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Neurosteroids—Endogenous Regulators of Seizure Susceptibility and Role in the Treatment of Epilepsy

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INTRODUCTION

The term neurosteroid was coined in 1981 by the French endocrinologist Etienne-Emile Baulieu to refer to steroids that are synthesized de novo in the nervous system from cholesterol independently of the peripheral steroidogenic endocrine glands.1 Paul and Purdy then characterized neuroactive steroids as "natural or synthetic steroids that rapidly alter the excitability of neurons by binding to membrane-bound receptors such as those for inhibitory and (or) excitatory neurotransmitters."2 This chapter is concerned with the anticonvulsant and antiepileptogenic properties of neurosteroid-related steroid molecules (i.e., related to endogenously synthesized steroids) that meet the Paul and Purdy definition of neuroactive steroids by virtue of their pharmacological actions as positive allosteric modulators of γ-aminobutyric acid A (GABA_A) receptors. The effect of these steroids on GABA_A receptors occurs through a direct action on GABA_A receptors and is not related to interactions with classical steroid hormone receptors that regulate gene transcription. Indeed, GABA_A receptor modulatory...
neurosteroids are not themselves active at intracellular steroid receptors. We will make scant mention of other endogenous neurosteroids that exert other types of pharmacological actions, such as inhibition of GABA_\text{A_1} receptors or effects on excitatory amino acid receptors (pregnenolone sulfate is an example of such a steroid), although it is conceivable that neurosteroids with such actions could play a role in regulating seizure susceptibility.

It has been known since the 1940s, from the pioneering work of Hans Selye, that naturally occurring steroids such as the ovarian steroid progesterone and the adrenal steroid deoxycorticosterone (DOC) can exert anesthetic and anticonvulsant actions.\(^9\) Recognizing that some steroids could produce such acute central nervous system effects, researchers at the pharmaceutical company Glaxo identified the synthetic steroid alphaxalone as having anesthetic properties. In the early 1970s, alphaxalone was marketed as a component of the intravenous anesthetic agent Althesin, which also included the less potent anesthetic steroid alphadalone acetate, said to increase the solubility of alphaxalone.\(^4\) Several years later, the mechanism of action of alphaxalone was defined: it was found to enhance synaptic inhibition via an action on GABA_\text{A_1} receptors.\(^5\)\(^6\) A major advance occurred when naturally occurring metabolites of progesterone and DOC were also found to enhance and directly activate GABA_\text{A_1} receptors.\(^7\) It was speculated that the anesthetic and hypnotic properties of progesterone and DOC known since the time of Selye were due to their conversion in the body to these metabolites, respectively: allopregnanolone (3α-hydroxy-5α-pregnan-20-one) and allotetralhydrodeoxycorticosterone (3α,21-dihydroxy-5α-pregnan-20-one; THDOC). At the time, it was recognized that the enzymes required for the conversion of the steroid hormone precursors to their active A-ring reduced metabolites are present in brain so that part of the synthesis of the GABA_\text{A_1} receptor active steroids could occur locally. Therefore, the neuroactive steroids allopregnanolone and THDOC came to be referred to as neurosteroids even though it was not believed at the time that their synthesis occurred independently of peripherally synthesized precursor steroid hormones. We now know that all of the enzymes required for the synthesis of the GABA_\text{A_1} receptor active steroids from cholesterol are present in the brain.\(^8\) It is well recognized that these steroids readily cross the blood-brain barrier. While it is likely that locally synthesized GABA_\text{A_1} receptor active steroids play a role in modulating circuit excitability, there is little information on the relative importance of de novo local synthesis versus peripheral production of either the active GABA_\text{A_1} receptor modulatory steroids (e.g., allopregnanolone or THDOC) or their precursor hormones (e.g., progesterone or DOC), which are converted locally by brain 5α-reductase and 3α-hydroxysteroidoxidoreductase (3α-HSOR). Nevertheless, it is common to refer to GABA_\text{A_1} receptor modulatory steroids such as allopregnanolone and THDOC as neurosteroids, and we will follow that practice here. This chapter reviews the potential roles of such neurosteroids as endogenous modulators of seizure susceptibility and also the more limited evidence that they can, under certain circumstances, influence epileptogenesis (transformation of the brain to an epileptic state). The discussion of these topics serves as a prelude to the main objective of this chapter, which is to review the evidence supporting the utility of exogenously administered neurosteroid-related agents in the treatment of epilepsy.

**DIVERSITY OF NEUROSTEROIDS AND THEIR BIOSYNTHESIS**

A variety of GABA_\text{A_1} receptor modulatory neurosteroids are known to be synthesized endogenously (Figs. 77-1 and 77-2). The best recognized of these are the pregnane neurosteroids allopregnanolone and THDOC, which are produced via sequential A-ring reduction of the steroid hormones progesterone and its 21-hydroxylated derivative deoxycorticosterone by 5α-reductase and 3α-HSOR isoenzymes. In the periphery, the steroid precursors are mainly synthesized in the gonads, adrenal gland, and feto-placental unit, but as noted above, synthesis of both of these neurosteroids likely occurs in the brain from cholesterol or from peripherally derived intermediates including the steroid hormone precursors. A-ring reduction can also occur in peripheral tissues such as reproductive endocrine tissues, liver, and skin that are rich in the two reducing activities.\(^9\) Since neurosteroids are highly lipophilic and can readily cross the blood-brain barrier, neurosteroids
synthesized in peripheral tissues accumulate in the brain and can influence brain function.

The 5β-isomers of allopregnanolone and THDOC have GABA_A receptor modulatory activity that is only modestly less potent than that of the corresponding 5α-epimers. A steroid 5β-reductase enzyme is distributed widely in vertebrates, including in the gonads, liver, and brain. However, whether progesterone or DOC is a substrate, and the extent to which 5β-reduced epimers of allopregnanolone and THDOC are produced endogenously, is unclear.

The androgenic steroid testosterone differs from progesterone by virtue of a 17-alcohol that replaces the 17-acetyl in progesterone. Testosterone is a substrate for both 5α-reductase and 5β-reductase isoenzymes.

![Diagram of biochemical pathways of neurosteroid biosynthesis](image.png)

**Figure 77-1.** Biochemical pathways of neurosteroid biosynthesis. Cholesterol is trafficked by STAR (steroidogenic acute regulatory protein) and TSP (translocator protein; 18 kDa) to the inner mitochondrial membrane, where it is converted to pregnenolone by P450scc (cytochrome P450 side-chain cleavage). Pregnenolone is the precursor for progesterone that can undergo the two sequential A-ring reduction steps catalyzed by 5α-reductase and 3α-HSOR (3α-hydroxysteroid oxidoreductase) to form the neurosteroid allopregnanolone. Alternatively, in the zona reticularis of the adrenal cortex, P450c21 (cytochrome P450 21-hydroxylase) converts progesterone to DOC (deoxycorticosterone), which is the precursor for the neurosteroid THDOC (4-androsten-3α,17β-diol). In the brain, this reaction appears to be mainly catalyzed by CYP2D isoenzymes, which can also convert allopregnanolone to THDOC.

![Diagram of peripheral steroidogenesis showing side pathways for the production of known neurosteroids](image.png)

**Figure 77-2.** General scheme of peripheral steroidogenesis showing side pathways for the production of known neurosteroids. Steroids known to act on GABA_A receptor in a fashion similar to that of the prototype neurosteroids allopregnanolone and allosteroid hydroxydeoxycorticosterone are boxed.
The product of 5α-reduction of testosterone, 5α-dihydrotestosterone, is hormonally more active than testosterone itself. However, subsequent 3α-reduction leads to 5α-androstenediol (5α-androstane-3α,17β-diol), which is comparable in potency and efficacy to alloprogesterone as a GABA_1 receptor-positive modulator. Thus, 5α- and 5β-Androstenediol are further metabolized by 17β-hydroxysteroid dehydrogenase to androsterone and etiocholanolone, respectively, which are considered the major excreted metabolites of testosterone. These compounds and their conjugates are present at high concentrations. Androsterone and etiocholanolone also have GABA_1 receptor-positive modulatory activity and represent endogenous neurosteroids. Collectively, the various GABA_1 receptor modulatory steroids that lack the pregnane 17β-ethyl moiety, such as 5α-androstenediol, androsterone, and etiocholanolone, can be considered androsterone neurosteroids. Finally, substantial amounts of androstenediol, the 16-un-saturated form of 5α-androstenediol, are present in mammals, including humans. This compound is considered to be a pheromone that increases sexual receptivity in pigs and possibly other species. Androstenedol also is a GABA_1 receptor-positive modulator that has similar efficacy but is modestly less potent than alloprogesterone.

PRODUCTION OF NEUROSTEROIDS IN THE BRAIN AND THEIR LOCALIZATION TO PRINCIPAL NEURONS

In addition to peripheral production, it is clear that neurosteroids can be formed from steroid hormone precursors (such as progesterone, DOC, and perhaps testosterone) locally in the brain. 5α-Reductase activity has been identified in both neurons and glial cells in rodent and sheep brain, in regions, such as the neocortex and hippocampus, that are relevant to epilepsy. 3α-HSOR is also expressed widely in the brain. In humans, both enzymes have been found in neocortex and hippocampus. Thus, it is likely that neurosteroids can be formed from their parent steroid hormone precursors directly in the brain. Steroid precursors readily enter the brain, so pools of peripherally synthesized precursors are available for local neurosteroid biosynthesis. In peripheral tissues, 5α-reductase is believed to be the rate-limiting step in the production of neurosteroids because 3α-HSOR is a more ubiquitous enzyme; the same situation likely applies in brain.

It is likely that neurosteroids can be produced locally in brain not only from their steroid hormone precursors but also from more elementary steroid precursors such as cholesterol or pregnenolone. The rate-limiting step in steroidogenesis is the conversion of cholesterol to pregnenolone by the mitochondrial enzyme P450scc (cytochrome P450 cholesterol side-chain cleavage enzyme; CYP11A). Access of cholesterol to P450scc requires StAR (steroidogenic acute regulatory protein), which functions to transfer cholesterol from the outer mitochondrial membrane to the inner membrane where P450scc is located. Translocator protein 18 kD (TSPO), formerly called peripheral or mitochondrial benzodiazepine receptor, likely functions as a complex with StAR. Both proteins are expressed widely in peripheral tissues and in the brain. Activation of TSPO by certain ligands facilitates the intramitochondrial flux of cholesterol and thereby increases the availability of cholesterol to P450scc, enhancing pregnenolone synthesis and ultimately neurosteroid production.

The observation that TSPO ligands enhance neurosteroid production not only confirms the key role of TSPO in neurosteroidogenesis, but also suggests that such ligands may have therapeutic utility as an alternative to exogenously administered neurosteroids in situations where it is desirable to increase the level of brain neurosteroids.

In addition to P450scc, 3β-hydroxysteroid dehydrogenase, an enzyme required for the conversion of pregnenolone to progesterone, has been demonstrated in the brain. Thus, the enzymes necessary for in situ synthesis of progesterone from cholesterol are present in the brain.

In the adrenal, P450c21 (cytochrome P450 21-hydroxylase) converts progesterone to DOC, which is the precursor for the neurosteroid THDOC. The brain also possesses 21-hydroxylase activity, but it expresses only very small amounts of P450c21 mRNA and protein. It appears that cytochrome P450 2D (CYP2D) isoforms, in particular CYP2D4, present in brain can 21-hydroxylate progesterone to form DOC, which can then be converted to
THDOC. In addition, allopregnanolone itself is a substrate for CYP2D4, so that in brain, allopregnanolone may be converted directly to THDOC. Allopregnanolone and THDOC persist in the brain after adrenalectomy and gonadectomy or after pharmacological suppression of adrenal and gonadal steroid synthesis, confirming that these two key neurosteroids can be synthesized independently of peripherally produced steroid hormone precursors. However, the regulatory mechanisms underlying neurosteroid biosynthesis in the brain remain unclear.

In studies with mouse and rat brain, in situ hybridization with mRNA probes to 3α-reductase and 3α-HSOR indicates that the two mRNAs colocalize to glutamatergic principal neurons and not GABAergic inhibitory neurons or glial cells within neocortex, hippocampus, amygdala, and other brain regions. Immunohistochemistry with an antisera raised against allopregnanolone that also recognizes THDOC confirms that the neurosteroids are concentrated in principal neurons, predominantly in cell bodies and thick dendrites. The highly restricted distribution of neurosteroids to principal neurons suggests that they are mainly derived from local synthesis and not from the circulation, although it is clear that peripheral neurosteroids, as previously noted, readily cross the blood-brain barrier. It is remarkable that brain neurosteroids are localized to the neurons that contain their targets (GABA A receptors). This observation is consistent with the notion that neurosteroids function in an autocrine fashion in which they reach their targets by lateral membrane diffusion.

NEUROSTEROID MODULATION OF GABA A RECEPTORS

In electrophysiological studies, allopregnanolone and THDOC at aqueous concentrations in the range 10–1500 nM enhance the activation of GABA A receptors by GABA. At higher concentrations, the steroids directly activate the receptor in the absence of GABA. In addition, like other positive allosteric modulators of GABA A receptors, neurosteroids exert allosteric effects on these receptors such that there is enhancement of the binding of [3H]flunitrazepam, a benzodiazepine receptor agonist, and [3H]muscimol, a specific GABA A site agonist, as well as inhibition of the binding of [3H]t-butylbicycloorthobenzoate (TBPS), a cage convulsant and noncompetitive GABA A receptor antagonist. Neurosteroid enhancement of GABA A receptors occurs through increases in both channel open frequency and channel open duration. Thus, neurosteroids greatly enhance the probability of GABA A receptor chloride channel opening, thereby enhancing GABA A receptor-mediated inhibition.

The effects of neurosteroids on GABA A receptors occur by binding to discrete sites on the receptor-channel complex that are located within the transmembrane domains of the α and β subunits. The binding sites for neurosteroids are distinct from the recognition sites for GABA, benzodiazepines, and barbiturates. Although the exact location of neurosteroid binding has not been mapped, it has been proposed that there are two distinct sites for neurosteroids that act as positive modulators: one for allosteric enhancement of GABA and another for direct activation of the receptor. Using site-directed mutagenesis, it has been shown that a highly conserved glutamine at position 241 in the M1 domain (toward the intracellular side) of the α subunit plays a key role in neurosteroid modulation of GABA responses and is believed to contribute to the binding site for modulation. Additional nearby residues in the M4 domain of the same α subunit (tyrosine 410 and asparagine 407, which are located more toward the extracellular side) have also been proposed to contribute to the binding site. Other investigators have found that mutations in serine 240 and tryptophan 245 of the α subunit interfere with neurosteroid potentiation. Studies with structurally diverse steroids have led to the conclusion that the steroid binding pocket on the α subunit is more correctly viewed as a hydrophobic surface that can accommodate steroid molecules of different structures. Direct activation of the receptor, in contrast, has been proposed to be due to binding at a site on the interface between β and α subunits formed by a threonine at position 236 in the α subunit and a tyrosine at position 284 in the β subunit. However, more recent models of
the GABA<sub>3</sub> receptor have questioned whether these residues reside at the β subunit-α subunit interface. A photo-incorporable analog of the anesthetic etomidate appears to bind at the interface, but binding of this ligand is not competitively inhibited by neurosteroids. In fact, neurosteroids (at concentrations that produce direct receptor activation) enhance binding, presumably due to allosteric effects transmitted upon interaction with a different site on the receptor. The newer topology models do not bring into proximity the residues in the β and α subunits proposed to constitute the site for direct activation. Therefore, at present, the location of this site is uncertain.

A range of steroid structures have activity as positive modulators of GABA<sub>3</sub> receptors in line with the hydrophobic surface binding site model. Nevertheless, there are certain strict structural requirements for neurosteroid-positive modulation. A hydrogen bond-donating 3α-hydroxy group on the steroid A-ring and a hydrogen bond-accepting group (typically a keto moiety) on the D-ring at either C20 of the pregnane steroid side chain or C17 of the androstan ring system are critical for positive modulatory activity at GABA<sub>3</sub> receptors. The orientation of the C5 hydroxy group only modestly influences potency.

Studies with recombinant GABA<sub>3</sub> receptor isoforms indicate that neurosteroids act on most subunit combinations. This distinguishes neurosteroids from benzodiazepines, which only act on GABA<sub>3</sub> receptors that contain γ2 subunits and do not contain α4 or α6 subunits. In general, the specific α subunit type may influence neurosteroid efficacy, whereas the γ subunit type may affect both the efficacy and potency of neurosteroid modulation.

GABA<sub>3</sub> is a relatively low-efficacy agonist of GABA<sub>3</sub> receptors in which the δ subunit replaces the more common γ2 subunit, even though it binds with high affinity to such δ-subunit-containing receptors. Neurosteroids therefore have an opportunity to markedly enhance the current generated by δ-subunit-containing GABA<sub>3</sub> receptors even in the presence of saturating GABA concentrations. Consequently, GABA<sub>3</sub> receptors that contain the δ subunit are highly sensitive to neurosteroid-induced potentiation of GABA responses and mice lacking δ subunits show drastically reduced sensitivity to neurosteroids. GABA<sub>3</sub> receptors containing δ subunits exhibit low desensitization, and they are located nonsynaptically (perisynaptically/extrasynaptically) since the γ2 subunit is required for synaptic targeting. These properties cause them to be prime candidates for mediating tonic GABA<sub>3</sub> receptor current that is activated by ambient concentrations of GABA in the extracellular space. Ambient GABA is believed to result from spillover of synaptically released GABA; concentrations would increase with high levels of activity of GABAergic interneurons, as occurs during seizures. Tonic GABA<sub>3</sub> receptor current causes a steady inhibition of neurons and reduces their excitability. Neurosteroids could therefore have a general role in setting the level of excitability and might specifically potentiate tonic inhibition during seizures when ambient GABA may rise. Overall, the robust effect of neurosteroids is likely to be due to their action on both synaptic and perisynaptic/extrasynaptic GABA<sub>3</sub> receptors.

Although neurosteroids are viewed as high-potency modulators of GABA<sub>3</sub> receptors since they are effective at concentrations in the mid-nanomolar to low-micromolar range in aqueous solution, recent studies indicate that neurosteroid binding to the GABA<sub>3</sub> receptor is actually of low affinity (K<sub>a</sub> ~1 mM). The high effective potency of neurosteroids results from partitioning of the lipophilic steroids within the plasma membrane, such that the concentrations presented to the receptor are orders of magnitude greater. Neurosteroids access the GABA<sub>3</sub> receptor from the lipophilic plasma membrane. The nonspecific accumulation and removal of the neurosteroids from the membrane are the major factors determining the rates of neurosteroid action when applied to cells via aqueous solution; rates of binding and unbinding to the receptor are only secondary factors. It is noteworthy that intracellular delivery through the plasma membrane is compatible with the autocrine mechanism discussed above, in which the neurosteroids act on the GABA<sub>3</sub> receptors in the same neurons in which they are produced.

As noted, at high concentrations (>10 μM), neurosteroids can directly activate GABA<sub>3</sub> receptor channels in the absence of GABA<sub>3</sub>. In this respect, neurosteroids resemble barbiturates but not benzodiazepines. Given the high concentrations required, whether direct
actions are relevant to the role of endogenous neurosteroids or to the pharmacological actions of exogenously administered neurosteroid-related agents is not well understood.

ANTICONVULSANT AND ANTEIPILEPTOGENIC EFFECTS OF NEUROSTEROIDS

Exogenously administered neurosteroids, like other agents that act as positive GABA$_A$ receptor modulators, exhibit broad-spectrum anticonvulsant effects in diverse rodent seizure models. They protect against seizures induced by GABA$_A$ receptor antagonists including pentylentetrazol (PTZ) and bicuculline, and they are effective against pilocarpine-induced limbic seizures and seizures in kindled animals. However, neurosteroids may exacerbate generalized absence seizures. The potencies of neurosteroids in models where they confer seizure protection vary largely in accordance with their activities as positive allosteric modulators of GABA$_A$ receptors. Thus, allopregnanolone has roughly equal potency to THDOC, but androstenediol, androstenedione, and etiocholanolone are somewhat less potent. Like other anticonvulsant agents that act on GABA$_A$ receptors, neurosteroids are inactive or only weakly active against electrically induced tonic extension seizures elicited according to the maximal electroshock (MES) protocol that is widely used for drug screening. However, they are active in the 6 Hz model in mice in which limbic-like seizures are induced by electrical stimulation of lower frequency and longer duration than in the MES test. In general, neurosteroids have comparable potencies in the 6 Hz and PTZ models. Neurosteroids are also highly effective in suppressing seizures due to withdrawal of GABA$_A$ receptor modulator drugs including neurosteroids and benzodiazepines (diazepam), and also due to other types of agents such as ethanol, which may act in part through GABA$_A$ receptors. In contrast to benzodiazepines, where utility in the chronic treatment of epilepsy is limited by tolerance, anticonvulsant tolerance is not obtained with neurosteroids. Thus, neurosteroids have the potential to be used in the chronic treatment of epilepsy, and this has been borne out in clinical trials (see below).

The mechanisms responsible for tolerance to benzodiazepines are not known. However, factors such as uncoupling of the allosteric linkage between the GABA and benzodiazepine sites and changes in receptor subunit turnover with switching of subunits may be contributing mechanisms. Neurosteroids do not act on the benzodiazepine site of GABA$_A$ receptors, and they are able to modulate all isoforms of GABA$_A$ receptors, even those that contain benzodiazepine-insensitive a4 and a6 subunits or do not include the obligatory a2 subunit required for benzodiazepine sensitivity. Thus, it is clear that neurosteroids can act on GABA$_A$ receptors where the proposed benzodiazepine tolerance mechanisms have been invoked. Surprisingly, while chronic neurosteroid exposure does not lead to anticonvulsant tolerance, chronic neurosteroid exposure does lead to tolerance to benzodiazepines. Thus, it appears that the same plastic changes that underlie benzodiazepine tolerance are brought into play by chronic neurosteroid exposure. However, neurosteroids, acting at distinct sites on GABA$_A$ receptors and exhibiting effects on the full range of GABA$_A$ receptor isoforms, do not exhibit anticonvulsant tolerance.

The sulfated neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate, which act as GABA$_A$ receptor antagonists, are pro-convulsant when administered at high doses into the brain, producing seizures and status epilepticus. Compelling evidence that such steroids exist endogenously in the brain is lacking, and in any case it is unlikely that they exist at sufficiently high concentrations to exert pro-convulsant effects, so the physiological relevance is unclear. However, it is known that the seizure-facilitating effects of these steroids can be blocked by coadministration of allopregnanolone or other neurosteroids that positively modulate GABA$_A$ receptors.

In addition to anticonvulsant activity, there is some limited evidence that endogenous neurosteroids play a role in regulating epileptogenesis. Following pilocarpine-induced status epilepticus in the rat, the neurosteroidogenic enzyme P450scc is upregulated for several weeks, suggesting that neurosteroidogenesis may be increased. Ordinarily, rats develop spontaneous recurrent seizures following a latent period of similar duration to the period during which P450scc is elevated. Inhibiting neurosteroid synthesis with...
finasteride accelerated the onset of spontaneous recurrent seizures, suggesting that endogenous neurosteroids play a role in restraining epileptogenesis or at least that they inhibit the expression of seizures. Exogenous treatment with neurosteroids or with progesterone, which serves as a precursor for neurosteroid synthesis, has also been reported to delay the occurrence of epileptogenesis in some situations. In fact, progesterone may impair epileptogenesis in kindling models, even at doses that do not affect seizure expression. If endogenous neurosteroids can be confirmed as endogenous regulators of epileptogenesis, neurosteroids themselves or modulators of neurosteroid disposition could potentially have disease-modifying therapeutic activity.

ROLE OF ENDOGENOUS NEUROSTEROIDS IN THE MODULATION OF SEIZURES
Endogenous neurosteroids may play a role in the physiological regulation of seizure susceptibility in individuals with epilepsy. We will discuss several such situations: catamenial epilepsy, stress, temporal lobe epilepsy, and alcohol withdrawal. However, it is noteworthy that there is no evidence that alterations in neurosteroid levels in the absence of preexisting epilepsy can induce epileptogenesis.

Neurosteroids in Catamenial Epilepsy
Catamenial epilepsy, the cyclical occurrence of seizure exacerbations during particular phases of the menstrual cycle in women with preexisting epilepsy, is a specific form of pharmaco-resistant epilepsy. Catamenial seizure exacerbations affect up to 70% of women of childbearing age with epilepsy. Although there are several forms of catamenial epilepsy, neurosteroids have been implicated only in the seizure exacerbations that occur in the most common situation, which is when women with normal menstrual cycles experience seizure exacerbations in the perimenstrual period. It is hypothesized that withdrawal of progesterone-derived neurosteroids leads to enhanced brain excitability predisposing to seizures.

During the menstrual cycle, circulating progesterone levels are low in the follicular phase but rise in the midluteal phase for about 10 to 11 days before declining in the late luteal phase. Circulating allopregnanolone levels parallel those of its parent progesterone. Circulating THDOC levels also fluctuate during the menstrual cycle, with higher levels in the luteal phase. Overall, the serum levels of THDOC are lower than those of allopregnanolone, so THDOC is likely to be less relevant to catamenial epilepsy, although it could contribute. An important unanswered question is whether the local brain synthesis of neurosteroids also fluctuates.

In addition to withdrawal of the anti-convulsant effects of neurosteroids in association with the fall in progesterone at the time of menstruation, plasticity in GABA_A receptors, the targets of neurosteroid action, could also play a role in the enhanced brain excitability that is presumed to underlie the increase in seizure susceptibility in perimenstrual catamenial epilepsy. The precise changes in brain GABA_A receptor subunit expression occurring during the human menstrual cycle have not been determined. However, it is now well recognized that prolonged exposure to allopregnanolone in rats causes increased expression of the a4 GABA_A receptor subunit in hippocampus, resulting in decreased benzodiazepine sensitivity of GABA_A receptor currents. Although a4 can coassemble with y2 to form synaptic GABA_A receptors, it preferentially coassembles with d to form nonsynaptic (perisynaptic/extrasynaptic) GABA_A receptors. Treatment of rats with allopregnanolone results in transient increased expression of the d subunit in hippocampus and increased benzodiazepine-insensitive tonic current. Progesterone also increases d subunit expression, likely as a result of conversion to allopregnanolone. The relevance of the increased d subunit expression for catamenial epilepsy is unclear, as d subunit increases may be transitory and followed by reduced expression with chronic exposures, as in pregnancy or in the prolonged luteal phase of the human menstrual cycle. Therefore, an important consequence of the incorporation of the normally low-abundance a4 subunit into synaptic GABA_A receptors is that synaptic currents generated by these receptors have accelerated decay kinetics, so that there is less total charge transfer, which results in
reduced inhibition. GABA$_A$ receptor-modulating neurosteroids cause a prolongation of the decay of GABA-mediated synaptic currents. Consequently, in the presence of high levels of allopregnanolone during the luteal phase, the acceleration due to $\delta$4 substitution is balanced. However, when neurosteroids are withdrawn at the time of menstruation, synaptic inhibition is diminished from normal, resulting in enhanced excitability, which, among other effects, predisposes to seizures. Indeed, chronic exposure to neurosteroids also is accompanied by down-regulation of $\delta$ subunit expression and perisynaptic/extrasynaptic GABA$_A$ receptors. This change is believed to be a compensatory mechanism, which would avoid excessive sedation caused by high neurosteroid levels acting on sensitive $\delta$ subunit-containing GABA$_A$ receptors. At the time of neurosteroid withdrawal, $\delta$ subunit expression rapidly recovers. However, if recovery is not sufficiently fast, there could be an enhancement of excitability due to a reduction in tonic inhibition mediated by perisynaptic/extrasynaptic GABA$_A$ receptors in the relative absence of neurosteroids.

A rodent model has been developed to simulate the hormonal changes that are believed to be relevant to perimenstrual catamenial epilepsy. Rodents have a 4 to 5 day estrous cycle, and studies of fluctuations in seizure susceptibility in cycling female rodents have not led to results that are relevant to the human menstrual cycle. In order to provide a model that more closely mimics the human situation, a condition of pseudopregnancy was induced in rats by sequential gonadotropin treatment. This resulted in prolonged high circulating levels of estrogen and progesterone similar to those that occur in the luteal phase of the 28 day human menstrual cycle. Then, to simulate the withdrawal of allopregnanolone that occurs in conjunction with the fall in progesterone levels at the time of menstruation, the animals were treated with finasteride 11 days after the initiation of gonadotropin treatment.

The neurosteroid withdrawal model of catamenial epilepsy was used to investigate therapies for perimenstrual catamenial epilepsy. A key result is that conventional antiepileptic drugs, including benzodiazepines and valproate, have reduced potency in protecting against seizures during the period of enhanced seizure susceptibility following neurosteroid withdrawal. This pharmacoresistance seems to mimic the situation in women with catamenial epilepsy in which breakthrough seizures occur despite treatment with antiepileptic drugs. In contrast to the results with conventional antiepileptic drugs, neurosteroids, including allopregnanolone, THDOC, and their $\beta$-isomers, were found to have enhanced activity in the perimenstrual catamenial epilepsy model.

This suggested a “neurosteroid replacement” approach to treat catamenial seizure exacerbations. A neurosteroid could be administered in a “pulse” prior to menstruation and then withdrawn or continuously administered throughout the month. While intermittent administration at the time of increased seizure vulnerability is rational, continuous administration would avoid withdrawal of the therapeutic agent, which itself could predispose to seizures. This factor, as well as the practical difficulty many women experience in predicting the time of their menstrual periods, suggests that continuous administration is preferred. The neurosteroid would be administered at low doses to avoid sedative side effects. Such low doses are expected to contribute little anticonvulsant activity during most of the menstrual cycle. Patients would still require treatment with conventional antiepileptic medications. However, during the period of enhanced seizure susceptibility at the time of menstruation, the increased potency of the neurosteroid would confer protection against perimenstrual seizure exacerbations. It is noteworthy that while the anticonvulsant activity of neurosteroids increases in conjunction with neurosteroid withdrawal, there is no corresponding increase in side effects (mainly sedation), at least as assessed by a measure of motor impairment. Therefore, enhanced side effects, which would negate the potential of the therapeutic approach, would not be expected to occur.

To determine whether the enhanced activity of neurosteroids is due to pharmacokinetic or pharmacodynamic factors, brain and plasma levels of the neurosteroid ganaxolone ($3\alpha$-hydroxy-3$\beta$-methyl-5$\alpha$-pregnan-20-one, discussed below) were determined with a liquid chromatography-mass spectrometric method. Control and neurosteroid withdrawn animals received a single dose of ganaxolone (7 mg/kg, subcutaneously), resulting in an elevation in PTZ threshold that peaked at 30 min and returned to baseline at 120–180 min. Ganaxolone caused a markedly greater (1.8-fold) elevation of the PTZ threshold in the withdrawn animals than in controls.
indicating a greater sensitivity to the anticonvulsant effects of ganaxolone. Surprisingly, plasma and brain ganaxolone levels were reduced in withdrawn animals (69% of control levels). Adjusting for the reduced brain levels, the pharmacodynamic sensitivity to ganaxolone was enhanced 2.3-fold in the withdrawn animals compared with controls. There was a significant increase in clearance (CL) of ganaxolone in the withdrawn animals, which accounts for the reduced plasma and brain levels. Ganaxolone levels reached a peak more slowly in brain \( T_{\text{max-brain}} \approx 30 \text{ min} \) than in plasma \( T_{\text{max-plasma}} \approx 15 \text{ min} \); the \( T_{\text{max-brain}} \) value corresponds with the peak elevation in seizure threshold. These studies confirmed the enhanced anticonvulsant activity of ganaxolone in the rat model of catamenial epilepsy. The enhanced activity occurs in the face of decreased plasma and brain ganaxolone levels, indicating a marked increase in pharmacodynamic sensitivity.

Recently, studies have been conducted with the catamenial epilepsy model in female rats that experienced a prolonged bout of status epilepticus induced by lithium-pilocarpine treatment, resulting in a chronic epileptic state with spontaneous recurrent seizures. Epileptic animals in the catamenial epilepsy model exhibited about six seizures per day, each lasting approximately 1 min. When neurosteroids were withdrawn by treatment with finasteride, an enormous (more than 10-fold) increase in seizure frequency was observed. In contrast, finasteride did not induce seizures in normal animals. However, it did induce an increase in seizures in epileptic rats that were not treated with gonadotropins, albeit of smaller magnitude than that in the pseudopregnant animals. The observation that inhibition of the synthesis of endogenous neurosteroids in nonepileptic animals did not lead to seizures indicates that neurosteroid reductions are not epileptogenic. This is consistent with the observation that finasteride does not cause seizures in humans who do not have epilepsy. Finasteride is used clinically for the treatment of benign prostatic hypertrophy and male pattern hair loss. Seizures have not been reported as an adverse effect of the drug. While it is clear that finasteride does not provoke seizures in the general population, there are no prospective studies to determine whether inhibition of 5α-reductase by finasteride influences seizure susceptibility in individuals with epilepsy. There is a single anecdotal report of a woman with epilepsy taking finasteride for male pattern baldness who experienced an increase in seizure frequency and severity in association with finasteride use. The doses of finasteride used clinically are in the range of 1–5 mg/day, which are far less than the doses of 30–100 mg/kg used in rats to inhibit brain neurosteroid synthesis. Furthermore, in humans, finasteride is selective for the type 2 5α-reductase isoform and is less active on the type 1 enzyme that is the isoform predominantly present in the brain. This selectivity is not observed with the rat enzymes. In sum, finasteride, as administered clinically in humans, probably does not block neurosteroidogenesis sufficiently to influence seizure susceptibility under most circumstances. Also, individuals with congenital 5α-reductase deficiency, caused by a mutation in the 5α-reductase type 2 gene (a condition with ambiguous genitalia), do not exhibit epilepsy. While neurosteroid reduction by itself does not lead to epilepsy, it is apparent that endogenous neurosteroids do modulate seizure susceptibility in epileptic animals.

Although it has been assumed that the effect of finasteride on seizure susceptibility is mediated through inhibition of peripheral neurosteroidogenesis, ovariectomized epileptic animals also exhibited large increases in seizure frequency following finasteride treatment, indicating that a major effect of the drug may be to influence neurosteroid synthesis in the brain. Whatever the site of action of finasteride, treatment with exogenous allopregnanolone was found to rapidly terminate the finasteride-induced exacerbation of seizures, providing additional evidence that the increase in seizure frequency is due to a finasteride-induced reduction in neurosteroids and not some other action of the drug. More importantly, it supports the concept that neurosteroid replacement may be useful in the treatment of seizures associated with neurosteroid fluctuations, such as catamenial epilepsy. In catamenial epilepsy, breakthrough seizures occur despite treatment with antiepileptic drugs. Previous studies (reviewed in ref. 88) and the new results from Lawrence et al. support the potential of neurosteroids as a novel treatment approach for these pharmaco-resistant seizures.

Although neurosteroids seems to be the most direct approach to the treatment of catamenial
Neurosteroids and Stress-Induced Seizure Fluctuations

The availability of neurosteroids is increased during physiological stress. Stress results in the hypothalamic release of corticotropin-releasing hormone (CRH), which liberates adrenocorticotropic hormone (ACTH) from the anterior pituitary. Along with cortisol, ACTH also enhances the synthesis of adrenal DOC, which is released into the circulation and can serve as a precursor for synthesis of the neurosteroid DOC (Fig. 77-1). In contrast to allopregnanolone, which is present in the brain even after adrenalectomy and gonadectomy, THDOC appears to be derived nearly exclusively from adrenal sources. Plasma and brain levels of THDOC and allopregnanolone rise rapidly following acute stress. Acute stressors such as swimming, foot shock, or carbon dioxide exposure elicit an increase in allopregnanolone and THDOC concentrations in plasma and in brain. Plasma levels of THDOC normally fluctuate between 1 and 5 nM, but increase to 15–30 nM following acute stress and might reach 40–60 nM during pregnancy. In contrast, allopregnanolone levels during the third trimester of pregnancy typically reach 70–160 nM and have been measured as high as 220 nM. 

Stress-induced neurosteroids have been demonstrated to elevate the seizure threshold. Stress-induced seizure protection could be due to circulating neurosteroids synthesized in peripheral tissues or to those produced locally in the brain. However, the effects of swim stress-induced increases in seizure threshold and THDOC levels in rats were abolished in adrenalectomized animals, implicating adrenal-derived THDOC. Despite stress-induced seizure protection in animals, patients and clinicians are not likely to recognize a reduction in seizure frequency associated with stress. Indeed, stress has been reported to trigger seizure activity in persons with epilepsy. During stressful episodes adrenal hormone levels are expected to fluctuate, and it may simply be the withdrawal of THDOC during such fluctuations that is associated with seizure provocation. Alternatively, other unidentified hormonal factors with proconvulsant activity may be responsible for stress-induced increases in seizures. However, chronic stress of the type experienced by patients with epilepsy likely has different endocrinological consequences than acute stress. The effects on seizures of fluctuations in neurosteroid levels in chronic stress remain to be studied.

Neurosteroids in Temporal Lobe Epilepsy

Sexual and reproductive dysfunction are common among persons with epilepsy. In particular, men with temporal lobe epilepsy (TLE) often have diminished libido and sexual potency that is associated with low testosterone levels. This hypogonadal state has been attributed to the effects of certain hepatic enzyme-inducing antiepileptic drugs, or alternatively—given the extensive connections between temporal lobe structures such as the amygdala and hypothalamic nuclei that
govern the production and secretion of gonadotropin releasing hormone—to suppression of the hypothalamic-pituitary-gonadal axis by limbic seizures. There is evidence that serum androgen levels normalize after temporal lobe surgery that results in successful seizure control but not in patients who continue to have seizures, supporting the view that seizures are responsible for the hypoandrogenic state.\textsuperscript{9,10} Testosterone, as noted previously, is a precursor for at least three neurosteroids with anticonvulsant properties: 5α-androstane-3α,17α-diol, androsterone, and etiocholanolone.\textsuperscript{9,11} There is evidence that serum levels of at least two of these steroids (androsterone and etiocholanolone) are reduced in men with epilepsy compared with control subjects.\textsuperscript{10} It is conceivable that reduced levels of such anticonvulsant neurosteroids lead to an enhanced propensity for seizures and that neurosteroid replacement might be a useful therapeutic approach.

Certain biological factors in TLE may influence the sensitivity to endogenous neurosteroids and could have an impact on the efficacy of exogenous neurosteroids used in epilepsy therapy. Studies in a status epilepticus model of TLE have shown a striking reduction in δ subunit-containing GABA\textsubscript{A} receptors in the dentate gyrus,\textsuperscript{11,12} suggesting that neurosteroid effects on nonsynaptic GABA\textsubscript{A} receptors may be reduced. In addition, in dentate gyrus granule cells, neurosteroid modulation of synaptic currents is diminished and δ4 subunit-containing receptors are present at synapses.\textsuperscript{13} All of these changes may facilitate seizures in epileptic animals but may reduce the efficacy of endogenous neurosteroids. The expression of neurosteroidogenic enzymes such as P450scc\textsuperscript{73} and 3α-HSOR\textsuperscript{11,19} appears to be elevated in the hippocampus in animals and human subjects affected by TLE. If local neurosteroidogenesis is enhanced, this may counteract in part the epileptogenesis-induced changes. However, the effect of withdrawal of neurosteroids, as might occur in catamenial epilepsy or with stress, could be enhanced.

Neurosteroids and Alcohol Withdrawal Seizures

Systemic administration of moderate doses (1–2.5 g/kg) of ethanol causes increases in plasma and brain neurosteroids that may contribute to many of the behavioral effects of ethanol in rodents.\textsuperscript{115} This effect of ethanol is believed to be due to activation of the hypothalamic-pituitary-adrenal axis. As is the case in the catamenial epilepsy model, chronic ethanol-induced elevations in neurosteroids lead to an enhancement in the anticonvulsant actions of the neurosteroids allopregnanolone and THDOC.\textsuperscript{116} These effects are associated with increases in the sensitivity of GABA\textsubscript{A} receptors to neurosteroids.\textsuperscript{117} Endogenous neurosteroids may protect against ethanol withdrawal seizures. However, ethanol induction of allopregnanolone is diminished in tolerant and dependent animals. Reduced availability of allopregnanolone under such circumstances may be a factor that predisposes to alcohol withdrawal seizures. As is the case with catamenial epilepsy, neurosteroid replacement could conceivably be useful in the treatment of alcohol withdrawal seizures, given that current pharmacological approaches are not entirely satisfactory.\textsuperscript{117}

GANAXOLONE AS A NOVEL NEUROSTEROID-BASED ANTIPILEPTIC DRUG

Ganaxolone, the synthetic 3β-methyl derivative of allopregnanolone,\textsuperscript{118} is the only neurosteroid that has been evaluated for the treatment of epilepsy in humans.\textsuperscript{75,118} Allopregnanolone itself has been administered to humans at low doses intravenously (0.05–0.09 mg/kg) and found to be largely free of side effects except for sedation.\textsuperscript{10,115} However, it has been proposed that allopregnanolone can undergo back conversion by 3β-HSOR isoenzymes to a hormonally active intermediate (dihydropregesterone).\textsuperscript{112} The 3β-methyl substituent of ganaxolone eliminates this back conversion, potentially avoiding hormonal side effects. Other than this theoretical advantage, ganaxolone has pharmacological properties similar to those of the natural neurosteroid from which it is derived.

Preclinical Studies

Ganaxolone has protective activity in diverse rodent seizure models, including clonic seizures induced by the chemoconvulsants pentylenetetrazol, bicuculline, flurothyld
t-butylbicycloorthobenzoate, and aminophylline; limbic seizures in the 6 Hz model; amygdala- and cocaine-kindled seizures; and cornelia kindled seizures (Table 77-1).\textsuperscript{64,65,158} In chronically treated rats, tolerance does not occur to the anticonvulsant activity of ganaxolone.\textsuperscript{67} In addition, a recent study in female amygdala-kindled mice demonstrated suppression of behavioral and electrographic seizures with a median effective dose ($ED_{50}$) of 6.6 mg/kg.\textsuperscript{157}

Animal pharmacokinetic studies have found that ganaxolone has a large steady-state volume of distribution (6.5, 7.0, 19.5, and 3.5 L/kg in mice, rats, rabbits, and dogs, respectively), indicating that it distributes extensively into tissues.\textsuperscript{78} Studies with radioactive ganaxolone in rats have found that ganaxolone (and its metabolites) are concentrated in tissues including the brain (brain-to-plasma concentration ratio between 5 and 10). Ganaxolone is highly bound to human plasma proteins (>99%). It is extensively metabolized to at least 16 different compounds; the primary metabolite is 16α-hydroxyganaxolone, which likely results from the action of CYP3A4. This primary metabolite is inactive in the PTZ seizure model and is 25-fold weaker than ganaxolone in inhibiting \textsuperscript{[35S]}TBPS binding. Ganaxolone is a CYP3A4 autoinducer in rodents but not in dogs or humans; chronic exposure to high doses in female rats does cause liver hypertrophy. Metabolites of ganaxolone are eliminated in the urine (13%–23%) and feces (65%–76%) in rats and dogs; the corresponding values in male healthy volunteers are 25% and 69%, respectively. Because of its aqueous insolubility, orally administered ganaxolone is poorly absorbed. To provide more consistent bioavailability, the steroid has been administered as a submicron particulate suspension and in a proprietary solid formulation.

Animal safety studies have demonstrated little evidence of target organ or systemic toxicity with either single-dose or multiple-dose ganaxolone treatment. In studies on pre- and postnatal development in mice, rats, and dogs, ganaxolone did not affect fetal implantation, viability, or growth and development from birth to weaning and was not teratogenic. Genotoxicity tests have not demonstrated any mutagenic or clastogenic potential for ganaxolone. Oral administration of ganaxolone to conscious dogs at a dose of 10 mg/kg did not reveal changes in cardiovascular hemodynamics.

### Clinical Safety and Efficacy Studies

Over the past decade, ganaxolone has been studied in various clinical trials to assess its efficacy and safety in the treatment of epilepsy. More than 900 subjects have received the drug at doses of up to 1875 mg/day in adults and up to 54 mg/kg/day in children in Phase 1 normal volunteer studies, epilepsy trials, and also clinical trials for migraine. Single oral doses of 50–1600 mg in healthy volunteers results in peak plasma concentrations of 14 to as high as 460 ng/mL. Overall, the drug is safe and well tolerated. The most common side effect is

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**Table 77-1 Anticonvulsant Profile of Ganaxolone in Mouse Seizure Models**

<table>
<thead>
<tr>
<th>Seizure Model</th>
<th>$ED_{50}$ Value (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentylentetrazol</td>
<td>3.5 (2.1–5.8)</td>
<td>Reddy and Rogawski\textsuperscript{127}</td>
</tr>
<tr>
<td>Pentylentetrazol kindling</td>
<td>4.1 (2.7–6.4)</td>
<td>Cassar et al.\textsuperscript{125}</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>4.6 (3.2–6.8)</td>
<td>Carter et al.\textsuperscript{118}</td>
</tr>
<tr>
<td>Fluorothyl</td>
<td>5.0 (ND)</td>
<td>Liptáković et al.\textsuperscript{123}</td>
</tr>
<tr>
<td>6 Hz</td>
<td>6.3 (4.0–9.8)</td>
<td>Kaminski et al.\textsuperscript{112}</td>
</tr>
<tr>
<td>Amygdala kindling</td>
<td>6.6 (5.1–9.7)</td>
<td>Reddy and Rogawski\textsuperscript{127}</td>
</tr>
<tr>
<td>t-Butylbicycloorthobenzoate</td>
<td>11.7 (8.8–15.7)</td>
<td>Carter et al.\textsuperscript{118}</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>11.5 (8.1–16.3)</td>
<td>Carter et al.\textsuperscript{118}</td>
</tr>
<tr>
<td>Cocaine kindling</td>
<td>17.0 (ND)</td>
<td>Kaminski et al.\textsuperscript{128}</td>
</tr>
<tr>
<td>Maximal electroshock</td>
<td>29.7 (25.3–34.8)</td>
<td>Carter et al.\textsuperscript{118}</td>
</tr>
<tr>
<td>N-methyl-D-aspartate</td>
<td>&gt;30 (ND)</td>
<td>Carter et al.\textsuperscript{118}</td>
</tr>
<tr>
<td>Stricynus</td>
<td>&gt;40 (ND)</td>
<td>Carter et al.\textsuperscript{118}</td>
</tr>
</tbody>
</table>

Numbers in parentheses are 95% confidence intervals. ND, not determined. $ED_{50}$ is the dose estimated to produce seizure protection in 50% of mice. The $ED_{50}$ value in the rotarod test of motor toxicity in mice was 33.4 (50.9–39.4) mg/kg.\textsuperscript{119}
versus 2%). Seven percent of the subjects in those in the placebo group discontinued treatment, whereas indicated that ganaxolone maintains its efficacy over time. Adverse events reported by at least 5% of patients, and at least twice as common in the ganaxolone group compared to the placebo group, were dizziness, fatigue (both 16% versus 8%), and somnolence (13% versus 2%). Seven percent of the subjects in the ganaxolone treatment group and 6% of those in the placebo group discontinued treatment due to adverse events.

CONCLUSIONS

Neurosteroids are endogenous modulators of neural excitability that are believed to have a role in the regulation of seizure susceptibility in the setting of preexisting epilepsy. Menstrual and stress-related fluctuations in seizures may in part be related to changes in brain neurosteroid levels. In addition, men with TLE who have suppression of the hypothalamic-pituitary-gonadal axis may have a reduction in testosterone-derived neurosteroids that could worsen seizures.

Treatment with exogenously administered natural neurosteroids or synthetic analogs such as ganaxolone may be beneficial to treat partial seizures. Further studies are required to determine if neurosteroid replacement is a useful therapeutic approach for seizure exacerbations related to endogenous neurosteroid fluctuations, such as in catamenial epilepsy and stress. In the future, agents that influence the endogenous synthesis of neurosteroids, such as Tspo ligands, may find utility as an alternative to neurosteroids themselves in the treatment of epilepsy.131

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DISCLOSURE STATEMENT

D.S.R. has no conflicts of interest to disclose. M.A.R. is a consultant to Sage Therapeutics and a scientific founder and has served as consultant to Marinus Pharmaceuticals, the current sponsor of ganaxolone.

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