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Neurosteroid regulation of $GABA_A$ receptors: a role in catamenial epilepsy

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Abstract

The female reproductive hormones progesterone and estrogen regulate network excitability. Fluctuations in the circulating levels of these hormones during the menstrual cycle cause frequent seizures during certain phases of the cycle in women with epilepsy. This seizure exacerbation, called catamenial epilepsy, is a dominant form of drug-refractory epilepsy in women of reproductive age. Progesterone, through its neurosteroid derivative allopregnanolone, increases γ-aminobutyric acid type-A receptor (GABAR)-mediated inhibition in the brain and keeps seizures under control. Catamenial seizures are believed to be a neurosteroid withdrawal symptom, and it was hypothesized that exogenous administration of progesterone to maintain its levels high during luteal phase will treat catamenial seizures. However, in a multicenter, double-blind, phase III clinical trial, progesterone treatment did not suppress catamenial seizures. The expression of GABARs with reduced neurosteroid sensitivity in epileptic animals may explain the failure of the progesterone clinical trial. The expression of neurosteroid-sensitive δ subunit-containing GABARs is reduced, and the expression of α4γ2 subunit-containing GABARs is upregulated, which alters the inhibition of dentate granule cells in epilepsy. These changes reduce the endogenous neurosteroid control of seizures and contribute to catamenial seizures.

Keywords

Catamenial epilepsy; progesterone; neurosteroids; $GABA_A$ receptors

Introduction

Epilepsy is a neurological disorder characterized by recurrent unprovoked seizures. The occurrence of seizures is mostly unpredictable; however, cyclicity in seizure incidence is observed in women of reproductive age. The seizure clustering characteristic of catamenial epilepsy occurs due to menstrual cycle-linked hormonal changes. Female reproductive hormones, progesterone and estrogen, modulate network excitability through their

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neuroactive derivatives called neurosteroids. This review focuses on the role of neurosteroid regulation of seizures via modulation of GABARs.

A temporal pattern of seizure clustering, called catamenial epilepsy, is observed in as many as 30% of women of reproductive age with epilepsy (Frye, 2008;Reddy and Rogawski, 2009;Reddy, 2013;Bazan et al., 2005;Herzog et al., 2004;Duncan et al., 1993;Herzog et al., 1997;Herzog, 2008;Reddy and Rogawski, 2009;Herzog et al., 2015). Catamenial seizures are seen in all types of epilepsy, but, are more prevalent in patients with temporal lobe epilepsy (TLE) (Taubøll et al., 1991;El-Khayat et al., 2008;Duncan et al., 1993;Quigg et al., 2008) and represent a major form of drug refractory epilepsy in women. Based on the time of seizure exacerbation, catamenial seizures can be divided into three patterns; seizure clustering during the perimenstrual phase is classified as type I or C1 and increased seizure incidence during the follicular phase is classified as type II or C2, whereas seizure exacerbation during the luteal phase is classified as type III or C3 (Herzog et al., 1997) (Fig. 1). The fluctuations in progesterone and estrogen levels during the menstrual cycle underlie these patterns of seizure clustering. Estrogen levels rise during the follicular phase and reach a peak at the time of ovulation. On the other hand, progesterone levels rise following ovulation and decline just before the end of the cycle. Progesterone typically exerts anticonvulsant actions, whereas estrogen is a proconvulsant (see below), and the cyclic changes in the levels of progesterone and estrogen are responsible for the different patterns of catamenial seizures. Type I seizure clustering occurs due to withdrawal form high progesterone levels, whereas a high ratio of estrogen to progesterone during the follicular phase is proposed to cause type II seizure clustering (Herzog et al., 1997). In contrast, type III seizures occur due to an inadequate luteal phase in which progesterone levels do not rise to the levels normally seen in healthy women (Herzog et al., 1997). Perimenstrual seizure exacerbation is the most commonly observed pattern of catamenial seizures (Quigg et al., 2008). A clinical diagnosis of catamenial epilepsy is made if the seizure frequency during a particular phase is greater than twice that seen during other phases (Herzog, 2015). The clinical aspects of catamenial epilepsy are discussed elsewhere in this issue. Here, we will focus on the molecular mechanisms regulating catamenial seizures.

Neurosteroids

Progesterone is metabolized by glia in the brain to compounds called neurosteroids, which can alter inhibitory and excitatory neurotransmission. In addition to progesterone, stress steroids are also a substrate for neurosteroid synthesis. Neurons and glia can also synthesize neurosteroids from cholesterol (Fig. 2), and the neurosteroid levels in the brain can be augmented independent of the levels of circulating steroid hormones (Corpechot et al., 1993;Purdy et al., 1991;Baulieu, 1998). Conversion of cholesterol to allopregnanolone involves multiple steps regulated by an array of enzymes. Cytoplasmic cholesterol is first transported to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR, also called translocator protein (TSPO) or peripheral benzodiazepine receptors), then enzyme P450 side-chain cleavage breaks it down to progesterone, which can enter the allopregnanolone synthetic pathway (Rupprecht et al., 2009;Korneyev et al., 1993;Papadopoulos et al., 2006;Le et al., 1987). The enzymes involved in neurosteroid synthesis are expressed in almost all regions of the brain, as their mRNA and protein can be

detected in the principal neurons of the cortex, hippocampus, thalamus, amygdala, and hypothalamus (King et al., 2002;Stoffel-Wagner et al., 2000;Stoffel-Wagner et al., 2003;Stoffel-Wagner, 2003;Petratos et al., 2000;Melcangi et al., 1998;Stoffel-Wagner, 2001;Ibanez et al., 2003;Kimoto et al., 2002). Interestingly GABAergic interneurons are devoid of neurosteroid synthetic enzymes (Agís-Balboa et al., 2006); since neurosteroids synthesized within interneurons could dampen their activity and affect GABA release on principal neurons, the absence of neurosteroid synthetic machinery in interneurons could function to protect the activity of interneurons. Many species of amphibians, birds, and mammals appear to express neurosteroid synthetic enzymes in the brain, suggesting that endogenous neurosteroids may regulate excitability across vertebrates (Do Rego et al., 2009).

Impaired neurosteroid synthesis in the brain can cause epilepsy. Patients of infantile-onset epileptic encephalopathy protocadherin-19 female-limited epilepsy (PCDH19-FE) have lower serum neurosteroid levels and altered expression of some of the neurosteroid synthetic enzymes (Tan et al., 2015). Whether neurosteroid levels in patients with acquired epilepsy are also lower has not been studied. However, many women with epilepsy experience disorders of reproductive endocrine function (Herzog et al., 1986), which may limit the availability of circulating progesterone for allopregnanolone synthesis. A recent study has found that serum neurosteroid levels are reduced in patients in status epilepticus, which is a disorder characterized by self-sustaining seizures that can last for days to months (Meletti et al, 2017). Serum neurosteroid levels also increase as pregnancy progresses in women (Pennell et al, 2015). A similar measurement of serum neurosteroid levels in epilepsy patients could enhance our understanding of how spontaneous seizures affect endogenous neurosteroid synthesis.

The reproductive cycle of female epileptic animals is also affected (Scharfman et al., 2009;Amado et al., 1987), and the experimental animals may provide a useful system to understand whether endogenous neurosteroid synthetic machinery is impaired by spontaneous seizures. Acquired epilepsy develops following inciting brain insults such as febrile seizures, brain trauma, infection, status epilepticus, and brain tumors. Blockade of endogenous neurosteroid synthesis hastens the onset of spontaneous seizures (Joshi et al., 2017;Biagini et al., 2009;Biagini et al., 2010;Biagini et al., 2006). We found that even a transient blockade of neurosteroid synthesis can accelerate epileptogenesis. Treatment of animals with finasteride, an inhibitor of enzyme 5α-reductase that regulates the rate-limiting step in allopregnanolone synthesis, on a single day following status epilepticus led to an early onset of spontaneous seizures (Joshi et al., 2017). The expression of enzyme cytochrome P450 side-chain cleavage is transiently upregulated in the hippocampus following statue epilepticus (Biagini et al., 2009). Whether this plays a compensatory role is not known. A detailed characterization of the expression of neurosteroidogenic enzymes during epileptogenesis and in epileptic animals will enhance our understanding of epilepsy and provide insights into whether neurosteroid synthetic enzymes can be targeted for therapeutic purposes.

Neurosteroid regulation of seizures

Progesterone is an endogenous anticonvulsant agent, and an inverse correlation exists between progesterone levels and seizure frequency (Bäckström, 1976). Infusion of progesterone suppresses ictal activity in human EEG (Bäckström, 1984). Similarly, acute administration of progesterone also reduces the susceptibility to seizures evoked by chemical or electrical stimulation in experimental animals (Frye et al., 2002;Frye and Scalise, 2000;Kokate et al., 1999a;Reddy et al., 2004;Reddy and Ramanathan, 2012). Multiple lines of evidence show that the anticonvulsant effects of progesterone are mediated through its conversion to allopregnanolone. Progesterone cannot exert anticonvulsant effects in animals treated with finasteride or in mice that lack the expression of enzyme 5α-reductase (Frye et al., 2002;Kokate et al., 1999a;Reddy et al., 2004). Furthermore, administration of allopregnanolone or the stress steroid derivative tetrahydrodeoxycorticosterone can suppress seizures evoked by chemical or electrical stimulation on their own (Frye and Scalise, 2000;Kokate et al., 1994;Kokate et al., 1996;Kokate et al., 1999a;Reddy et al., 2004). Progesterone and neurosteroids also increase the threshold to kindling (Carter et al., 1997;Holmes and Weber, 1984;Reddy et al., 2010;Edwards et al., 2001;Holmes and Weber, 1984;Reddy and Ramanathan, 2012).

All of the above studies were performed in naïve animals; since seizures can affect the expression of neurosteroid synthetic enzymes as well as their cellular targets, it is important to study the anticonvulsant effects of neurosteroids in epileptic animals. Reddy and colleagues (2011) have developed an animal model of catamenial epilepsy in which high progesterone levels are induced by sequential treatment with pregnant mare serum gonadotropin (PMSG) followed by human chorionic gonadotropin (β-HCG); neurosteroid withdrawal, similar to that observed during the perimenstrual period in women, is then triggered by administration of finasteride. Lawrence and colleagues used this model of catamenial seizures in epileptic animals to determine the effects of elevated progesterone levels and subsequent neurosteroid withdrawal on the frequency of spontaneous seizures (Lawrence et al., 2010). They found that PMSG and β-HCG treatment induced a 2-3-fold increase in serum progesterone levels; however, the frequency of spontaneous seizures did not differ between the treated and untreated animals (Lawrence et al., 2010). Furthermore, finasteride treatment not only caused a dramatic increase in seizure frequency in PMSG and β-HCG-treated animals, it also increased seizure frequency in animals with basal levels of progesterone. Thus, in contrast to the findings in non-epileptic female animals, a chronic rise in progesterone levels did not suppress spontaneous seizures in epileptic animals.

The findings of Lawrence and colleagues are similar to the findings of a recent clinical trial of progesterone therapy for catamenial seizures. Since catamenial seizures are believed to be a neurosteroid withdrawal disorder, elevating progesterone levels by twice daily treatment for 14 days was proposed to suppress catamenial seizures (Herzog et al., 2012). However, in this phase III, double-blind, multicenter trial, an equal fraction of women treated with progesterone and placebo treatment reported a reduction in seizure frequency. Thus, chronic progesterone treatment failed to suppress catamenial seizures in patients.

There are reports of a few clinical studies showing suppression of catamenial seizures by progesterone treatment and one anecdotal study, in which a woman on progesterone therapy experienced more seizures when she was treated with finasteride for male pattern baldness (Herzog, 1995;Herzog and Frye, 2003). There may be some protective effect in women with an identified type I seizure exacerbation pattern (Herzog and Frye, 2014). One factor that could contribute to the reduced efficacy of neurosteroids and the failure of the clinical trial is that the expression of GABARs, which are targets of neurosteroids in the brain (see below), is altered. Thus, additional studies are necessary to gain insight into acute versus the chronic effects of progesterone in epileptic and non-epileptic animals.

Neurosteroids and GABARs

The anticonvulsant effects of neurosteroids are mediated via their action on GABARs, which are GABA-gated chloride channels. Activation of GABARs leads to chloride influx in a majority of instances and causes membrane hyperpolarization that dampens excitation. GABARs are assembled from various subunits α, β, γ, δ, and ε, and are expressed throughout the brain and spinal cord (Sieghart, 2006;Whiting, 2003). Some of the subunits have multiple isoforms, whereas others are represented by a single isoform; for example, there are six α subunits (α1 to α6), three β subunits (β1 to β3), and 3 γ subunits (γ1 to γ3), whereas the δ and ε subunits have only one isoform (Whiting et al., 1999). A majority of GABARs are composed of 2 α, 2 β and a γ, δ, or ε subunit (Baumann et al., 2001), and there is region-specific expression of receptors with a specific subunit assembly. For example, α4βxδ subunit-containing receptors are expressed on the extrasynaptic membrane of hippocampal dentate granule cells (DGCs) and thalamic nuclei, whereas α6βxδ subunit-containing receptors are expressed on cerebellar granule cells (Sur et al., 1999;Bencsits et al., 1999;Quirk et al., 1994;Pirker et al., 2000). In contrast, hilar interneurons express α1βxδ subunit-containing receptors (Glykys et al., 2007;Milenkovic et al., 2013;Peng et al., 2004). The γ2 subunits exhibit substantial diversity in partnering with α subunits; receptors containing γ2 subunits that contain α1, α2, α4, or α5 subunits are found under pathophysiological conditions (Glykys et al., 2008;Rajasekaran et al., 2010;Sieghart and Sperk, 2002). Receptors containing only α and β subunits also appear to be expressed on cultured hippocampal pyramidal neurons (Mortensen and Smart, 2006); whether these receptors are expressed *in vivo* is unclear.

Each subunit is made up of an ‘N’ terminal extracellular domain followed by four transmembrane domains and a short ‘C’ terminal extracellular domain (Fig. 3). The 2nd transmembrane domain lines the ion-channel pore. The intracellular loop joining the transmembrane domains 3 and 4 contains sites for phosphorylation by different kinases such as protein kinase C and protein kinase A (Jacob et al., 2008). This region also interacts with proteins such as GABAR-associated protein (GABARAP) (Jacob et al., 2008). GABAR subunit composition regulates the surface membrane localization and dynamics of trafficking of the receptors. The γ2 subunit-containing receptors are clustered at the synapses through their interaction with gephyrin (Essrich et al., 1998;Sun et al., 2004;Nusser et al., 1998;Zhang et al., 2007;Alldred et al., 2005). On the other hand, receptors lacking γ subunits are restricted to the peri- or extrasynaptic membrane (Nusser et al., 1998;Wei et al., 2003;Sun et al., 2004).

Synaptic and extrasynaptic GABARs mediate distinct forms of inhibition, phasic and tonic inhibition, respectively (Nusser and Mody, 2002;Mtchedlishvili and Kapur, 2006;Glykys and Mody, 2007b;Glykys and Mody, 2007a;Glykys et al., 2008). Release of GABA at the synaptic cleft triggers synaptic currents that can be measured as spontaneous or miniature inhibitory post-synaptic currents (Fig. 4). On the other hand activation of extrasynaptic receptors leads to a persistent background current that can be measured in terms of the holding current or the membrane noise (Nusser and Mody, 2002;Mtchedlishvili and Kapur, 2006) (Fig. 4). The δ subunit-containing GABARs desensitize slowly and incompletely (Saxena and Macdonald, 1994); thus, once opened, these receptors remain open for a long time. By virtue of these unique properties, the δ subunit-containing receptors mediate a major fraction of the tonic current, which is substantially attenuated in mice lacking δ subunit expression (Maguire et al., 2005;Stell et al., 2003).

The subunit composition also determines the pharmacological properties of GABARs (Olsen et al., 2007;Olsen and Sieghart, 2009;Sieghart, 2006). The GABA affinity of δ subunit-containing receptors is higher than the γ2 subunit-containing GABARs (Saxena and Macdonald, 1994). Thus, these receptors are activated by the low nanomolar concentrations of GABA that spill over the synaptic cleft and/or are released by glia (Glykys and Mody, 2007b). The δ subunit-containing receptors are also more sensitive to alcohol and neurosteroids than those containing a γ2 subunit (Olsen et al., 2007;Olsen and Sieghart, 2009;Sieghart, 2006). In contrast, the δ subunit-containing GABARs are insensitive to benzodiazepines, which are the widely used anticonvulsant agents that allosterically activate γ2 subunit-containing receptors that do not contain α4 or α6 subunits (Olsen et al., 2007;Olsen and Sieghart, 2009;Sieghart, 2006).

Neurosteroids can activate many GABAR subtypes expressed in the brain (Majewska et al., 1986;Maitra and Reynolds, 1998;Belelli et al., 2002;Puia et al., 1993;Puia et al., 1990). Neurosteroids are lipophilic in nature and can access the binding site from the inside or outside of the cell (Akk et al., 2005;Chisari et al., 2010). The neurosteroid binding site is localized in the interphase between the α and β subunits (Hosie et al., 2006), and there is a stringent structural requirement for the binding of neurosteroids to the receptors (Wittmer et al., 1996). Site-directed mutagenesis studies have helped identify residues that are critical for neurosteroid binding (Hosie et al., 2006). The α1 subunit residue T236 is critical for direct activation of GABARs, whereas the residue Q241 is important for allosteric modulation and activation. Furthermore, residues N407 and Y410 are also involved in binding to neurosteroids. In addition to these residues in the α1 subunit, residue T284 of the β2 subunit is also involved i n the direct activation of GABARs (Hosie et al., 2006). The binding of neurosteroids to residues between TM1 and TM4 of the α1 subunit is proposed to lead to an allosteric modulation of GABARs, whereas neurosteroid binding to a site between α and β subunits leads to direct receptor activation. Recently, another site F301, in the β3 subunits, has also been identified to bind to neurosteroids, although the physiological role of this interaction is currently unclear (Chen et al., 2012). Neurosteroids at nanomolar concentrations act as positive allosteric modulators, whereas at micromolar concentrations, these agents activate the receptors even in the absence of GABA (Callachan et al., 1987;Puia et al., 1990). The residue T236 is involved in the allosteric action, whereas the residue Q241 is involved in the direct agonist-like action (Hosie et al., 2006).

GABARs with known subunit composition expressed in exogenous systems such as HEK293 cells have been valuable in understanding the mechanism of neurosteroid action. One such study found that neurosteroids could enhance the current evoked by low concentrations of GABA but not the current evoked by saturating concentrations of GABA (Bianchi and Macdonald, 2003), indicating that neurosteroids increase the GABA efficacy of these receptors. This is particularly important for δ subunit-containing receptors, as the GABA efficacy of these receptors is low and neurosteroids convert GABA from a partial to full agonist at these receptors. The neurosteroid potentiation of GABARs occurs by increasing the frequency of channel opening and prolonging the duration of the channel open time (Twyman and Macdonald, 1992;Bianchi et al., 2002;Ramakrishnan and Hess, 2010). Furthermore, neurosteroids potentiate the δ subunit-containing receptors more than the γ2 subunit-containing receptors (Wohlfarth et al., 2002). These findings from an exogenous expression system are supported by findings in δ-subunit knockout animals. In these animals, the sedative and anxiolytic effects of neurosteroids were attenuated (Mihalek et al., 1999). Furthermore, neurosteroids also fail to enhance the tonic current of DGCs of δ-subunit knockout animals (Stell et al., 2003).

Neurosteroid regulation of GABARs in epilepsy

Removal of neurosteroid control partially underlies the recurrent spontaneous seizures seen in epileptic animals. Because the hippocampus is primarily involved in seizure generation in temporal lobe epilepsy, changes in GABAR expression and pharmacology have been extensively studied in animal models of TLE. The GABARs expressed on DGCs of epileptic animals have reduced neurosteroid sensitivity, in addition to other changes in the pharmacological properties of these receptors (Rajasekaran et al., 2010;Zhang et al., 2007;Brooks-Kayal et al., 1998;Gonzalez et al., 2013;Mtchedlishvili et al., 2001;Sun et al., 2007;Gibbs, et al., 1997;Joshi et al., 2017). Both the synaptic and extrasynaptic GABARs expressed on DGCs of epileptic animals have reduced neurosteroid sensitivity (Mtchedlishvili et al., 2001). Neurosteroids at physiological concentrations fail to enhance the synaptic and tonic GABAR currents in DGCs of epileptic animals (Rajasekaran et al., 2010;Zhang et al., 2007;Sun et al., 2007). The ongoing neurosteroid synthesis shape GABAR currents, and neurosteroids exert a control on network excitability through GABARs (Keller et al., 2004;Stell et al., 2003;Walker and Semyanov, 2008). Our studies revealed that δ-GABAR expression is reduced as early as 4 days following SE, whereas γ2 subunit upregulation coincided with the onset of spontaneous seizures between 10-14 days following SE (Joshi et al., 2017). Thus, the reduced neurosteroid control during the latent period could contribute to the dentate gating function, which is compromised during epileptogenesis (Heinemann et al., 1992;Lothman et al., 1992;Pathak et al., 2007;Stringer and Lothman, 1989). Furthermore, even though the tonic current appears to be unaltered and the synaptic currents are augmented in epileptic animals, the inhibition of DGCs is likely to be reduced *in vivo*. The neurosteroid sensitivity of GABARs expressed on cortical neurons of kindled animals is also reduced, and this reduction is associated with dephosphorylation of GABARs (Gavrilovici et al., 2006;Kia et al., 2011). Whether neurosteroid modulation of other principal neurons of the trisynaptic circuit as well as that of thalamic neurons, which also express δ-GABARs, is altered in epilepsy is currently not known.

Altered GABAR expression in epileptic animals underlies the reduced neurosteroid sensitivity of these receptors (Raol et al., 2006;Rajasekaran et al., 2010;Zhang et al., 2007;Sperk et al., 1998;Tsunashima et al., 1997;Brooks-Kayal et al., 1998;Gonzalez et al., 2013;Lund et al., 2008;Peng et al., 2004). Some of these alterations have also been observed in tissue resected from epilepsy patients (Loup et al., 2000;Loup et al., 2006;Palma et al., 2005). The expression of δ and α1 subunit mRNA and protein is reduced in DGCs of epileptic animals, whereas that of α4 and γ2 subunits is increased (Raol et al., 2006;Rajasekaran et al., 2010;Zhang et al., 2007;Sperk et al., 1998;Tsunashima et al., 1997;Peng et al., 2004). The reduction in δ and α1 subunit expression occurs before the onset of spontaneous seizures and could play a role in epileptogenesis (Joshi et al., 2017;Peng et al., 2004). The NMDA receptor activation that occurs during status epilepticus in experimental animals appears to reduce the δ subunit expression, whereas the reduced α1 subunit expression is triggered by activation of the JAK/STAT pathway (Lund et al., 2008;Joshi et al., 2017). Preventing the reduction in α1 subunit expres s ion also suppresses the onset of spontaneous seizures (Raol et al., 2006). Whether blocking the δ subunit expression also prevents epileptogenesis has not been tested. However, mice lacking δ subunit expression have an increased seizure susceptibility, indicating that the δ subunit may play a role in epileptogenesis (Spigelman et al., 2002). In addition, we have shown that activation of ERK1/2 plays a role in the NMDA-triggered reduction in δ subunit expression in cultured hippocampal neurons (Joshi and Kapur, 2013). ERK1/2 are activated during SE and following recurrent spontaneous seizures, and animals with a constitutive activation of ERK1/2 develop epilepsy (Berkeley et al., 2002;Garrido et al., 1998;Kim et al., 1994;Houser et al., 2008;Nateri et al., 2007). These findings suggest that NMDAR activation during SE could increase ERK1/2 signaling, resulting in down-regulation of δ subunit expression and reduced neurosteroid sensitivity of the tonic current.

Potentially compensatory changes in GABARs are seen in epileptic animals; the most prominent alteration includes upregulation of α4γ2 subunit-containing receptors (Rajasekaran et al., 2010;Zhang et al., 2007;Lund et al., 2008). However, the upregulation of these receptors does not occur until the onset of spontaneous seizures (Joshi et al., 2017;Peng et al., 2004). Furthermore, these receptors are not as sensitive to neurosteroids as α4δ or α1γ2 subunit-containing receptors (Belelli et al., 2002;Rajasekaran et al., 2010;Sun et al., 2007;Zhang et al., 2007). The differential time course of changes in GABAR expression is likely to make the brain vulnerable during the epileptogenic period (Pathak et al., 2007). Removal of neurosteroid control of excitability could be one of the mechanisms that contribute to epileptogenesis. Indeed, in one study, daily treatment of animals with finasteride, which inhibits the enzyme 5α-reductase and blocks the ongoing neurosteroid synthesis led to an early onset of epilepsy (Biagini et al., 2009). We found that δ subunit expression is reduced on the 4th day following SE, and a single day of endogenous neurosteroid synthesis blockade on the 4th day following SE accelerated epileptogenesis (Joshi et al., 2017).

Notably, these changes may not be uniform in all models of epilepsy. For example, in the controlled cortical impact model of traumatic brain injury, the tonic current was enhanced, and the expression of the δ subunit increased (Mtchedlishvili et al., 2010;Kharlamov et al., 2011). Furthermore, the frequency of spontaneous seizures may also affect the expression of

GABAR subunits (González et al., 2015). The expression of some of the proteins, such as gephyrin and glutamate receptor-interacting protein (GRIP), associated with GABAR trafficking and/or surface membrane anchoring is also reduced following SE (Gonzalez et al., 2013). Whether their expression is also down-regulated in epileptic animals is currently not known.

Neurosteroid regulation of GABAR trafficking and expression

The number of receptors expressed at the surface membrane is regulated by constitutive endocytosis and insertion. We and others have shown that the rate of insertion of synaptic γ2 subunit-containing receptors is rapid, and new receptors can appear at the surface membrane within minutes (Joshi and Kapur, 2009;Joshi et al., 2013b;Bogdanov et al., 2006). In contrast, the insertion of δ subunit-containing receptors is slower than that of γ2 subunit-containing receptors. Interestingly, GABARs containing α4βxδ subunits are inserted at the surface membrane at a faster rate than the receptors containing α1βxδ subunits (Joshi et al., 2013b), which suggests that α subunits influence the trafficking of δ subunit-containing GABARs. In contrast, the rate of insertion of α4βxγ2 or α1βxγ2 subunit-containing r eceptors is similar; thus, the γ2 subunit plays a dominant role in regulating the rate of insertion. The rate of internalization of γ2 subunit-containing receptors is also faster than those containing δ subunits (Joshi and Kapur, 2009). The mechanisms regulating trafficking of synaptic receptors have been extensively studied (Michels and Moss, 2007). In contrast, the mechanisms that regulate trafficking of δ subunit-containing receptors are poorly understood. Neurosteroids appear to enhance cell surface expression of GABARs through receptor phosphorylation (Abramian et al., 2014). However, these studies were performed in α4β3 subunit-containing receptors, and whether neurosteroid regulation of surface expression of δ and γ2 subunit-containing receptors is identical or distinct is not known.

Progesterone, estrogen, and neurosteroids also influence GABAR subunit expression. Estrus cycle-linked fluctuations in progesterone alter the hippocampal expression of δ and γ2 subunits (Maguire et al., 2005;Wu et al., 2013). High progesterone levels correlate with a greater expression of δ subunit-containing GABARs and a lower expression of γ2 subunit-containing GABARs. These estrus cycle-linked fluctuations in GABAR expression persist in animals lacking progesterone receptor expression or when progesterone receptors are blocked but are prevented if allopregnanolone synthesis is inhibited by treatment of animals with finasteride (Maguire and Mody, 2007;Wu et al., 2013). Thus, neurosteroids appear to regulate δ subunit expression (Follesa et al., 2004). In accordance with the role of tonic current and neurosteroids in the regulation of network excitability (Semyanov et al., 2004;Walker and Semyanov, 2008), these changes in the expression of δ subunit expression influence seizure susceptibility. The mice in the diestrus stage that have higher progesterone levels and a greater expression of δ subunit-containing receptors are less susceptible to seizures than mice in the estrus stage of the cycle (Maguire et al., 2005).

Puberty, pregnancy and postpartum are also associated with considerable changes in the hormonal milieu. In accordance with the high progesterone levels during pregnancy, the expression of the δ subunit is also increased during pregnancy, and that of the γ2 subunit is reduced (Sanna et al., 2009;Concas et al., 1998;Concas et al., 1999). The expression of both

of these subunits returns to baseline following pregnancy. In contrast, the expression of the α4 subunit is increased following postpartum as a result of withdrawal from high progesterone and neurosteroid levels (Sanna et al., 2009;Concas et al., 1998;Concas et al., 1999;Smith et al., 1998a;Smith et al., 1998b).

Sulfated steroids

In contrast to the anticonvulsant effects of reduced neurosteroids, sulfated neurosteroids exert an excitatory, proconvulsant action. The effects of the sulfated progesterone derivative, pregnanolone sulfate (PS), on seizures and neurotransmission have been studied *in vitro* and *in vivo*. Administration of PS increases the convulsant potency of NMDA, reduces the concentration of PTZ necessary to trigger convulsions, and decreases the latency to PTZ-evoked seizures (Kokate et al., 1999b;Reddy and Kulkarni, 1998;Maione et al., 1992;Reddy and Kulkarni, 1998). An intracerebroventricular infusion of PS causes seizures, whereas its infusion into the hippocampus causes prolonged seizures of status epilepticus (Kokate et al., 1999b;Williamson et al., 2004). Sulfated steroids reduce GABA release from presynaptic terminals and affect inhibition (Mtchedlishvili and Kapur, 2003). Other studies have shown that sulfated steroids may also potentiate NMDARs (Majewska and Schwartz, 1987;Majewska et al., 1988;Wu et al., 1991).

Proconvulsant effects of estrogens

Estrogens have a proconvulsant effect (Veliskova and DeSantis, 2013;Veliskova, 2006;Veliskova, 2007;Frye, 2008;Reddy, 2013). A positive relationship has been observed between the levels of estrogens and seizures in women with epilepsy (Backstrom, 1976). Administration of estrogens to experimental animals also increases seizure susceptibility (Woolley and Timiras, 1962;Woolley, 2000;Edwards et al., 1999), whereas blocking estrogen synthesis can even suppress prolonged seizures of status epilepticus (Sato and Woolley, 2016). These excitatory actions of estrogens are mediated through increased glutamatergic transmission (Smith et al., 1987;Smith et al., 1988;Smejkalova and Woolley, 2010;Oberlander and Woolley, 2016). Estrogens enhance the expression of the GluA1 subunit of AMPA receptors via activation of estrogen receptor (ER)-β (Liu et al., 2008;Tada et al., 2015). Estrogens also increase the number of dendritic spines that harbor glutamatergic synapses on CA1 neurons (Woolley and McEwen, 1992;Woolley and McEwen, 1993). In addition, acute application of estrogen to hippocampal slices also suppressed the amplitude of GABAR-mediated IPSCs of CA1 pyramidal neurons by decreasing the probability of GABA release (Huang and Woolley, 2012). Thus, the proconvulsant effects of estrogen involve the reduction of inhibition and activation of excitatory mechanisms.

Conclusions

Menstrual cycle-linked fluctuations in estrogen and progesterone, which exert proconvulsant and anti-convulsant effects respectively, underlie catamenial seizure exacerbation. The progesterone derivative allopregnanolone exerts anticonvulsant actions via potentiation of GABAR-mediated inhibitory transmission in the brain. However, the neurosteroid sensitivity

of GABARs is reduced in epilepsy. The mechanisms that trigger the reduction in the expression of neurosteroid-sensitive δ-GABARs in epilepsy are not known. Identifying these mechanisms can provide additional therapeutic targets to alleviate catamenial seizures.

Furthermore, despite catamenial epilepsy being primarily a neurosteroid-withdrawal disorder, prolonged progesterone treatment has failed to exert beneficial effects. Thus, additional studies are warranted to understand the effects of acute and prolonged progesterone treatment in the brain. While progesterone effects mediated by neurosteroids have been extensively studied over the last few decades, the effects mediated via progesterone receptors remain underexplored. Progesterone receptors are ligand-activated nuclear hormone receptors that can regulate gene expression (Conneely et al., 1987;Mani and Oyola, 2012;Singh and Su, 2013). These receptors are widely expressed in the brain (Mitterling et al., 2010), and their signaling may be significant when progesterone treatment is performed over a longer period of time.

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Highlights

- Female reproductive cycle-linked seizure exacerbation
- Anticonvulsant effects of progesterone and allopregnanolone
- Neurosteroid potentiation of $GABA_A$ receptor-mediated inhibition
- Expression of $GABA_A$ receptors with reduced neurosteroid sensitivity in DGCs of epileptic animals

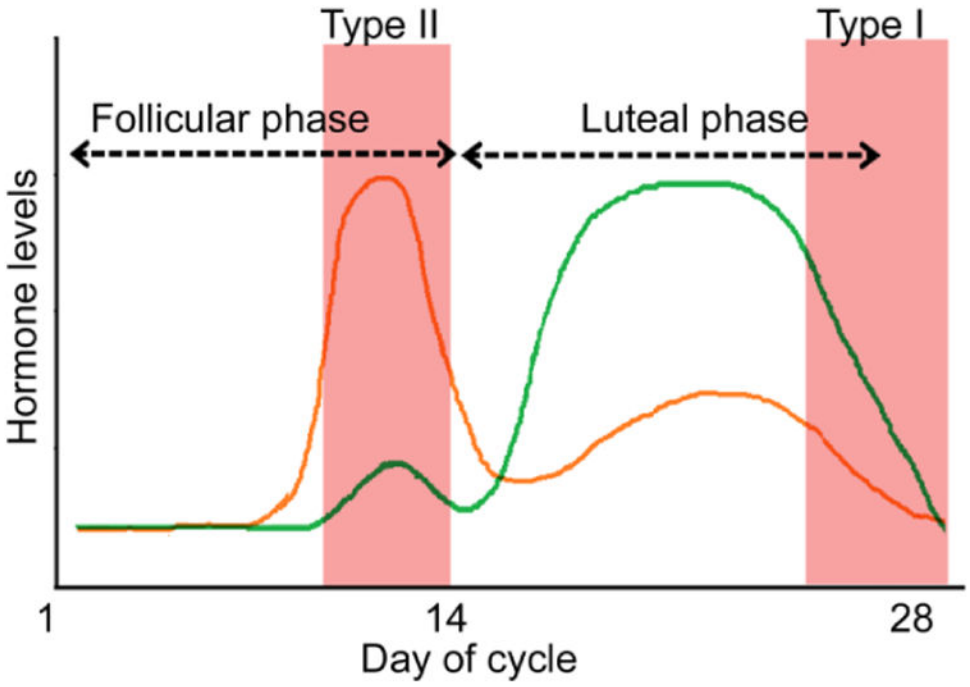

Figure 1. Catamenial seizure exacerbation and the menstrual cycle

Serum progesterone (green) and estrogen (orange) levels fluctuate during a typical 28-day long menstrual cycle. Estrogen levels rise during the follicular phase, whereas progesterone levels rise during the luteal phase. A mid-cycle high estrogen-to-progesterone ratio underlies type II seizure exacerbation. On the other hand, withdrawal from high progesterone levels at the end of the cycle leads to the increased seizure frequency in type I seizures.

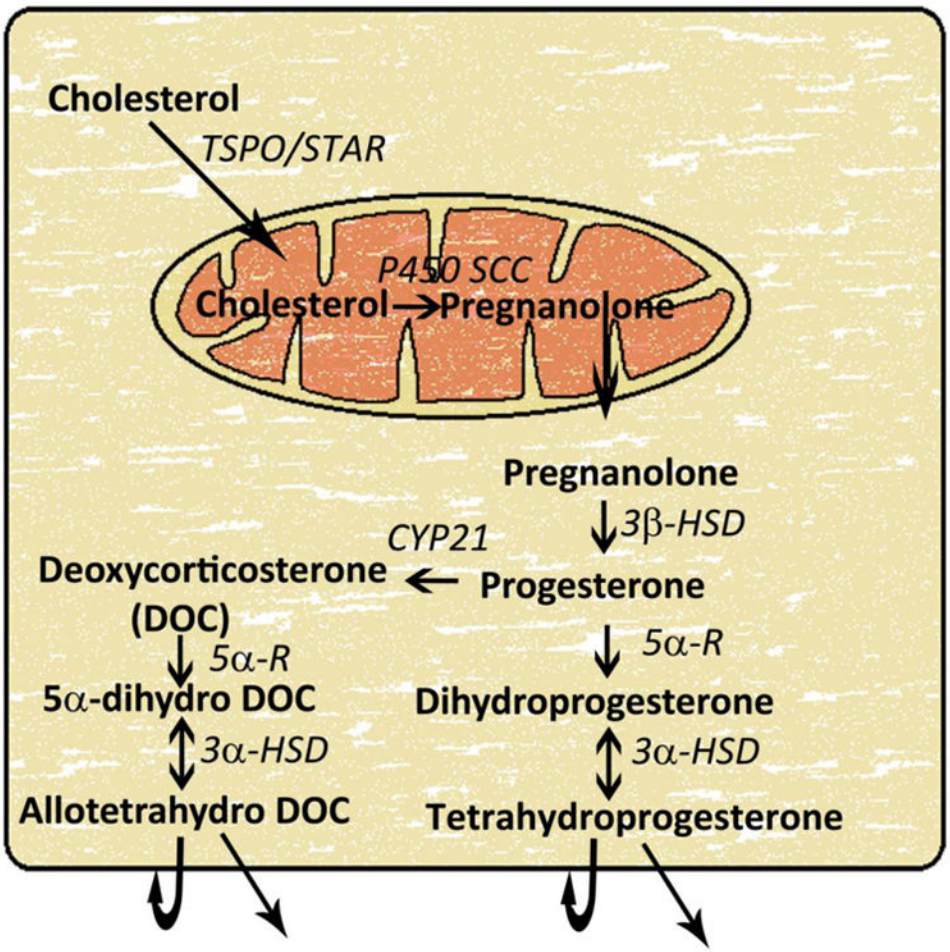

Figure 2. Neurosteroid synthetic pathway in the brain

Cytoplasmic cholesterol is transferred to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR), also called translocator protein (TSPO), or peripheral benzodiazepine receptors. In the mitochondria, cholesterol is converted to pregnanolone by enzyme cytochrome P450 side-chain cleavage (P450scc) in a rate-limiting step. Pregnanolone is transferred back to the cytoplasm and gets converted to progesterone by enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD). Circulating progesterone can also enter the neurosteroid synthetic pathway at this step. Subsequently, progesterone is converted to dihydroprogesterone by the enzyme 5α-reducatse (5α-R) in the 2nd rate-limiting step in this biosynthetic pathway. Finally, dihydroprogesterone is converted to tetrahydroprogesterone (THP), also called allopregnanolone, by the enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD). Progesterone can also be converted to deoxycorticosterone and then to allotetrahydrodeoxycorticosterone (THDOC). Both allopregnanolone and allotetrahydrodeoxycorticosterone can activate $GABA_A$ receptors expressed in the same cell or surrounding cells.

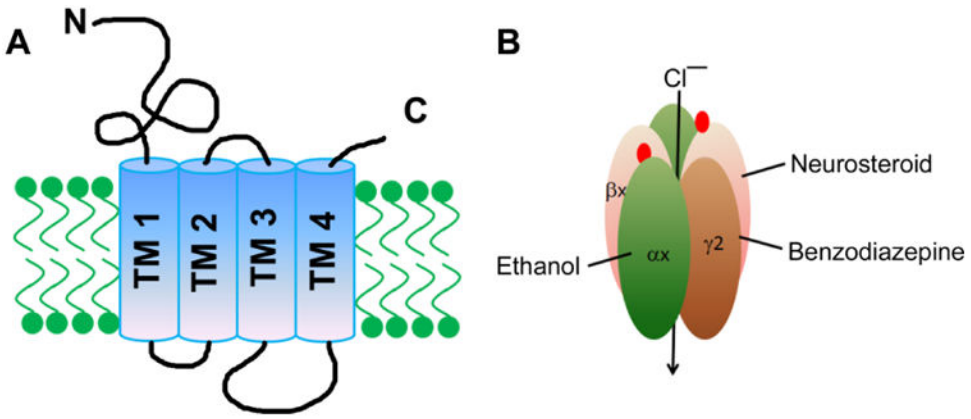

Figure 3. $GABA_A$ receptor subunit composition and pharmacology

A: A schematic diagram illustrating the structure of GABAR subunits. Each subunit is made up of an "N" terminal extracellular domain, four transmembrane domains (TM1-4), and a short "C" terminal extracellular domain. The intracellular loop between TM3 and TM4 contains residues that play a critical role in the surface membrane trafficking of these receptors. The Cys-loop in the "N" terminal extracellular domain is shown in red. B: Structure of a GABAR showing GABA (red circles) binding sites between the α and β subunits, the benzodiazepine binding site between the α and γ subunits, and the neurosteroid binding site.

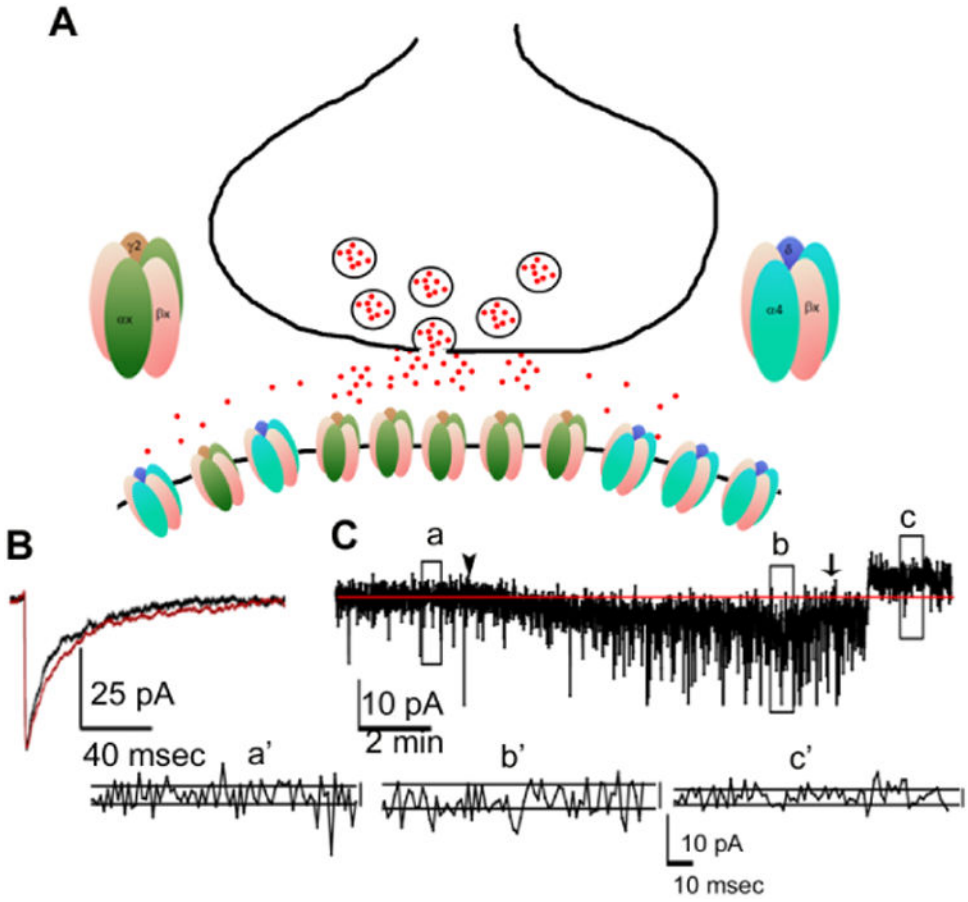

Figure 4. Neurosteroid modulation of synaptic and extrasynaptic receptors of DGCs

A: A schematic diagram showing the expression of γ2 subunit-containing receptors at the synaptic and extrasynaptic sites on the membrane of DGCs where they are exposed to GABA released from the presynaptic terminal. The excess GABA in the synaptic cleft can spread to extrasynaptic sites to activate α4βxδ subunit-containing receptors. **B:** A representative averaged miniature inhibitory post-synaptic current showing prolongation of decay after application of allopregnanolone (10 nM, red trace). **C:** A trace illustrating allopregnanolone modulation of tonic current. Allopregnanolone (10 nM) bath application was started at the arrowhead and it increased the holding current. Once a stable response was obtained, bath application of the GABAR blocker picrotoxin (50 μM) was started (arrow), and it reduced the holding current. Traces below (a′, b′ and c′) show membrane noise, which is another measure of tonic current, at baseline (a), after application of allopregnanolone (b), and after application of picrotoxin (c).