repressor Kelch-like ECH associated protein 1 (Keap1) upon exposure to increased ROS levels in the cell. Nrf2 binds to the antioxidant response element (ARE) in the promoter of 3α-HSD3 and upregulates its expression to meet the detoxification demands of the cell. Both TSPO and 3α-HSD3—enzymes responsible for allopregnanolone synthesis—are upregulated in response to these stressors, highlighting a role for allopregnanolone in stress response. OMM: outer mitochondrial membrane; IMM: inner mitochondrial membrane; IMS: intermembrane space; 3α-HSD3: 3α-hydroxysteroid dehydrogenase type 3 (aka AKR1C2); PXR: pregnane X receptor
inability to masculinize the external genitalia. Late masculinization of patients with mutations in 5α-R2 also may result as 5α-R1 activity increases during puberty. Of note, a polymorphism of 5α-R2 has been associated with increased risk for PTSD in males only. 5α-R deficiency in males with PTSD also has been characterized by a decrease in the ratio of 5α-DHP to progesterone in CSF; the sum of CSF allopregnanolone and pregnanolone levels were, in turn, strongly and negatively correlated with PTSD symptoms. No mutations of the 5α-R1 gene have been recorded so far in humans, possibly because dysfunctional phenotypes have gone undetected. However, experimental reduction of 5α-R1 expression in male rodents results in reduced allopregnanolone levels and a behavioral phenotype characterized by anxiety, resistance to sedation by GABA receptor-agonistic drugs, aggression, and enhanced contextual fear conditioning resistant to extinction and extinction retention. Phenomena consistent with PTSD in humans. 5α-R1/R2 inhibitors (e.g., dutasteride and finasteride) are commonly used in the treatment of male pattern baldness or benign prostatic hyperplasia, with a more controversial role in treating prostate cancer. One study suggested that prolonged use of finasteride reduced allopregnanolone concentrations in CSF. Off-target side effects of finasteride (which has submicromolar potency for 5α-R1, but sub-nanomolar potency for 5α-R2) seen in a subpopulation of males have included depression, suicidal ideation, reduced sexual interest, increased aggression, chronic pain and metabolic disturbances—conditions commonly comorbid with PTSD and major depression. Clinical relevant changes in 3α-HSD3 expression have not been well characterized. Studies have implicated 3α-HSD3 transcription in prostate and breast cancer. Reduced expression of 3α-HSD3 has been associated with hirsutism. It also has been associated with Niemann-Pick disease type C, as allopregnanolone treatment in a mouse model of Niemann-Pick type C delays symptom onset. Female-specific 3α-HSD3 deficiency, suggested by low CSF allopregnanolone levels and a low ratio of the sum of CSF allopregnanolone and pregnanolone (measured together due to technical limitations) to 5α-DHP, has been reported in premenopausal women with PTSD in association with increased negative mood and PTSD reexperiencing symptoms. The mechanisms by which this enzyme may become dysregulated and the range of clinical conditions resulting from such dysregulation need further investigation.

**Pharmacological Implications**

Pharmacological approaches to altering allopregnanolone availability or engagement with GABA receptors have primarily targeted neuropsychiatric pathologies. Small molecule ligands for TSPO have been shown to suppress anxiety and contextual fear in animal models of PTSD in association with increasing levels of allopregnanolone. These ligands (PK11195, YL-IPA08, and AC-5216) were thought to increase the transfer of cholesterol from the OMM to IMM by TSPO, thereby driving allopregnanolone synthesis. A link between the administration of these ligands and GABA receptor activity also has been demonstrated, most likely due to the effects of elevating allopregnanolone. However, given that TSPO may not play a significant role in steroidogenesis, the mechanism by which these ligands act to increase allopregnanolone in mouse models is uncertain. Given that TSPO is upregulated at areas of brain injury of varied etiologies and is used as a marker of neuroinflammation, it could be speculated that there is some commonality in the pathways of stress response leading to allopregnanolone synthesis rather than directly affecting steroidogenesis. The newer knockout mouse model studies seem to support this approach, with one study demonstrating significantly decreased capacity for cellular respiration in conditions of increased stress among mice treated with finasteride in comparison to wild type.

Selective serotonin reuptake inhibitors increase neurosteroidogenesis at doses below those that block serotonin reuptake. An increase in allopregnanolone levels in the CSF of depressed patients following fluoxetine or fluoxetine treatment correlated with decreases in depressive symptoms. Fluoxetine, sertraline, and paroxetine were shown to increase the affinity of 3α-HSD3 for 5α-DHP in vitro, thereby increasing the rate of allopregnanolone synthesis from 5α-DHP. However, another study suggested that fluoxetine acts in female, but not male, rats by inhibiting the conversion of allopregnanolone back into 5α-DHP. SSRIs also have shown efficacy in some subpopulations with PMDD, a disorder associated with low allopregnanolone levels in some but not all studies. However, recent studies have also demonstrated the efficacy of a GABA receptor modulating steroid antagonist in treating PMDD, as well as dutasteride, which prevented luteal phase increases in plasma allopregnanolone. These studies thus highlight the biological heterogeneity of PMDD.

The pharmacological use of allopregnanolone itself has been under investigation for several neuropsychiatric disorders: status epilepticus, traumatic brain injury, benign essential tremor, major depression, post-partum depression, chronic pain, Alzheimer’s disease, autism, and cocaine craving. Clinical trials of ganaxolone, a 3β-methylated analog of allopregnanolone with similar GABAergic properties are underway for treatment of focal epilepsies, female pediatric epilepsy, and fragile X syndrome. Ganaxolone also was recently investigated in PTSD, but trough drug levels in plasma.
were low and prevented a conclusive assessment of its efficacy.\textsuperscript{167} In addition, it is possible that chronic administration of allopregnanolone or allopregnanolone analogs is not therapeutic in PTSD.

Indeed, support for the therapeutic effects of intermittent (rather than steady state) administration of allopregnanolone has been demonstrated in rodent models of Alzheimer’s disease\textsuperscript{14,168} as well as in preclinical models of Niemann-Pick type C disease, traumatic brain injury, diabetic neuropathy, nerve injury, multiple sclerosis, and Parkinson’s disease.\textsuperscript{14} In PTSD, too high steady-state allopregnanolone levels would be expected to suppress reactivity of the sympathetic nervous system and hypoallopregnanolone levels would be expected to suppress extinction and extinction retention.\textsuperscript{163}

The enhancement of long-term depression (LTD) and long-term potentiation (LTP) interference by slowly rising intraneuronal levels of allopregnanolone and pregnanolone (and perhaps their sulfated metabolites) in response to NMDA receptor activation\textsuperscript{22} during extinction learning may enhance consolidation of new learning and prevent the reconsolidation of non-reinforced fear conditioned associations.\textsuperscript{120} The gradual development of LTP interference under such conditions is thought to protect newly modulated synapses from further excitation during memory consolidation.\textsuperscript{170} This may apply whether synapses are newly strengthened by LTP (e.g., as the new safe context is learned under arousing treatment conditions), or weakened by LTD (e.g., due to non-reinforcement of the previously acquired association between contextual cues and the previously experienced unconditioned stimulus). It should be noted, however, that these findings in allopregnanolone deficient rodents contrast with the studies in wild-type rodents in which acute allopregnanolone administration has been shown to negatively impact hippocampus-dependent novel object recognition, spatial learning and memory consolidation, and contextual fear conditioning.\textsuperscript{7,171}

Finally, it should be noted that dramatic changes in the target receptor specificity of these GABA\textsubscript{A}ergic neurosteroids can result from small variations in their structure. The 3\(\beta\)-hydroxylated isomer of allopregnanolone (known as isopregnanolone, isoallopregnanolone, or epiallopregnanolone) has GABA\textsubscript{A} receptor subunit selective antagonist effects\textsuperscript{172,173} and thus diminishes some but not all effects of allopregnanolone.\textsuperscript{173–175} The 3\(\beta\)-hydroxylated isomer of pregnanolone (epipregnanolone) also negatively modulates GABA\textsubscript{A} receptor function and blocks allopregnanolone effects, while the sulfated metabolites of allopregnanolone, pregnanolone, and epipregnanolone allosterically antagonize both GABA\textsubscript{A} and NMDA receptor function.\textsuperscript{176,177} In contrast, epiallopregnanolone sulfate facilitates NMDA receptor function.\textsuperscript{178}

Given the variable receptor effects of these neurosteroid isomers and metabolites, the relationship of allopregnanolone and pregnanolone to clinical phenomena of interest may vary depending on the (often unmeasured) presence of these isomers and metabolites—an important area for future research. In addition, several of these neurosteroids impact other receptor classes\textsuperscript{178} so that treatments aimed at altering their synthesis may have unanticipated off-target effects.

**Conclusion**

Allopregnanolone and pregnanolone play significant roles in a range of neuropsychiatric disorders. As these neurosteroids must be produced de novo in response to stress or other stimulatory demands, manipulating the pharmacokinetics of their synthesis constitutes a promising therapeutic approach and requires an understanding of enzymes involved in their synthesis and upstream molecular regulation. As noted above, much is known about the initial steps in steroidogenesis, but less is known about synthetic steps specific to allopregnanolone and pregnanolone synthesis. Further elucidation of these mechanisms and exploitation of the steps in the steroidogenic process already detailed may lead to novel therapeutics for a multitude of conditions. In addition, future pharmaceutical research should identify subpopulations of individuals for whom allopregnanolone and pregnanolone synthesis or levels should be augmented versus constrained and determine whether treatment is best delivered continuously or episodically. To harness the protective effects of these treatments and to reduce harm, it may also be necessary to both individualize drug dose and precisely time dosing relative to disorder-specific neurophysiological processes inherent to recovery.\textsuperscript{14,162,163}

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References

1. Carver CM, Reddy DS. Neurosteroid interactions with synaptic and extrasynaptic GABA<sub>A</sub> receptors: Regulation of subunit plasticity, phasic and tonic inhibition, and neuronal network excitability. *Psychopharmacol* 2013; 230(2): 151–188.

2. Guennoun R, Labombarda F, Gonzalez Deniselle MC, et al. Progesterone and allopregnanolone in the central nervous system: Response to injury and implication for neuroprotection. *J Steroid Biochem Mol Biol* 2015; 146: 48–61.

3. Peters JA, Kirkness EF, Callachan H, et al. Modulation of the GABA<sub>A</sub> receptor by depressant barbiturates and pregnane steroids. *Brit J Pharmacol* 1988; 94(4): 1257–1269.

4. Pinna G, Uznova V, Matsumoto K, et al. Brain allopregnanolone regulates the potency of the GABA<sub>A</sub> receptor agonist muscimol. *Neuropharmacology* 2009; 3(3): 440–448.

5. Puia G, Santi M, Vicini S, et al. Neurosteroids act on recombinant human GABA<sub>A</sub> receptors. *Neuron* 1990; 4(5): 759–765.

6. Hosie AM, Wilkins ME, Da Silva HMA, Smart TG. Endogenous neurosteroids regulate GABA<sub>A</sub> receptors through two discrete transmembrane sites. *Nature* 2006; 444(7118): 486–489.

7. Frye CA, Sturgis JD. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. *Neurobiol Learn Mem* 1995; 64(1): 83–96.

8. He J, Evans CO, Hoffman SW, et al. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol* 2004; 189(2): 404–412.

9. Rasmussen AM, Marx CE, Pineles SL, et al. Neuroactive steroids and PTSD treatment. *Neurosci Lett* 2017; 649: 156–163.

10. Rasmussen AM, Pineles SL. Neurotransmitter, peptide, and steroid hormone abnormalities in PTSD: biological endophenotypes relevant to treatment. *Curr Psychiatry Rep* 2018; 20(7): 52.

11. Rossetti MF, Cambiasso MJ, Holschbach MA, et al. Estrogens and progestagens: synthesis and action in the brain. *J Neuroendocrinol* 2016; 28: 1–11.

12. Griffin LD, Gong W, Verot L, et al. Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. *Nat Med* 2004; 10(7): 704–711.

13. Adeosun SO, Hou X, Jiao Y, et al. Allopregnanolone reinstates tyrosine hydroxylase immunoreactive neurons and motor performance in an MPTP-lesioned mouse model of Parkinson’s disease. *PLoS One* 2012; 7(11): e50040.

14. Brinton RD. Neurosteroids as regenerative agents in the brain: therapeutic implications. *Nat Rev Endocrinol* 2013; 9(4): 241–250.

15. Pineles SL, Nilhni YI, Pinna G, et al. PTSD in women is associated with a block in conversion of progesterone to the GABA<sub>A</sub>ergic neurosteroids allopregnanolone and pregnanolone measured in plasma. *Psychoneuroendocrinology* 2018; 93: 133–141.

16. Pineles SL, Nilhni YI, King MW, et al. Extinction retention and the menstrual cycle: Different associations for women with posttraumatic stress disorder. *J Abnorm Psychol* 2016; 125(3): 349–355.

17. Bixo M, Ekberg K, Poromaa IS, et al. Treatment of premenstrual dysphoric disorder with the GABA<sub>A</sub>/receptor modulating steroid antagonist Sepranolone (UC1010)—a randomized controlled trial. *Psychoneuroendocrinology* 2017; 80: 46–55.

18. Gulinello M, Gong QH, Li X, Smith SS. Short-term exposure to a neuroactive steroid increases 5α GABA<sub>A</sub>receptor subunit levels in association with increased anxiety in the female rat. *Brain Res* 2001; 910(1–2): 55–66.

19. Gulinello M. Anxiogenic effects of neurosteroid exposure: sex differences and altered GABA<sub>A</sub> receptor pharmacology in adult rats. *J Pharmacol Exp Ther* 2003; 305(2): 541–548.

20. Smith SS, Ruderman Y, Frye C, Homanics G, Yuan M. Steroid withdrawal in the mouse results in anxiogenic effects of 3α,5β-THP: A possible model of premenstrual dysphoric disorder. *Psychopharmacology* 2006; 186(3): 323–333.

21. Martinez PE, Rubinow DR, Nieman LK, et al. 5α-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.

22. Izumi Y, O’dell KA, Zorumski CF. Metaplastic LTP inhibition after LTD induction in CA1 hippocampal slices involves NMDA receptor-mediated neurosteroidogenesis. *Physiol Rep* 2013; 1(5): 1–10.

23. Purdy RH, Morrow AL, Moore PH, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci* 1991; 88(10): 4553–4557.

24. Miller WL. Disorders in the initial steps of steroid hormone synthesis. *J Steroid Biochem Mol Biol* 2017; 165: 18–37.

25. Liu J, Heikkilä P, Meng QH, Kahri AI, Tikkanen MJ, Voutilainen R. Expression of low and high density lipoprotein receptor genes in human adrenals. *Eur J Endocrinol* 2000; 142(6): 677–682.

26. Miller WL, Bose HS. Early steps in steroidogenesis: intracellular cholesterol trafficking. *J Lipid Res* 2011; 52(12): 2111–2135.

27. Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009; 29(4): 431–438.

28. Miller WL. Role of mitochondria in steroidogenesis. *Pediatr Adrenal Dis* 2010; 20(6): 1–19.

29. Kwon HJ, Abi-Mosleh L, Wang ML, et al. Structure of Niemann-Pick type C2 disease. *Cell MolLife Sci* 2007; 137(7): 1213–1224.

30. Xu S, Benoff B, Liou HL, et al. Structural basis of sterol binding by NPC1 reveals distinct subdomains for binding and transfer of cholesterol. *Cell* 2009; 137(7): 23525–23531.

31. Goedeke L, Fernandez-Hernando C. Regulation of cholesterol homeostasis. *Cell Mol Life Sci* 2012; 69(6): 915–930.

32. Illingworth DR, Kenny TA, Orwell ES. Adrenal function in heterozygous and homozygous hypobetalipoproteinemia. *J Clin Endocrinol Metab* 1982; 54(1): 27–33.
33. Clark BJ. The mammalian START domain protein family in lipid transport in health and disease. *J Endocrinol* 2012; 212(3): 257–275.

34. Rodriguez-Aguado D, Calderon-Dominguez M, Ren S, et al. Subcellular localization and regulation of StarD4 protein in macrophages and fibroblasts. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2011; 1811(10): 597–606.

35. Castillo AF, Orlando U, Hellenberger KE, et al. The role of mitochondrial fusion and STAR phosphorylation in the regulation of STAR activity and steroidogenesis. *Mol Cell Endocrinol* 2014; 408: 73–79.

36. de Brito OM, Scorrano L. Mitofusin-2 regulates mitochondrial and endoplasmic reticulum morphology and tethering: The role of Ras. *Mitochondrion* 2009; 9(3): 222–226.

37. Soto EA, Kliman HJ, Strauss JF, et al. Gonadotropins and cyclic adenosine 3′,5′-monophosphate (cAMP) alter the morphology of cultured human granulosa cells. *Biol Reprod* 1986; 34(3): 559–569.

38. Duarte A, Poderoso C, Cooke M, et al. Mitochondrial fusion is essential for steroid biosynthesis. *PLoS One* 2012; 7(9): 1–12.

39. Bose HS, Lingappa VR, Miller WL. Rapid regulation of steroidogenesis by mitochondrial protein import. *Nature* 2002; 417(6884): 87–91.

40. Granot Z, Melamed-Book N, Bahat A, et al. Turnover of STAR protein: roles for the proteasome and mitochondrial proteases. *Mol Cell Endocrinol* 2007; 265–266(Suppl.): 51–58.

41. Miller WL, Strauss JF. Molecular pathology and mechanism of action of the steroidogenic acute regulatory protein, STAR. *J Steroid Biochem Mol Biol* 1999; 69(1–6): 131–141.

42. Tsujishita Y, Hurley JH. Structure and lipid transport mechanism of a STAR-related domain. *Nat Struct Biol* 2000; 7(5): 408–414.

43. Artemenko IP, Zhao D, Hales DB, et al. Mitochondrial processing of newly synthesized steroidogenic acute regulatory protein (STAR), but not total STAR, mediates cholesterol transfer to cytochrome P450 side chain cleavage enzyme in adrenal cells. *J Biol Chem* 2001; 276(49): 46583–46596.

44. Bose M, Whittal RM, Miller WL, Bose HS. Steroidogenic activity of STAR requires contact with mitochondrial VDAC1 and phosphate carrier protein. *J Biol Chem* 2008; 283(14): 8837–8845.

45. Prasad M, Kaur J, Pawlak KJ, Bose M, Whittal RM, Bose HS. Mitochondria-associated endoplasmic reticulum membrane (MAM) regulates steroidogenic activity via steroidogenic acute regulatory protein (STAR)-voltage-dependent anion channel 2 (VDAC2) interaction. *J Biol Chem* 2015; 290(5): 2604–2616.

46. Marriott K-SC, Prasad M, Thapliyal V, Bose HS. Sigma-1 receptor at the mitochondrial-associated endoplasmic reticulum membrane is responsible for mitochondrial metabolic regulation. *J Pharmacol Exp Ther* 2012; 343(3): 578–586.

47. Midzak A, Zirkin B, Papadopoulos V. Translocator protein: pharmacology and steroidogenesis. *Biochem Soc Trans* 2015; 43(4): 572–578.

48. Anholt RRH, Pedersen PL, De Souza EB, Snyder SH. The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane. *J Biol Chem* 1986; 261(2): 576–583.

49. Gauthier J, Campanela M. TSPO: kaleidoscopic 18-kDa amid biochemical pharmacology, control and targeting of mitochondria. *Biochem J* 2016; 473(2): 107–121.

50. Gut P, Zweckstetter M, Banati RB. Lost in translocation: the functions of the 18-kD translocator protein. *Trends Endocrinol Metab* 2015; 26(7): 349–356.

51. Li F, Liu J, Zheng Y, Garavito RM, Ferguson-Miller S. Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism. *Science* 2015; 347: 555–558.

52. Fan J, Papadopoulos V. Evolutionary origin of the mitochondrial cholesterol transport machinery reveals a universal mechanism of steroid hormone biosynthesis in animals. *PLoS One* 2013; 8(10): 1–20.

53. Rodriguez-Aguado D, Ren S, Wong E, et al. Intracellular cholesterol transporter StarD4 binds free cholesterol and increases cholesteryl ester formation. *J Lipid Res* 2008; 49(7): 1409–1419.

54. Papadopoulos V, Amri H, Boujrad N, et al. Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. *Steroids* 1997; 62(1): 21–28.

55. Morohaku K, Pelton SH, Daugherty DJ, Butler WR, Deng W, Selvaraj V. Translocator protein/peripheral benzodiazepine receptor is not required for steroid hormone biosynthesis. *Endocrinology* 2014; 155(1): 89–97.

56. Tu LN, Morohaku K, Manna PR, et al. Peripheral benzodiazepine receptor/translocator protein global knock-out mice are viable with no effects on steroid hormone biosynthesis. *J Biol Chem* 2014; 289(40): 27444–27454.

57. Banati RB, Middleton RJ, Chan R, et al. Positron emission tomography and functional characterization of a complete PBR/TSPO knockout. *Nat Commun* 2014; 5: 1–12.

58. Fan J, Campioli E, Midzak A, Culty M, Papadopoulos V. Conditional steroidal cell-targeted deletion of TSPO unveils a crucial role in viability and hormone-dependent steroid formation. *Proc Natl Acad Sci* 2015; 112(23): 7261–7266.

59. Mukai S, Okamoto M, Yamano T, et al. Cholesterol accumulation in adrenocortical mitochondria after ACTH-stimulation. *Endocrinol Jpn* 1984; 31(2): 177–184.

60. Stevens VL, Xu T, Lambeth JD. Cholesterol pools in rat adrenal mitochondria: Use of cholesterol oxidase to infer a complex pool structure. *Endocrinology* 1992; 130(3): 1557–1563.

61. Tuckey RC, Cameron KJ. Catalytic properties of cytochrome P-450scC purified from the human placenta: comparison to bovine cytochrome P-450scC. *Biochim Biophys Acta (BBA)/Protein Struct Mol Biol* 1993; 1163(2): 185–194.

62. Simard J, Ricketts ML, Gingras S, et al. Molecular biology of the 3β-hydroxysteroid dehydrogenase/Δ5-44 isomerase gene family. *Endoc Rev* 2005; 26(4): 525–582.

63. Thomas JL, Bose HS. Regulation of human 3-beta-hydroxysteroid dehydrogenase type-2 (3βHSD2) by molecular chaperones and the mitochondrial environment affects steroidogenesis. *J Steroid Biochem Mol Biol* 2015; 151: 74–84.

64. Thomas JL, Boswell EL, Scaccia LA, Pletnev V, Umland TC. Identification of key amino acids responsible for the substantially higher affinities of human type 1...
3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD1) for substrates, coenzymes and inhibitors relative to human 3β-HSD2. *Bioorg Chem* 2005; 280(22): 21321–21328.

65. Pawlak KJ, Prasad M, Thomas JL, Whittal RM, Bose HS. Inner mitochondrial translocase Tim50 interacts with 3β-hydroxysteroid dehydrogenase type 2 to regulate adrenal and gonadal steroidogenesis. *J Biol Chem* 2011; 286(45): 39130–39140.

66. Prasad M, Thomas JL, Whittal RM, et al. Mitochondrial 3β-hydroxysteroid dehydrogenase enzyme activity requires reversible pH-dependent conformational change at the intermembrane space. *J Biol Chem* 2012; 287(12): 9534–9546.

67. Thomas JL, Frieden C, Nash WE, et al. An NADH-induced conformational change that mediates the sequential 3β-hydroxysteroid dehydrogenase/isomerase activities is supported by affinity labeling and the time-dependent activation of isomerase. *J Biol Chem* 1995; 270(36): 21003–21008.

68. Rajapaksha M, Prasad M, Thomas JL, et al. Chaperones rejuvenate folding and activity of 3-β-hydroxysteroid dehydrogenase 2. *ACS Chemical Biology* 2013; 8: 1000–1008.

69. Traish AM. 5α-Reductases in human physiology: an unfolding story. *Endocr Pract* 2012; 1(1): 1–38.

70. El-Awady MK, El-Garf W, El-Houssieny L. Steroid 5α-reductase mRNA type 1 is differentially regulated by androgens and glucocorticoids in the rat liver. *Endocr J* 2004; 51(1): 37–46.

71. Roselli CE, Finn TJ, Ronnekleiv-Kelly SM, Tanchuck MA, Kaufman KR, Finn DA. Localization of brain 5α-reductase messenger RNA in mice selectively bred for high chronic alcohol withdrawal severity. *Alcohol* 2011; 45(8): 763–772.

72. Agis-Balboa RC, Pinna G, Pibiri F, et al. Down-regulation of neurosteroid biosynthesis in corticobulbar circuits mediates social isolation-induced behavior in mice. *Proc Natl Acad Sci* 2007; 104(47): 18736–18741.

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

74. Torres JM, Gomez-Capilla JA, Ortega E. Quantitative reverse-transcriptase polymerase chain reaction assay for mRNA levels of steroid 5α-reductase isoforms. *Analytical Biochem* 2002; 307(1): 177–180.

75. Torres JM, Ortega E. Differential regulation of steroid 5α-reductase isoforms expression by androgens in the adult rat brain. *FASEB J* 2003; 17(11): 1428–1433.

76. Torres JM, Ortega E. Steroid 5α-reductase isoforms in the adult female rat brain: central role of dihydrotestosterone. *J Mol Endocrinol* 2006; 36: 239–245.

77. Poletti A, Coscarella A, Negri-Cesi P, Colciago A, Celotti F, Martini L. 5α-reductase isoforms in the central nervous system. *Steroids* 1998; 63: 246–251.

78. Aumüller G, Eicheler W, Renneberg H, Adermann K, Vilja P, Forssmann WG. Immunocytochemical evidence for differential subcellular localization of 5α-reductase isoenzymes in human tissues. *Acta Anat* 1996; 156(4): 241–252.

79. Eicheler W, Tuohimaa P, Vilja P, Adermann K, Forssmann WG, Aumüller G. Immunocytochemical localization of human 5α-reductase 2 with polyclonal antibodies in androgen target and non-target human tissues. *J Histochem Cytochem* 1994; 42: 667–675.

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

81. Normington K, Russell DW. Tissue distribution and kinetic characteristics of rat steroid 5α-reductase isozyme. *J Biol Chem* 1992; 267(27): 19548–19554.

82. Morgan CA, Wang S, Mason J, et al. Hormone profiles in humans experiencing military survival training. *Biol Psychiatry* 2000; 47(10): 891–901.

83. Bhattacharyya AK, Wang M, Rajagopalan K, et al. Analysis of the steroid binding domain of rat steroid 5α-reductase (isozyme-1) the steroid D-ring binding domain of 5α-reductase. *Steroids* 1999; 64(3): 197–204.

84. Wang M, Bhattacharyya AK, Taylor MF, et al. Site-directed mutagenesis studies of the NADPH-binding domain of rat steroid 5α-reductase (isozyme-1): Analysis of aromatic and hydroxylated amino acid residues. *Steroids* 1999; 64(5): 356–362.

85. Chen M, Jin Y, Penning TM. The rate-determining steps of aldo-keto reductases (AKRs), a study on human steroid 5β-reductase (AKR1D1). *Chem Biol Interact* 2015; 234: 360–365.

86. Charbonneau A, The VL. Genomic organization of a human 5β-reductase and its pseudogene and substrate selectivity of the expressed enzyme. *Biochim Biophys Acta - Gene Struct Exp* 2001; 1517(2): 228–235.

87. Palermo M, Marazzi MG, Hughes BA, Stewart PM, Clayton PT, Shackleton CHL. Human Δ4-3-oxosteroid 5β-reductase (AKR1D1) deficiency and steroid metabolism. *Steroids* 2008; 73(4): 417–423.

88. Drury JE, Di Costanzo L, Penning TM, Christianson DW. Inhibition of human steroid 5β-reductase (AKR1D1) by finasteride and structure of the enzyme-inhibitor complex. *J Biol Chem* 2009; 284(30): 19786–19790.

89. Wenners A, Hartmann F, Jochens A, et al. Stromal markers AKR1C1 and AKR1C2 are prognostic factors in primary human breast cancer. *Int J Clin Oncol* 2015; 21(3): 548–556.

90. Dufort I, Labrie F. Human types 1 and 3 3α-le-hydroxysteroid dehydrogenases: differential lability and tissue distribution. *J Clin Endocrinol Metab* 2001; 86(2): 841–846.

91. Penning TM, Jin Y, Steckelbroeck S, et al. Structure-function of human 3α-hydroxysteroid dehydrogenases: Genes and proteins. *Mol Cell Endocrinol* 2004; 215(1–2): 63–72.

92. Jin Y, Stayrook SE, Albert RH, et al. Crystal structure of human type III 3β-hydroxysteroid dehydrogenase/bile acid binding protein complexed with NADP+ and ursodeoxycholate. *Biochem* 2001; 40: 10161–10168.

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

94. Dunn JF, Nisula BC, Rodbard D. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J Clin Endocrinol Metab* 1981; 53(1): 58–68.
95. Hammond GL. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. J Endocrinol 2016; 230: R13–R25.

96. Chan D, Slaunwhite WR. The binding of a synthetic progestin, R5020, to transcortin and serum albumin. J Clin Endocrinol Metab 1977; 44: 983–985.

97. Selby C. Sex hormone binding globulin: origin, function and clinical significance. Ann Clin Biochem An Int J Biochem Lab Med 1990; 27(6): 532–541.

98. Sigel E, Steinmann ME. Structure, function, and modulation of GABAA receptors. J Biol Chem 2012; 287(48): 40224–40234.

99. Thomas P, Pang Y. Membrane progesterone receptors: evidence for neuroprotective, neurosteroid signaling and neuroendocrine functions in neuronal cells. Neuroendocrinology 2012; 96(2): 162–171.

100. Frye CA, Koonce CJ, Walf A, et al. Novel receptor targets for production and action of allopregnanolone in the central nervous system: a focus on progesterone xenobiotic receptor. Front Cell Neurosci 2014; 8: 106.

101. Lamba V, Yasuda K, Lamba JK, et al. PXR (NR1I2): transcriptional regulation of steroidogenic genes. Endocrinol Exp 2016; 7: 24.

102. Ruggiero C, Lalli E. Impact of ACTH signaling on transcriptional regulation of steroidogenic genes. Front Endocrinol 2016; 7: 24.

103. Lavoie HA, King SR. Transcriptional regulation of steroidogenic genes: STARD1, CYP11A1 and HSD3B. Exp Biol Med 2009; 234(8): 880–907.

104. Gévy NY, Lalli E, Sassone-Corsi P, Murphy BD. Regulation of Niemann-Pick C1 gene expression by the 3'S-cyclic adenosine monophosphate pathway in steroidogenic cells. Mol Endocrinol 2003; 17: 704–715.

105. Lagor WR, De Groh ED, Ness GC. Diabetes alters the occupancy of the hepatic 3-hydroxy-3-methylglutaryl-CoA reductase promotor. J Biol Chem 2005; 280(44): 36601–36608.

106. Hovik EA, Lewis AE, Aumo L, Bakke M. Molecular aspects of steroidogenic factor 1 (SF-1). Mol Cell Endocrinol 2010; 315(1–2): 27–39.

107. Rasmussen MK, Ekstr B, Zamaratskaia G. Regulation of 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase: A review. Int J Mol Sci 2013; 14(9): 17926–17942.

108. Bassett MH, Suzuki T, Sasano H, et al. The orphan nuclear receptor NGFI-B regulates transcription of 3α-hydroxysteroid dehydrogenase: Implications for the control of adrenal functional zonation. J Biol Chem 2004; 279(36): 37622–37630.

109. Kurakula K, Koenis DS, van Tiel CM, de Vries CJM. NR4A nuclear receptors are orphan but not lonesome. Biochim Biophys Acta 2014; 1843(11): 2543–2555.

110. Sewer MB, Dammer EB, Jagarlapudi S. Transcriptional regulation of adrenocortical steroidogenic gene expression. Drug Metab Rev 2007; 39(2–3): 371–388.

111. Volakakis N, Kadkhodaei B, Joodmardi E, et al. NR4A orphan nuclear receptors as mediators of CREB-dependent neuroprotection. Proc Natl Acad Sci 2010; 107(27): 12317–12322.

112. Whalin ME, Boujad N, Papadopoulos V, Krueger KE. Studies on the phosphorylation of the 18 kDa mitochondrial benzoiazepine receptor protein. J Recept Res 1994; 14: 217–228.

113. Spanic T, Fabjan T, Majdic G. Expression levels of mRNA for neurosteroidogenic enzymes 17β-HSD, 5α-reductase, 3α-HSD and cytochrome P450 aromatase in the fetal wild type and SF-1 knockout mouse brain. Endocr Res 2015; 40(1): 44–48.

114. Melangi RC, Celotti F, Castano P, Martini L. Intracellular signalling systems controlling the 5 alpha-reductase in glial cell cultures. Brain Res 1992; 585(1–2): 411–415.

115. Morita K, Arimochi H, Tsuuro Y. Adrenergic activation of steroid 5α-reductase gene expression in rat C6 glioma cells: involvement of cyclic AMP/protein kinase A-mediated signaling pathway. J Mol Neurosci 2004; 22: 205–212.

116. Cagetti E, Pinna G, Guidotti A, Baicy K, Olsen RW. Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. Neuropsychopharmacology 2004; 46(4): 570–579.

117. Nagaya N, Acca GM, Maren S. Allopregnanolone in the bed nucleus of the stria terminalis modulates contextual fear in rats. Front Behav Neurosci 2015; 9: 1–10.

118. Pibiri F, Nelson M, Guidotti A, Costa E, Pinna G. Decreased corticolumbic allopregnanolone expression during social isolation enhances contextual fear: A model relevant for posttraumatic stress disorder. Proc Natl Acad Sci 2008; 105: 5567–5572.

119. Pinna G, Dong E, Matsumoto K, et al. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc Natl Acad Sci 2003; 100: 2035–2040.

120. Pinna G, Rasmusson AM. Up-regulation of neurosteroid biosynthesis as a pharmacological strategy to improve behavioural deficits in a putative mouse model of post-traumatic stress disorder. J Neuroendocrinol 2012; 24: 102–116.

121. Zhang L-M, Qiu Z-K, Zhao N, et al. Anxiolytic-like effects of YL-IPA08, a potent ligand for the translocator protein (18 kDa) in animal models of post-traumatic stress disorder. Int J Neuropsychopharmacol 2014; 17(10): 1659–1669.

122. Locci A, Pinna G. Neurosteroid biosynthesis down-regulation and changes in GABA A receptor subunit composition: a biomarker axis in stress-induced cognitive and emotional impairment. Br J Pharmacol 2017; 74: 3226–3241.

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

124. Girdler SS, Lindgren M, Porcu P, Rubinow DR, Johnson JL, Morrow AL. A history of depression in women is associated with an altered GABAergic neuroactive steroid profile. Psychoneuroendocrinology 2012; 37: 543–553.
125. Rasmussen AM, Pinna G, Paliwal P, et al. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. Biol Psychiatry 2006; 60: 704–713.

126. Romeo E, Strehlle A, Spalletta G, et al. Effects of antidepressant treatment on neuroactive steroids in major depression. Am J Psychiatry 1998; 155: 910–913.

127. Sciolli-Salter E, Forman DE, Tun C, et al. Potential neurobiological benefits of exercise in chronic pain and post-traumatic stress disorder: pilot study. J Rehabil Research Dev 2016; 53: 95–106.

128. Strehlle A, Romeo E, Hermann B, et al. 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: 274–277.

129. Uzunova V, Sheline Y, Davis JM, et al. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci 1998; 95: 3239–3244.

130. Wang MI, Seippel LE, Purdy RH, et al. Relationship between symptom severity and steroid variation in women with premenstrual syndrome: study on serum pregnenolone, progesterone sulfate, 5 alpha-pregnane-3,20-dione and 3 alpha-hydroxy-5 alpha-pregnane-20-one. J Clin Endocrinol Metab 1996; 81: 1076–1082.

131. Burczynski ME, Lin HK, Penning TM. Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress: Implications for the alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. Cancer Res 1999; 59: 607–614.

132. Lou H, Du S, Ji Q, Stolz A. Induction of AKR1C2 by phase II inducers: identification of a distal consensus antioxidant response element regulated by NRF2. Mol Pharmacol 2006; 69: 1662–1672.

133. Palackal NT, Lee SH, Harvey RG, Blair IA, Penning TM. Activation of polycyclic aromatic hydrocarbon trans-dihydriodiol proximate carcinogens by human aldo-keto reductase (AKR1C) enzymes and their functional overexpression in human lung carcinoma (A549) cells. J Biol Chem 2002; 277: 24799–24808.

134. Gatmiff J, Campanella M. TSPO is a REDOX regulator of cell mitophagy. Biochem Soc Trans 2015; 43(4): 543–552.

135. Yahyapour R, Motievasili E, Rezaeyan A, et al. Reduction–oxidation (redox) system in radiation-induced normal tissue injury: molecular mechanisms and implications in radiation therapeutics. Clin Transl Oncol 2018; 20(8): 975–988.

136. Wu KC, Cui JY, Klaassen CD. Effect of graded Nrf2 activation on phase-I and -II drug metabolizing enzymes and transporters in mouse liver. PLoS One 2012; 7(7): e39006.

137. Kliever SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev 2002; 23: 687–702.

138. Frye CA, Koonce CJ, Walf AA. The pregnane xenobiotic receptor, a prominent liver factor, has actions in the midbrain for neurosteroid synthesis and behavioral/neural plasticity of female rats. Front Syst Neurosci 2014; 8: 60.

139. Laue L, Hoeg JM, Barnes K, Loriaux DL, Chrousos GP. The effect of mevinolin on steroidogenesis in patients with defects in the low density lipoprotein receptor pathway. J Clin Endocrinol Metab 1987; 64: 531–535.

140. Braamskamp MJAM, Kusters DM, Wiegman A, et al. Gonadal steroids, gonadotropins and DHEAS in young adults with familial hypercholesterolemia who had initiated statin therapy in childhood. Atherosclerosis 2015; 241(2): 427–432.

141. Hyyppä MT, Kronholm E, Virtanen A, Leino A, Jula A. Does simvastatin affect mood and steroid hormone levels in hypercholesterolemic men? A randomized double-blind trial. Psychoneuroendocrinology 2003; 28(2): 181–194.

142. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev 2011; 32(1): 81–151.

143. Simon NG, Mo Q, Hu S, Garippa C, Lu S-F. Hormonal pathways regulating intermale and interfemale aggression. Int Rev Neurobiol 2006; 73: 99–123.

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

145. Gillespie CF, Almli LM, Smith AK, et al. Sex dependent influence of a functional polymorphism in steroid 5α-reductase type 2 (SRD5A2) on post-traumatic stress symptoms. Am J Med Genet Part B Neuropsychiatr Genet 2018; 162(3): 283–292.

146. Tanchuck-Nipper MA, Ford MM, Hertberg A, Beadle-Bohling A, Cozzoli DK, Finn DA. Sex differences in etanol’s anxiolytic effect and chronic ethanol withdrawal severity in mice with a null mutation of the 5α-reductase type 1 gene. Behav Genet 2015; 45(3): 354–367.

147. Wang K, Fan D-D, Jin S, Xing N-Z, Niu Y-N. Differential expression of 5α-reductase isozymes in the prostate and its clinical implications. Asian J Androl 2014; 16(2): 274–279.

148. Caruso D, Abbiati F, Giatti S, et al. Patients treated for male pattern hair with finasteride show, after discontinuation of the drug, altered levels of neuroactive steroids in cerebrospinal fluid and plasma. J Steroid Biochem Mol Biol 2015; 146: 74–79.

149. 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.

150. Ji Q, Aoyama C, Nien Y-D, et al. Selective loss of AKR1C2 in prostate cancer and its role in DHT metabolism. Prostate 2003; 54(4): 275–289.

151. Steiner AZ, Chang L, Ji Q, et al. A Novel Mechanism for Hirsutism. In Vivo 2008; 93: 1298–1303.

152. Qiu ZK, Zhang LM, Zhao N, et al. Repeated administration of AC-5216, a ligand for the 18 kDa translocator protein, improves behavioral deficits in a mouse model of post-traumatic stress disorder. Prog Neuro-Psychopharmacology Biol Psychiatry 2013; 45: 40–46.
154. Rupprecht R, Papadopoulou V, Rammes G, et al. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov* 2010; 9(12): 971–988.

155. Zhang L-M, Qiu Z-K, Chen X-F, et al. Involvement of allopregnanolone in the anti-PTSD-like effects of AC-5216. *J Psychopharmacol*. 2016. http://jop.sagepub.com/cgi/doi/10.1177/0269881115625115. Accessed November 26, 2018.

156. Zhang LM, Qiu ZK, Zhao N, et al. Anxiolytic-like effects of YL-IPA08, a potent ligand for the translocator protein (18 kDa) in animal models of post-traumatic stress disorder. *Int J Neuropsychopharmacol* 2014; 29(4): 1659–1669.

157. Guilarte TR. TSPO in diverse CNS pathologies and psychiatric disease: A critical review and a way forward [published online ahead of print September 4, 2018]. *Pharmacol Ther*. 2018. doi:10.1016/j.pharmthera.2018.09.003.

158. Griffin LD, Mellon SH. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. *Proc Natl Acad Sci* 1999; 96(23): 13512–13517.

159. Fry JP, Li KY, Devall AJ, Cockcroft S, Honour JW, Griffin LD, Mellon SH. Selective serotonin reuptake inhibitors after neurotrauma in organotypic spinal cord cultures: A key role for progesterone receptors and steroidol modulators of GABA<sub>A</sub> receptors. *Neuropsychopharmacology* 2013; 48(1): 46–55.

160. Bäckström T, Haage D, Löfgren M, et al. Paradoxical effects of GABA<sub>A</sub>-selective the selective antagonist of the GABA<sub>A</sub>-receptor. *Digit Vetenskapliga Ark*. 2009. http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-27069. Accessed November 26, 2018.

161. Labombarda F, Ghoumari AM, Liere P, De Nicola AF, Schumacher M, Guennoun R. Neuron protection by steroids after neurotrauma in organotypic spinal cord cultures: A key role for progesterone receptors and steroidol modulators of GABAA receptors. *Neuropsychopharmacology* 2013; 71: 46–55.

162. Patte-Mensah C, Meyer L, Taleb O, Mensah-Nyagan AG. Potential role of allopregnanolone for a safe and effective therapy of neuropathic pain. *Prog Neurobiol* 2014; 113: 70–78.

163. Rasmusson AMPG. Ganaxolone improves behavioral deficits in a mouse model of post-traumatic stress disorder. *Front Cell Neurosci* 2014; 8: 256.

164. Kazdoba TM, Hagerman RJ, Zolkowska D, Rogawski MA, Crawley JN. Evaluation of the neuroactive steroid ganaxolone on social and repetitive behaviors in the BTBR mouse model of autism. *Psychopharmacology* 2016; 233(2): 309–323.

165. Milivojevic V, Fox HC, Sofuoglu M, Covault J, Sinha R. Effects of progesterone stimulated allopregnanolone on craving and stress response in cocaine dependent men and women. *Psychoneuroendocrinology* 2016; 65: 44–53.

166. Zaccara G, Schmidt D. Do traditional anti-seizure drugs have a future? A review of potential anti-seizure drugs in clinical development. *Pharmacol Res* 2016; 104: 38–48.

167. Rasmusson AM, Marx CE, Jain S, et al. A randomized controlled trial of ganaxolone in posttraumatic stress disorder. *Psychopharmacology* 2017; 234(15): 2245–2257.

168. Bengtsson SK, Johansson M, Bäckström T, Wang M. Chronic allopregnanolone treatment accelerates alzheimer’s disease development in AβPPSwPSEN1ΔE9mice. *J Alzheimer’s Dis* 2012; 31(1): 71–84.

169. Barbaccia M, Serra M, Purdy R, Biggio G. Stress and neuroactive steroids. *Int Rev Neurobiol* 2001; 46: 243–272.

170. Cantarero G, Tang B, O’Malley R, Salas R, Celnik P. Motor Learning Interference Is Proportional to Occlusion of LTP-Like Plasticity. *J Neurosci* 2013; 33(11): 4634–4641.

171. Johansson IM, Birzniece V, Lindblad C, Olsson T, Bäckström T. Allopregnanolone inhibits learning in the Morris water maze. *Brain Res* 2002; 934(2): 125–131.

172. Lundgren P, Strömberg J, Bäckström T, Wang M. Allopregnanolone-stimulated GABA-mediated chloride ion flux is inhibited by 3β-hydroxy-5α-pregnan-20-one (isoallopregnanolone). *Brain Res* 2003; 982(1): 45–53.

173. Öfverman C, Strömberg J, Birzniece V, Turkmen S. The progesterone metabolite isoallopregnanolone is a subunit-selective antagonist of the GABA<sub>A</sub>-receptor. *Diget Vetenskapliga Ark*. 2009. http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-27069. Accessed November 26, 2018.

174. Bäckström T, Wahlgren M, Wahlström K, Zhu D, Wang M De. Isoallopregnanolone; an antagonist to the anesthetic effect of allopregnanolone in male rats. *Eur J Pharmacol* 2005; 512(1): 15–21.

175. Bengtsson SKS, Nyberg S, Hedström H, et al. Isoallopregnanolone antagonize allopregnanolone-induced effects on saccadic eye velocity and self-reported sedation in humans. *Psychoneuroendocrinology* 2015; 52(1): 22–31.

176. Park-Chung M, Wu FS, Purdy RH, Malayev AA, Gibbs TT, Farb DH. Distinct sites for inverse modulation of N-methyl-D-aspartate receptors by sulfated steroids. *Mol Pharmacol* 1997; 52(6): 1113–1123.

177. Gibbs TT, Russek SJ, Farb DH. Sulfated steroids as endogenous neuromodulators. *Pharmacol Biochem Behav* 2006; 84(4): 555–567.

178. Rupprecht R, Di Michele F, Hermann B, et al. Neuroactive steroids: Molecular mechanisms of action and implications for neuropharmacology. *Brain Res Rev* 2001; 37(1–3): 59–67.