

# 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-aminobutyric acid (GABA) at GABA<sub>A</sub> 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 (3 $\alpha$ -hydroxy-5 $\alpha$ -pregnan-20-one) and pregnanolone (3 $\alpha$ -hydroxy-5 $\beta$ -pregnan-20-one) are neurosteroid metabolites of progesterone that equipotently and positively modulate the action of gamma-aminobutyric acid (GABA) at GABA<sub>A</sub> synaptic and extrasynaptic receptors. At nanomolar doses, these stereoisomers increase the effects of GABA on GABA<sub>A</sub> receptor-mediated chloride ion influx ~7 to 10 times, thus markedly enhancing the inhibitory impact of GABA on neuronal firing.<sup>1–6</sup> 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<sub>A</sub> receptor number and subunit

composition, further shaping the dynamic balance between inhibitory and excitatory neuronal signaling.<sup>1</sup>

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.<sup>7-11</sup> 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,<sup>2,12</sup> and lastly as interruption and reversal of neurodegenerative processes associated with Parkinson's and Alzheimer's disease.<sup>13,14</sup>

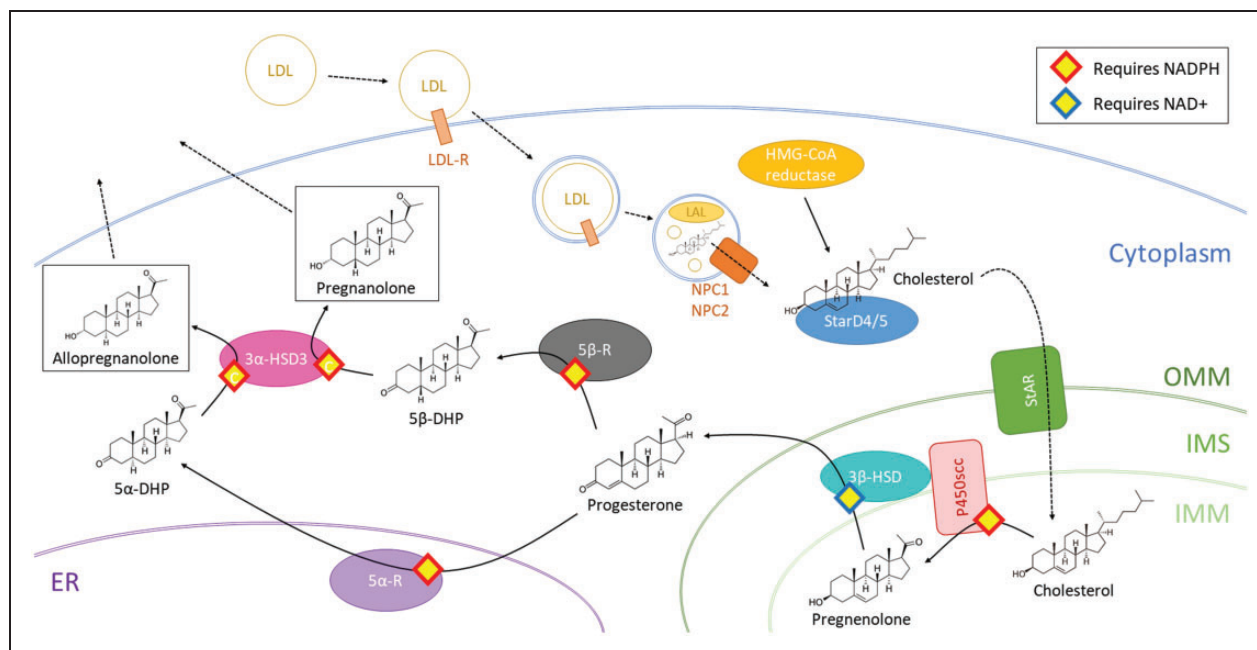
However, preclinical and clinical research suggests that high as well as low levels of these neurosteroids may be associated with phenotypically similar neuropsychiatric disorders.<sup>15-17</sup> Even apparently normal fluctuations in these neurosteroids that induce changes in GABA<sub>A</sub> receptor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

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<sup>22,23</sup>—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



**Figure 1.** 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; 5 $\alpha$ -DHP: 5 $\alpha$ -dihydroprogesterone; 5 $\beta$ -DHP: 5 $\alpha$ -dihydroprogesterone.

otherwise described below. Synthesis of pregnanolone follows similar steps with some differences described under the section “ $5\beta$ -reductase.”

### *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.<sup>24</sup>

It is thought that the majority of the cholesterol used in steroid biosynthesis originates from LDLs.<sup>24–26</sup> 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.<sup>27,28</sup> 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.<sup>24,27</sup> 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.<sup>28–30</sup>

Alternatively, cells may synthesize cholesterol de novo from three acetyl CoA molecules.<sup>31</sup> This reaction, catalyzed by 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, represents the committed, rate-limiting step for cholesterol biosynthesis; activated steroidogenic cells upregulate this enzyme.<sup>24,28</sup> 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.<sup>24,32</sup> 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.<sup>24,33</sup> 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.<sup>24,28</sup> 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).<sup>24,28,34</sup> 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.<sup>35–37</sup> The resulting domains of the endoplasmic reticulum, called mitochondria-associated membrane (MAM), are necessary for cholesterol transfer to steroidogenic mitochondria.<sup>35,38</sup>

### *Transport of Cholesterol Across the Mitochondrial Membrane*

The exact mechanism by which cholesterol is transported from the OMM to the inner mitochondrial membrane (IMM) is not fully understood, but some of the major steps and factors involved have been elucidated.

One of the most important proteins involved is StAR, which performs its cholesterol-transporting role only when anchored to the OMM.<sup>35,39</sup> StAR is first inserted into the OMM and then internalized into the mitochondrial matrix where it is degraded by mitochondrial proteases.<sup>35,40,41</sup> The half-life of a StAR molecule taking part in the one-way transport from the OMM to the mitochondrial matrix is around 4 to 5 h.<sup>35,40</sup> Structural analyses have identified a pocket in StAR capable of binding a single molecule of cholesterol.<sup>35,42</sup> However, each molecule of StAR supposedly transports hundreds of molecules of cholesterol, rather than the single cholesterol molecule suggested by its structural stoichiometry, suggesting yet unknown mechanisms by which StAR mediates transport of cholesterol.<sup>24,43</sup> StAR has been shown to interact with voltage-dependent anion channels 1 and 2 (VDAC1 and VDAC2) which are thought to regulate the processing of StAR as it passes from the OMM to the mitochondrial matrix.<sup>44,45</sup> Another protein in the  $\sigma$ -receptor family named  $\sigma$ -1 is thought to tether StAR and VDAC2 together to allow for this interaction in the MAM of the OMM and is essential to the proper action of StAR.<sup>46</sup>

Less is known about a controversial protein formerly proposed to play a role in mitochondrial cholesterol transport, the 18 kDa translocator protein (TSPO) originally characterized as the peripheral benzodiazepine receptor (PBR).<sup>47</sup> TSPO is anchored to the OMM and is estimated to constitute 5% to 10% of all proteins in the OMM of steroidogenic cells.<sup>48–50</sup> TSPO contains a cholesterol recognition/interaction amino acid consensus (CRAC) domain on its C-terminus, which extends out

into the cytosol.<sup>28,49,51</sup> TSPO is enriched at contact sites between the OMM and IMM.<sup>28</sup> Although TSPO is initially inserted into the OMM as a monomer, it is often found in polymeric form, in complex with many other proteins.<sup>28,49,52</sup>

One hypothesis suggests that the aforementioned proteins involved in cholesterol transport collaborate: StarD4/5 delivers cholesterol to the OMM where StAR is located.<sup>53</sup> Subsequently, StAR binds this cholesterol and was thought to transfer it to TSPO.<sup>28</sup> 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.<sup>28,52,54</sup> However, more recent studies with TSPO knockout mice demonstrated that loss of TSPO did not affect steroidogenesis or any gross aspect of development or behavior in comparison to wild-type mice, suggesting that TSPO does not play an essential role in this process.<sup>55–57</sup> However, further research has demonstrated that a reduction in TSPO does not affect gonadal steroidogenesis but significantly impairs adrenal steroidogenesis.<sup>58</sup> Given its intracellular localization to the OMM, high expression in steroidogenic 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 structural cholesterol<sup>28,59,60</sup> and undergo the first committed step for steroidogenesis described below.<sup>28,60</sup>

### P450 Side Chain Cleavage

P450 side chain cleavage (P450<sub>scc</sub>; also known as CYP11A1), located on the IMM, catalyzes the first committed step of steroidogenesis. P450<sub>scc</sub> transforms cholesterol 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 this enzyme).<sup>28,61</sup> P450<sub>scc</sub> is so crucial in committing cells to steroidogenesis that cells are defined as steroidogenic when they contain this protein.<sup>24</sup>

A pair of electrons is required for each of the three chemical reactions performed by P450<sub>scc</sub>.<sup>24</sup> P450<sub>scc</sub> activities thus require two co-factors: ferredoxin reductase, which receives electrons from NADPH (nicotinamide adenine dinucleotide phosphate), and ferredoxin, which shuttles electrons from ferredoxin reductase to P450<sub>scc</sub>.<sup>24,28</sup> The first reaction involves 22-hydroxylation of cholesterol bound to P450<sub>scc</sub>.<sup>24</sup> Due to the time required for the sequence of electron transport events, the binding of cholesterol, and the 22-hydroxylation of cholesterol, this first P450<sub>scc</sub> reaction constitutes the rate-limiting step in steroidogenesis in which approximately

20 molecules of cholesterol are processed by each P450<sub>scc</sub> molecule per second.<sup>24,61</sup> In the second step, the product of the first reaction undergoes 20-hydroxylation.<sup>24</sup> This is followed by the third step during which the C20-22 bond is cleaved to produce isocaproaldehyde and pregnenolone (Figure 1).<sup>24</sup>

### 3 $\beta$ Hydroxysteroid Dehydrogenase Type I and Type 2

In the context of allopregnanolone and pregnanalone synthesis, 3 $\beta$  hydroxysteroid dehydrogenase (3 $\beta$ -HSD) is responsible for transforming pregnenolone into progesterone.<sup>62</sup> 3 $\beta$ -HSD exists in two isoforms (3 $\beta$ -HSD1 and 3 $\beta$ -HSD2), which share 93% homology and a similar quaternary structure except for key residues that confer different ligand specificities.<sup>63</sup> The 3 $\beta$ -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.<sup>63</sup> However, the 3 $\beta$ -HSD1 isoform is found in non-steroidogenic tissues<sup>63</sup> and therefore may play a role in steroidogenesis in the central nervous system (CNS). This is important as 3 $\beta$ -HSD1 has a significantly higher affinity for common substrates, coenzymes, and inhibitors than 3 $\beta$ -HSD2<sup>64</sup> 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; 3 $\beta$ -HSD2 is discussed here, as it is more widely studied and has a well-delineated mechanism of action.

3 $\beta$ -HSD2 is localized to the IMM in close association with P450<sub>scc</sub> but performs its catalytic function in the intermembrane space because it requires a low pH environment to function.<sup>63,65,66</sup> 3 $\beta$ -HSD2 first performs a dehydrogenation reaction that converts NAD<sup>+</sup> (nicotinamide adenine dinucleotide) to NADH.<sup>62</sup> NADH induces a conformational change in 3 $\beta$ -HSD2, thus creating a catalytic site for the isomerization reaction that converts pregnenolone to progesterone.<sup>62,67</sup> The catalytic part of the protein can exist in three forms: a highly folded form at physiological pH, a partially folded form (molten globule with a high degree of secondary structure but no defined tertiary structure) at about pH 4.5, or a completely unfolded form at about pH 3.5.<sup>63,66</sup> 3 $\beta$ -HSD2 is most active when partially unfolded, a state stabilized by chaperone proteins that prevent 3 $\beta$ -HSD2 aggregation (in the case of denaturation) and facilitate recycling of the protein back to the less-active highly folded state during cell quiescence.<sup>63,66,68</sup> On both the OMM and IMM, 3 $\beta$ -HSD is tightly associated with P450<sub>scc</sub> and mitochondrial translocase proteins (Tom22, Tim50, and Tim23) that seem to be necessary for 3 $\beta$ -HSD2 function.<sup>63,65</sup> After synthesis, progesterone crosses the OMM into the cytosol via passive diffusion.<sup>28</sup>

### 5 $\alpha$ -Reductase Type 1 and Type 2 (5 $\alpha$ -R2)

5 $\alpha$ -Reductase (5 $\alpha$ -R) exists in three isoforms.<sup>69</sup> Type 1 (SRD5A1 or 5 $\alpha$ -R1) is the most abundant isoform in brain, but is also found in peripheral tissues, including the adrenal gland.<sup>70,71</sup> In the brain, 5 $\alpha$ -R1 has been colocalized with 3 $\alpha$ -hydroxysteroid dehydrogenase type 3 (also known as AKR1C2) (3 $\alpha$ -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.<sup>72,73</sup> 5 $\alpha$ -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.<sup>74–78</sup> 5 $\alpha$ -R2 is also expressed at high levels in spinal motor neurons and in the glomerulosa layer of the adrenal gland,<sup>79</sup> 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.<sup>23</sup> 5 $\alpha$ -R3 (SRD5A3) appears to be primarily responsible for mediating the N-glycosylation of proteins and does not seem to be active in steroidogenesis.<sup>69</sup>

Differing physiological characteristics of 5 $\alpha$ -R1 and 5 $\alpha$ -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 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT) and progesterone into 5 $\alpha$ -dihydroprogesterone (5 $\alpha$ -DHP), the immediate precursor for allopregnanolone.<sup>69</sup> 5 $\alpha$ -R1 has micromolar affinity for its steroid substrates (progesterone > testosterone > androstenedione > glucocorticoids) and is localized to the endoplasmic reticulum.<sup>79,80</sup> There it plays an important neuroprotective role by catabolizing the large quantities of steroids produced under conditions of stress<sup>81</sup> and certain reproductive phases in females, such as the luteal phase of the menstrual cycle and pregnancy.<sup>77</sup> In male rodents, 5 $\alpha$ -R1 activity increases markedly as testosterone levels decrease<sup>75</sup>—as occurs during intense stress in men.<sup>82</sup> In female rodents, testosterone has no effect on 5 $\alpha$ -R1 expression in the PFC, whereas 5 $\alpha$ -DHT markedly increases 5 $\alpha$ -R1 levels. In contrast, 5 $\alpha$ -R2 has nanomolar affinity for its substrates and thus may be critical in maintaining adequate resting allopregnanolone levels. In male rodents, 5 $\alpha$ -R2 levels fall markedly when testosterone levels decrease (as occurs during stress) and normalize when testosterone levels return to normal.<sup>75</sup> In contrast, a preliminary unconfirmed report suggests that 5 $\alpha$ -R2 expression in hippocampus may increase during acute stress.<sup>77</sup> Testosterone and 5 $\alpha$ -DHT have lesser effects on 5 $\alpha$ -R2 levels in females.<sup>76</sup>

Of note, potential effects of estradiol on 5 $\alpha$ -R levels have not been reported.

The mechanisms by which 5 $\alpha$ -R1 and 5 $\alpha$ -R2 reduce their steroid substrates have not been well elucidated. The hydrophobic nature of 5 $\alpha$ -R1 makes it difficult to solubilize in a form that retains metabolic activity<sup>83</sup>; thus, crystallography studies have not been possible. 5 $\alpha$ -R1 also lacks homology with other NADPH/steroid-binding enzymes.<sup>84</sup> 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.<sup>83,84</sup> 5 $\alpha$ -R1 works best at pH 5.0 to 8.0 and has a half-life of approximately 20 to 30 h.<sup>69</sup> Less is known about the chemical conditions under which 5 $\alpha$ -R2 activity is optimized.

### 5 $\beta$ -Reductase

5 $\beta$ -Reductase (5 $\beta$ -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 bile acid synthesis.<sup>85,86</sup> It shares homology with other AKR family members with a similar mechanism of action (e.g., 3 $\alpha$ -HSD, described below).<sup>85</sup> Northern blot studies on human tissues demonstrated abundant 5 $\beta$ -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.<sup>86</sup> This likely reflects a significant role for 5 $\beta$ -R in the inactivation of steroid hormones, given that two-thirds of these hormones, by mass, undergo transformation by 5 $\beta$ -R before excretion.<sup>87</sup>

Among other reactions, 5 $\beta$ -R catalyzes the reduction of progesterone to 5 $\beta$ -dihydroprogesterone (5 $\beta$ -DHP or 5 $\beta$ -pregnane-3-20-dione), the immediate precursor for pregnanolone and a potent activator of pregnane-X receptor (PXR, discussed later).<sup>88</sup> 5 $\beta$ -R requires NADPH, which induces a conformational change, and allows for binding of substrate.<sup>85</sup> Although the binding and release of NADPH and NADP<sup>+</sup> are generally the slowest steps in 5 $\beta$ -R-mediated reactions, 5 $\beta$ -R efficiency also depends on the substrate involved.<sup>85</sup> Human 5 $\beta$ -R demonstrates high efficiency for the reduction of progesterone, androstenedione, 17 $\alpha$ -hydroxyprogesterone, and testosterone; reduced efficiency for reduction of aldosterone and corticosterone; and poor efficiency for reduction of cortisol.<sup>86</sup>

### 3 $\alpha$ -Hydroxysteroid Dehydrogenase Type 3

3 $\alpha$ -HSD exists in four different isoforms (types 1, 2, 3, and 20a). Type 3 (associated with the AKR1C2 gene) is a cytosolic protein<sup>89</sup> found in steroidogenic tissues and the central nervous system.<sup>11,73,90,91</sup> Although 3 $\alpha$ -HSD3 has been primarily studied with regard to its role in

converting 5 $\alpha$ -DHT to 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ -diol), a very weak androgen and moderately potent GABAergic neuroactive steroid, it is also responsible for the conversion of 5 $\alpha$ -DHP and 5 $\beta$ -DHP to the potent GABAergic neurosteroid stereoisomers, allopregnanolone and pregnanolone, respectively.<sup>91</sup>

Among the 3 $\alpha$ -HSD family proteins, 3 $\alpha$ -HSD3 has the lowest catalytic efficiency, but the highest specificity for reducing 3-ketosteroids.<sup>91,92</sup> 3 $\alpha$ -HSD3 exists as a monomeric  $\alpha/\beta$  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.<sup>91,92</sup> 3 $\alpha$ -HSD3 binds NADPH before binding its steroid substrates; it then catalyzes the reduction of the substrate before releasing the steroid product.<sup>91</sup> The rate-limiting step in the 3 $\alpha$ -HSD3 conversion of substrate into product is the dissociation of NADP+ from the enzyme.<sup>91,93</sup>

### *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.<sup>73</sup> 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.<sup>94,95</sup> Albumin, the major serum protein, also carries a significant proportion of steroid hormones and has been shown to bind progesterone.<sup>96</sup> A less likely binding carrier is sex hormone-binding globulin, as it has high binding affinity only for 17 $\beta$ -hydroxysteroid hormones.<sup>97</sup>

The binding of allopregnanolone to the GABA<sub>A</sub> receptor hypersensitizes or prolongs the opening time of the chloride ion channel in the presence of GABA.<sup>1,2</sup> At a high enough concentration, allopregnanolone can also act as a direct agonist at this receptor.<sup>98</sup> Studies have identified allopregnanolone binding sites in the transmembrane domain of the GABA<sub>A</sub> receptor and suggest that allopregnanolone may access the receptor from an intracellular approach.<sup>1,73,98</sup> 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.<sup>2,99</sup> 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.<sup>2,100,101</sup> Other potential signaling partners for allopregnanolone have yet to be identified.

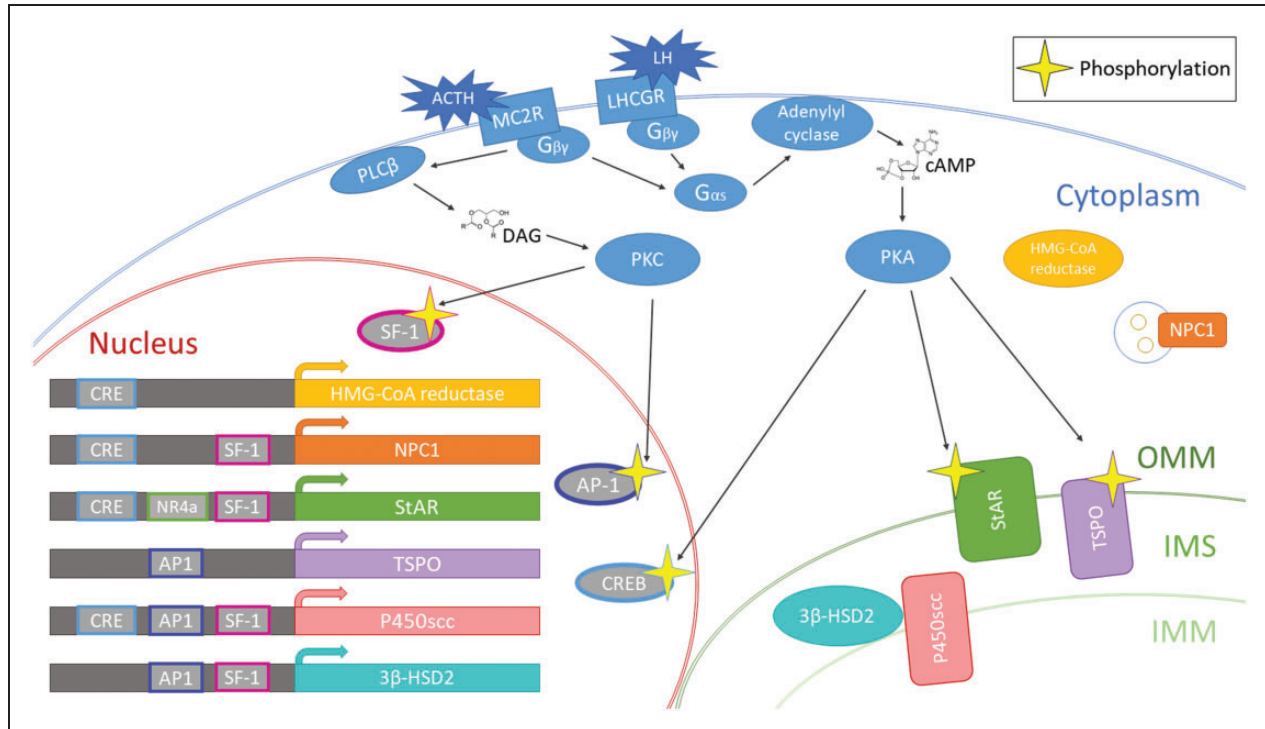
## **Common Pathways for Activating Steroidogenic Enzymes**

### *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.<sup>102</sup> Acutely, protein kinase A (PKA) phosphorylates StAR at Ser195, which doubles its enzymatic activity in transporting cholesterol from the OMM to the IMM.<sup>28,35</sup> TSPO is also a PKA substrate; phosphorylation of TSPO changes its ligand binding affinity and subsequently increases its activity.<sup>49,112</sup> 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 3 $\beta$ -HSD2 has been fairly well characterized due to their role in all steroid synthesis pathways, the regulatory mechanisms of 5 $\alpha$ -R, 5 $\beta$ -R, and 3 $\alpha$ -HSD3 are less well known. Recent research has shown that steroidogenic factor 1 (SF-1) regulates 5 $\alpha$ -R and 3 $\alpha$ -HSD differently: male murine SF-1 knockout models demonstrate decreased 5 $\alpha$ -R mRNA levels and, surprisingly, increased 3 $\alpha$ -HSD mRNA levels.<sup>113</sup> A few studies have demonstrated that male rat glial cells respond to increased cyclic adenosine monophosphate (cAMP) levels by enhancing de novo increases in 5 $\alpha$ -R mRNA levels and activity.<sup>114,115</sup> Similar experiments in male rodents show that 3 $\alpha$ -HSD does not increase in response to increases in cAMP.<sup>114</sup> 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



**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).<sup>103</sup> Upon ligand binding to MC2R or LHCGR, these receptors mediate a signaling cascade through their associated G-protein. The  $G_{\alpha s}$  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  $G_{\beta\gamma}$  subunit of the membrane-bound G-protein activates phospholipase C  $\beta$  (PLC $\beta$ ), 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.<sup>102</sup> CREs have been identified in the promoters of HMG-CoA reductase, NPC1, StAR, and P450scc.<sup>49,102,104,105</sup> PKC also has been shown to activate two transcription factors: steroidogenic factor 1 (SF-1) and activating protein 1 (AP-1).<sup>102</sup> SF-1 binding domains have been found in the promoters of NPC1, StAR, P450scc, and 3 $\beta$ -HSD2.<sup>102,106,107</sup> AP-1 binding domains have been found in the promoters of TSPO, P450scc and 3 $\beta$ -HSD2.<sup>49,62,102</sup> The Nuclear Receptor 4A (NR4A) family of transcription are involved in inflammation and have been shown to upregulate StAR and 3 $\beta$ -HSD2.<sup>108–111</sup> 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.

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.<sup>83,91</sup> 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 5 $\alpha$ -R1 and 3 $\alpha$ -HSD in brain.<sup>116</sup>—perhaps by increasing the activity of NADPH oxidase and reducing availability of NADPH.

### 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.<sup>117–121</sup> In contrast, compounds that induce neurosteroidogenesis or otherwise increase brain allopregnanolone levels reduce these negative behavioral

outcomes.<sup>9,122</sup> 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.<sup>15,123–130</sup> 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.<sup>49</sup>  $3\alpha$ -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.<sup>131–133</sup> 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.<sup>100,137</sup> 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.<sup>137</sup> 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.<sup>137</sup> Additionally, PXR drives the expression of phase II detoxification proteins such as glutathione-S-transferase as well as drug transporters such as organic anion-transporting polypeptide 2 (OATP2) and multidrug resistance-associated protein 2 (MRP2), which are responsible for excreting xenobiotic metabolites into the biliary system.<sup>137</sup> 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.<sup>100,138</sup> It is not known whether  $3\alpha$ -HSD3 is a target for PXR-induced upregulation, despite its role as a phase II detoxification protein.

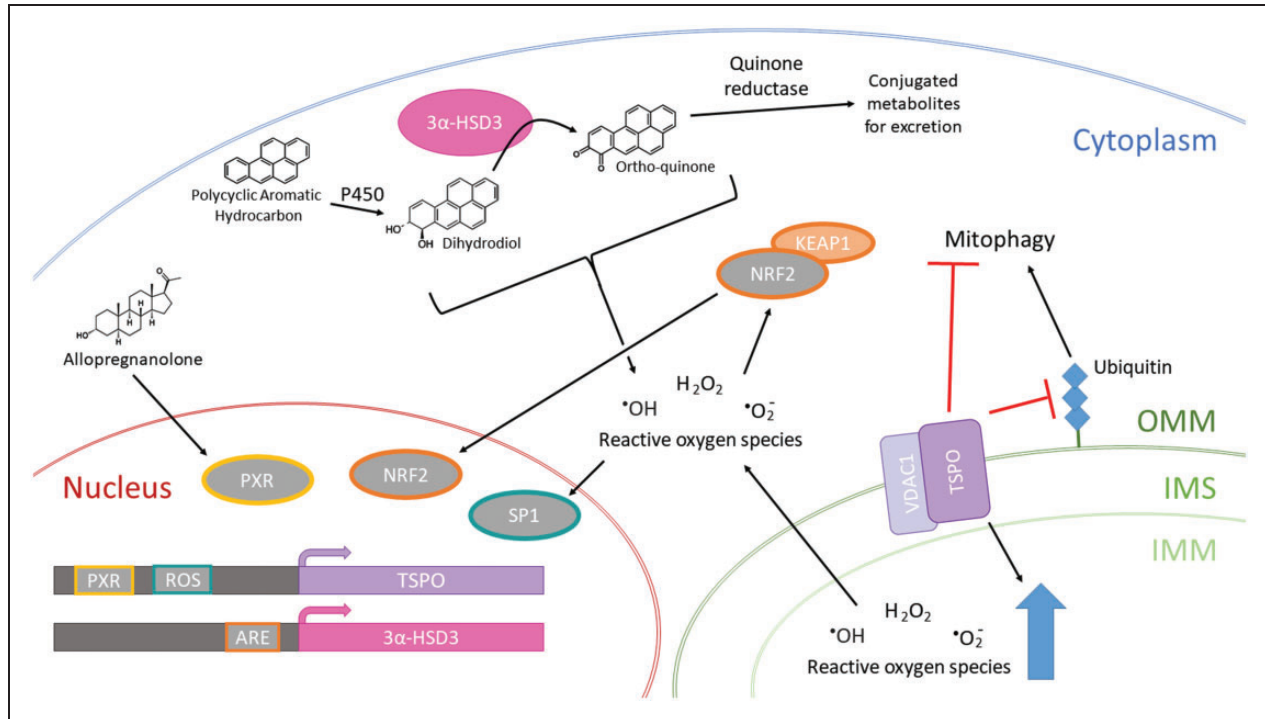
## Clinical Implications

### *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.<sup>24,26</sup> Wolman disease, one rare genetic disorder affecting LAL function, may lead to multi-organ failure and death in early childhood.<sup>24,26</sup> Cholesteryl ester storage disease is a milder, later-onset form of Wolman disease.<sup>24,26</sup> 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.<sup>24</sup> Niemann-Pick disease type C results from the mutation of NPC1, resulting in an inability to shuttle digested LDL products out of lysosomes.<sup>24,27</sup> Lysosomal glycosphingolipids and cholesterol accumulate to produce a variable phenotype that generally includes progressive neurodegeneration.<sup>24,26,28</sup> 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.<sup>139</sup> 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.<sup>140</sup> 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.<sup>141</sup>





**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 I (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.<sup>49,108,134</sup> It also has been shown to play a role in blocking ubiquitination of the mitochondria by other factors, thereby blocking mitophagy.<sup>134</sup> Among the regulatory elements that drive expression of the TSPO gene is an ROS response element in the promoter that binds specificity protein I (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.<sup>100</sup> On the other hand, 3 $\alpha$ -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 P450<sub>sc</sub>, which strictly plays a role in steroidogenesis; this family is also known as the CYP enzymes), to form a dihydrodiol.<sup>131–133</sup> 3 $\alpha$ -HSD3 then converts the dihydrodiol into an ortho-quinone, which is further conjugated to glutathione by a quinone reductase, thus producing a nontoxic metabolite for excretion from the body.<sup>131–133</sup> Both the dihydrodiol and the ortho-quinone intermediates are capable of producing ROS and are considered genotoxic.<sup>131,132</sup> However, the reaction pathway through 3 $\alpha$ -HSD3 is the major route for eliminating PAHs, making the synthesis of these intermediates necessary.<sup>135</sup> 3 $\alpha$ -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.<sup>132,136</sup> Nrf2 binds to the antioxidant response element (ARE) in the promoter of 3 $\alpha$ -HSD3 and upregulates its expression to meet the detoxification demands of the cell. Both TSPO and 3 $\alpha$ -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; 3 $\alpha$ -HSD3: 3 $\alpha$ -hydroxysteroid dehydrogenase type 3 (aka AKR1C2); PXR: pregnane X receptor

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.<sup>24,50,142</sup> Patients with defects in 3 $\beta$ -HSD are also diagnosed with CAH as 3 $\beta$ -HSD is required

for synthesis of nearly all steroidogenic end-products.<sup>63</sup> Less severe forms of 3 $\beta$ -HSD deficiency may present as hyperandrogenism in women, as progesterone precursors are routed into the androgen pathways.<sup>142</sup> 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.<sup>143</sup>

Severe mutations in 5 $\alpha$ -R2 result in male pseudohermaphroditism wherein patients with an XY genotype are unable to convert testosterone to 5 $\alpha$ -DHT, resulting in an

inability to masculinize the external genitalia.<sup>69,144</sup> Late masculinization of patients with mutations in 5 $\alpha$ -R2 also may result as 5 $\alpha$ -R1 activity increases during puberty.<sup>144</sup> Of note, a polymorphism of 5 $\alpha$ -R2 has been associated with increased risk for PTSD in males only.<sup>145</sup> 5 $\alpha$ -R deficiency in males with PTSD also has been characterized by a decrease in the ratio of 5 $\alpha$ -DHP to progesterone in CSF; the sum of CSF allopregnanolone and pregnanolone levels were, in turn, strongly and negatively correlated with PTSD symptoms.<sup>10</sup> No mutations of the 5 $\alpha$ -R1 gene have been recorded so far in humans, possibly because dysfunctional phenotypes have gone undetected.<sup>69</sup> However, experimental reduction of 5 $\alpha$ -R1 expression in male rodents results in reduced allopregnanolone levels and a behavioral phenotype characterized by anxiety, resistance to sedation by GABA receptor-active drugs, aggression, and enhanced contextual fear conditioning resistant to extinction and extinction retention,<sup>9,10,146</sup> phenomena consistent with PTSD in humans. 5 $\alpha$ -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.<sup>69,147</sup> One study suggested that prolonged use of finasteride reduced allopregnanolone concentrations in CSF.<sup>148</sup> Off-target side effects of finasteride (which has submicromolar potency for 5 $\alpha$ -R1, but sub-nanomolar potency for 5 $\alpha$ -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.<sup>149</sup>

Clinically relevant changes in 3 $\alpha$ -HSD3 expression have not been well characterized. Studies have implicated 3 $\alpha$ -HSD3 transcription in prostate and breast cancer.<sup>89,150,151</sup> Reduced expression of 3 $\alpha$ -HSD3 has been associated with hirsutism.<sup>152</sup> 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.<sup>12</sup> Female-specific 3 $\alpha$ -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 5 $\alpha$ -DHP, has been reported in premenopausal women with PTSD in association with increased negative mood and PTSD reexperiencing symptoms.<sup>125</sup> 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<sub>A</sub> 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.<sup>49,153–156</sup> 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.<sup>49,153,155,156</sup> A link between the administration of these ligands and GABA<sub>A</sub> receptor activity also has been demonstrated, most likely due to the effects of elevating allopregnanolone.<sup>153,155,156</sup> However, given that TSPO may not play a significant role in steroidogenesis,<sup>55–57</sup> 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,<sup>156</sup> 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 TSPO knockout mice in comparison to wild type.<sup>57</sup>

Selective serotonin reuptake inhibitors increase neurosteroidogenesis at doses below those that block serotonin reuptake.<sup>158</sup> An increase in allopregnanolone levels in the CSF of depressed patients following fluoxetine or fluvoxamine treatment correlated with decreases in depressive symptoms.<sup>129</sup> Fluoxetine, sertraline, and paroxetine were shown to increase the affinity of 3 $\alpha$ -HSD3 for 5 $\alpha$ -DHP in vitro, thereby increasing the rate of allopregnanolone synthesis from 5 $\alpha$ -DHP.<sup>158</sup> However, another study suggested that fluoxetine acts in female, but not male, rats by inhibiting the conversion of allopregnanolone back into 5 $\alpha$ -DHP.<sup>159</sup> 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<sub>A</sub> receptor modulating steroid antagonist<sup>17</sup> in treating PMDD, as well as dutasteride, which prevented luteal phase increases in plasma allopregnanolone.<sup>21</sup> 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.<sup>2,8,13,127,160–165</sup> Clinical trials of ganaxolone, a 3 $\beta$ -methylated analog of allopregnanolone with similar GABAergic properties are underway for treatment of focal epilepsies, female pediatric epilepsy, and fragile X syndrome.<sup>166</sup> Ganaxolone also was recently investigated in PTSD, but trough drug levels in plasma

were low and prevented a conclusive assessment of its efficacy.<sup>167</sup> 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<sup>14,168</sup> 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.<sup>14</sup> In PTSD, too high steady-state allopregnanolone levels would be expected to suppress reactivity of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis to novelty or stress,<sup>169</sup> thereby interfering with contextual learning and extinction, processes central to PTSD recovery. Notably, in rodents with experimentally induced allopregnanolone deficiency and related PTSD-like behaviors (including enhanced conditioned contextual fear and deficits in contextual fear extinction and extinction retention), administration of a single high dose of ganaxolone after the first extinction training session markedly improved the rate of extinction and extinction retention.<sup>163</sup>

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<sup>22</sup> during extinction learning may enhance consolidation of new learning and prevent the reconsolidation of non-reinforced fear conditioned associations.<sup>120</sup> The gradual development of LTP interference under such conditions is thought to protect newly modulated synapses from further excitation during memory consolidation.<sup>170</sup> 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.<sup>7,171</sup>

Finally, it should be noted that dramatic changes in the target receptor specificity of these GABAergic neurosteroids can result from small variations in their structure. The 3 $\beta$ -hydroxylated isomer of allopregnanolone (known as isopregnanolone, isoallopregnanolone, or epiallopregnanolone) has GABA<sub>A</sub> receptor subunit selective antagonist effects<sup>172,173</sup> and thus diminishes some but not all effects of allopregnanolone.<sup>173-175</sup> The 3 $\beta$ -hydroxylated isomer of pregnanolone

(epipregnanolone) also negatively modulates GABA<sub>A</sub> receptor function and blocks allopregnanolone effects, while the sulfated metabolites of allopregnanolone, pregnanolone, and epipregnanolone allosterically antagonize both GABA<sub>A</sub> and NMDA receptor function.<sup>176,177</sup> In contrast, epiallopregnanolone sulfate facilitates NMDA receptor function.<sup>178</sup>

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<sup>178</sup> 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.<sup>14,162,163</sup>

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