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Finasteride Inhibits the Disease-Modifying Activity of Progesterone in the Hippocampus Kindling Model of Epileptogenesis

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Abstract

Progesterone (P) plays an important role in seizure susceptibility in women with epilepsy. Preclinical and experimental studies suggest that P appears to interrupt epileptogenesis, which is a process whereby a normal brain becomes progressively epileptic due to precipitating risk factors. P has not been investigated widely for its potential disease-modifying activity in epileptogenic models. Recently, P has been shown to exert disease-modifying effects in the kindling model of epileptogenesis. However, the mechanisms underlying the protective effects of P against epileptogenesis remain unclear. In this study, we investigated the role of P-derived neurosteroids in the disease-modifying activity of P. It is hypothesized that 5 α -reductase converts P to allopregnanolone and related neurosteroids that retard epileptogenesis in the brain. To test this hypothesis, we utilized the mouse hippocampus kindling model of epileptogenesis and investigated the effect of finasteride, a 5 α -reductase and neurosteroid synthesis inhibitor. P markedly retarded the development of epileptogenesis and inhibited the rate of kindling acquisition to elicit stage 5 seizures. Pretreatment with finasteride led to complete inhibition of the P-induced retardation of limbic epileptogenesis in mice. Finasteride did not significantly influence the acute seizure expression in fully-kindled mice expressing stage 5 seizures. Thus, neurosteroids that potentiate phasic and tonic inhibition in the hippocampus, such as allopregnanolone, may mediate the disease-modifying effect of P, indicating a new role of neurosteroids in acquired limbic epileptogenesis and temporal lobe epilepsy.

Keywords

Progesterone; epileptogenesis; kindling; neurosteroid; finasteride; seizure

1. Introduction

Acquired limbic epilepsy or temporal lobe epilepsy (TLE) is caused by diverse precipitating factors such as brain injury, stroke, infections, or prolonged seizures. The mechanisms underlying the development of acquired TLE are not very well understood. The term 'epileptogenesis' is used to describe the complex plastic changes in the brain that, following

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a precipitating injury, convert a normal brain into a brain debilitated by recurrent seizures [1,2]. There is an unmet need for drugs that truly prevent the development of epilepsy (antiepileptogenic agents) or alter its natural course, delaying the appearance or severity of epileptic seizures (disease-modifying agents) in people at risk. Progesterone (P) plays an important role in women with epilepsy [3,4]. P has long been known to have antiseizure activity in animal models [5–7] and in clinical studies [8–11]. Therefore, it has been suggested that women with epilepsy are prone to seizures in response to decreased levels of P during perimenstrual periods [12,13]. The antiseizure properties of P are due to its conversion in the brain to allopregnanolone (3 α -hydroxy-5 α -pregnane-20-one) and other structurally-related neurosteroids [6,7,14,15]. Recent studies show that neurosteroids are mainly present in principal neurons in many brain regions that are relevant to acquired TLE including the hippocampus [16]. Neurosteroids rapidly alter neuronal excitability through direct interaction with synaptic and extrasynaptic GABA-A receptors in the hippocampus [17]. Although they act on all GABA-A-receptor subtypes, they have pronounced effects on the extrasynaptic δ -subunit containing GABA-A receptors that mediate tonic inhibition [18]. Therefore, allopregnanolone and related 5 α -reduced neurosteroids are powerful anticonvulsants in animal seizure models, and their synthetic analogs have been proposed for epilepsy therapy [19].

Neurosteroids are broad-spectrum anticonvulsants that confer seizure protection in various experimental models of evoked seizures in normal or healthy rats and mice [7,20–22]. They protect against seizures induced by GABA-A receptor antagonists, 6-Hz model, pilocarpine-induced limbic seizures, and seizures in kindled animals. Unlike benzodiazepines, tolerance to their actions does not occur during chronic administration in rats [19,23]. In addition to anticonvulsant activity, there is emerging evidence that neurosteroids play a role in regulating acquired epileptogenesis. Although the precise mechanisms underlying epileptogenesis remain unclear, epileptogenesis may involve an interaction of acute and delayed anatomic, molecular, and physiological events that are both complex and multifaceted. The initial precipitating factor activates diverse signaling events, such as inflammation, oxidation, apoptosis, neurogenesis and synaptic plasticity, which eventually lead to structural and functional changes in neurons [1,2]. These changes are eventually manifested as abnormal hyperexcitability and spontaneous seizures. In the rodent brain, the 5 α -reductase type I and II isozymes convert P to dihydroprogesterone, ultimately leading to the production of allopregnanolone and related neurosteroids, which may retard epileptogenesis by the interruption of one or more of the above events. In addition to catalyzing the rate-limiting step in the reduction of P, both isoforms of the 5 α -reductase enzyme are responsible for the conversion of testosterone and deoxycorticosterone to dihydroprogesterone and dihydrodeoxycorticosterone, respectively [13]. Finasteride, an irreversible inhibitor of 5 α -reductase, blocks the synthesis of all 5 α -reduced neurosteroids, including testosterone-derived androstenediol [24] and deoxycorticosterone-derived allotetrahydrodeoxycorticosterone (THDOC) [25] and thereby may affect epileptogenesis.

As a precursor of neurosteroids, P is an appealing agent for the intervention of epileptogenesis. Three observations support this contention. First, P's inhibitory effect on kindling was observed in rats [26] and mice [27]. Second, following pilocarpine-induced status epilepticus, the neurosteroidogenic enzyme cytochrome P450 side chain cleavage (P450_{scc}) was increased for several weeks, suggesting that it may be associated with the promotion of neurosteroidogenesis [28]. P450_{scc} is a critical enzyme involved in the conversion of cholesterol into pregnenolone, a precursor for several neurosteroids. Third, inhibiting neurosteroid synthesis with finasteride accelerated the onset of spontaneous recurrent seizures [29], suggesting that endogenous neurosteroids play a role in restraining epileptogenesis. However, P has not been investigated widely for its potential disease-modifying activity in epileptogenic models. Using the mouse kindling model, we previously

demonstrated that P inhibits the development of limbic epileptogenesis at doses that do not affect seizure expression and motor performance [27], indicating the disease-modifying activity of P. Kindling is a model of functional epileptogenesis, since seizures develop after repeated subthreshold stimulations applied in the hippocampus, amygdala or other brain regions, without induction of evident brain lesions [30]. However, the mechanisms underlying the protective effect of P against limbic epileptogenesis remain unclear.

In the present study, we sought to investigate the role of neurosteroids in the disease-modifying effect of P by using finasteride, a 5 α -reductase inhibitor that blocks the synthesis of P-derived and 5 α -reduced neurosteroids in the brain. Our results show for the first time that finasteride prevents P's inhibition of hippocampal kindling epileptogenesis, indicating that P-derived neurosteroids such as allopregnanolone may inhibit epileptogenesis, thus confirming the new role of neurosteroids in limbic epileptogenesis and TLE.

2. Materials and Methods

2.1 Animals

Adult female mice (3–5 months old) of C57BL6 strain weighing 25–30 g were used in the study. Animals were housed separately, four to a cage, and were fed mouse chow and water *ad libitum*. Animals were not checked for ovarian cycle stages due to a chronic experimental (kindling) protocol that lasted longer than the 5-day cycle. All procedures were performed in compliance with the *NIH Guide for the Care and Use of Laboratory Animals*, under a protocol approved by the Institutional Animal Care and Use Committee.

2.2. Hippocampus kindling epileptogenesis model

The kindling epileptogenesis was induced by daily hippocampal stimulations [2,27]. Mice were anesthetized by intraperitoneal injections of ketamine (100 mg/kg) and xylazine (20 mg/kg), and a bipolar electrode (Plastic-One, Inc.) was stereotactically implanted in the right ventral hippocampus (2.9 mm posterior, 3.0 mm lateral, and 3.0 mm below dura), and anchored with dental acrylic to three screws. After a recovery period of 7 days, the afterdischarge (AD) threshold was determined by application of a 1 s train of 1 ms biphasic rectangular pulses at 60 Hz beginning at 25 μ A. Additional stimulations, increasing by 25 μ A, were given at 5 min intervals until an AD lasting at least 5 s was detected using the digital EEG system (Astro-Med, West Warwick, RI). Kindling stimulations (1-ms pulses, 60 Hz frequency, 1 s duration) were applied following standard protocol, consisting of a once-daily stimulation at 125% of the AD threshold using an isolated pulse stimulator (A-M Systems). Stimulations were delivered daily until stage 5 seizures were elicited on three consecutive days. The AD duration was acquired from the hippocampal electrode using Axoscope 8.0 software (Axon Instruments). Behavioral seizures were rated according to the Racine's scale [31], as modified for the mouse: stage 0, no response or behavior arrest; stage 1, chewing or facial twitches; stage 2, chewing and head nodding (wet dog shakes); stage 3, forelimb clonus; stage 4, bilateral forelimb clonus and rearing; stage 5, falling.

2.3. Drug administration protocols

To examine the effect of P on epileptogenesis, P (25 mg/kg, s.c.) was given once daily, 30 min prior to stimulation sessions. The dose of P in the kindling model was based on our previously published dose-response study [27]. It is highly unlikely that P at such low dosage produces effects on seizures that are independent of its primary targets: binding to progesterone receptors and conversion to neurosteroids [7]. To study the role of neurosteroids in P's seizure protection, finasteride was used as the pharmacological tool. Finasteride (50 mg/kg, i.p.) was given 1 h before the P injection. Finasteride was given to control animals 1 h prior to kindling stimulations. Finasteride is an irreversible inhibitor of

both type I (brain) and type II (peripheral tissues) 5 α -reductase isozymes in rodents [32–36]. The dose of finasteride was selected from previous studies demonstrating its ability to maximally inhibit the biosynthesis of allopregnanolone [37,38]. In mice treated with P, allopregnanolone levels were reduced by 95% after finasteride treatment [37]. Finasteride did not have any significant effects except inhibition of 5 α -reductase enzyme. In rodents, it blocks both type I and II 5 α -reductase isozymes and therefore providing maximal inhibition of neurosteroids produced via the 5 α -reductase pathway [33–34]. Drug solutions for the injection were made in a 20% β -cyclodextrin solution, and additional dilutions were made using normal saline. To examine drug effects on seizure expression, finasteride and P were tested in fully-kindled animals expressing consistent stage 5 seizures. During each kindling session, the behavioral seizure score and the AD duration were recorded as indices of drug efficacy. P and finasteride were purchased from Steraloids (Newport, RI).

2.4. Data analysis

Group data was expressed as the mean $\pm$ standard error of the mean (SEM); differences in seizure stages between groups were compared with the nonparametric Kruskal-Wallis test followed by the Mann-Whitney *U*-test. Comparisons of the means of AD duration and rates of kindling between groups were made with one-way analysis of variance, followed by the unpaired two-tailed Student's *t*-test. In all statistical tests, the criterion for statistical significance was $p < 0.05$.

3. Results

3.1. Disease-modifying activity of P in the kindling epileptogenesis model

The development of kindling epileptogenesis in mice treated with vehicle, P (25 mg/kg), and finasteride (50 mg/kg) is shown in Fig.1. There was no significant group difference in the initial AD threshold (control: $78 \pm 10 \mu\text{A}$; P-treatment: $75 \pm 13 \mu\text{A}$; P+F-treatment: $68 \pm 19 \mu\text{A}$). In vehicle-treated control mice, there was a progressive increase in behavioral seizure intensity with all animals achieving stage 5 seizures after 13 stimulations (Fig.1A). P produced a marked retardation of development of kindling epileptogenesis, as evident by the significant ($p < 0.05$, Kruskal-Wallis followed by Mann-Whitney *U*-test) suppression of behavioral seizures in comparison to controls at the corresponding stimulation sessions (Fig. 1A). However, P (25 mg/kg) did not significantly affect the mean AD duration (Fig.1B; ($p > 0.05$, Student's *t*-test)). The number of stimulations for each stage of kindling in control and treatment groups is shown in Fig.2. The P-treatment group showed significant ($p < 0.05$, Student's *t*-test) suppression of the development of stage 3 and generalized (stage 4 and 5) seizures. The rate of kindling to elicit stage 5 seizures was significantly ($p < 0.05$, Student's *t*-test) inhibited in mice treated with P in comparison to the control group (Fig.3), indicating the disease-modifying effect of P on limbic epileptogenesis. These results are consistent with P's effect on kindling epileptogenesis in the conventional [27] and rapid [2] kindling protocols.

3.2. Finasteride blocks P's disease-modifying effect in the kindling epileptogenesis model

To determine whether the disease-modifying effect of P is due to its conversion to the neurosteroid allopregnanolone, mice were pretreated with the 5 α -reductase inhibitor finasteride (50 mg/kg, i.p.), which inhibits the synthesis of allopregnanolone from P [2]. As shown in Fig.1AB, finasteride significantly reversed the kindling-retarding effect of P in mice. Pretreatment with finasteride completely reversed the P-induced delay in reaching various stages of kindling (Fig.2), including induction of generalized stage 5 seizures (Fig. 3).

3.3. Effect of finasteride treatment in naïve and fully-kindled mice

In a naïve control group, finasteride (50 mg/kg, i.p.) was administered alone during kindling development. Finasteride did not significantly affect the AD threshold when tested 60-min after administration (control: $71 \pm 12 \mu\text{A}$ vs. F-treatment: $63 \pm 14 \mu\text{A}$). As might be expected, finasteride accelerated the acquisition of behavioral seizure stages during the kindling progression (Fig.3). However, when finasteride was given along with P, it completely prevented the disease-modifying effects of P in the kindling epileptogenesis model (Fig.1 & 3). In addition, the ability of finasteride (50 mg/kg, i.p.) to suppress kindled seizures was evaluated in fully-kindled mice expressing stage 5 seizures (Fig.4AB). Finasteride alone, or in combination with P, did not significantly affect the behavioral seizure expression (Fig.4A) or electrographic AD duration (Fig.4B) in fully-kindled mice. Finasteride did not significantly reduce the AD threshold (~11% decrease) in fully-kindled mice expressing stage 5 seizures.

4. Discussion

The key observation of this study is that the disease-modifying effect of P in the kindling model of epileptogenesis is blocked by a pretreatment of finasteride, which is consistent with a protective role of 5α -reduced neurosteroids in limbic epileptogenesis. Finasteride inhibition of P's kindling-retarding effect has not been reported previously. Finasteride is a 5α -reductase inhibitor that blocks the biosynthesis of endogenous neurosteroids such as allopregnanolone and related 5α -reduced steroids [32,33]. Consistent with animal studies, patients treated with finasteride had significantly reduced levels of neurosteroids [39]. Thus, it is suggested that inhibition of neurosteroids may accelerate the putative mechanisms that promote epileptogenesis in acquired TLE [2]. These results support the emerging notion that exogenous treatment with P delays the progression of acquired epileptogenesis [24,27].

However, there are certain caveats that must be considered in regards to the interpretation of these findings. First, the results apply only to the kindling model and protocol used in this study. Second, this study supports the disease-modifying potential of P to the extent that kindling development represents a model of limbic epileptogenesis. However, the relationship of this model to human TLE is not well validated. Third, the relevance of the dosages of P that were used in this study in relation to those required for true antiepileptogenic effect is uncertain. Fourth, we did not test P during earlier stages of kindling (stage 3) to ascertain whether this effect of P is because of antiseizure activity. Finally, it is likely that the effect of P on epileptogenesis may be model specific and false positive results are likely in the kindling model (e.g. levetiracetam) [40]. Therefore, further studies using spontaneous seizure models following kainic acid or pilocarpine-induced SE are needed to confirm antiepileptogenic efficacy of P. Nevertheless, our findings from multiple studies provide compelling evidence that P has disease-modifying or antiepileptogenic effect in the kindling model. To date, we tested P in three distinct kindling epileptogenesis protocols: (a) chronic P therapy in the conventional kindling [27]; (b) acute P therapy in the rapid kindling [2]; and (c) P therapy 30-min prior to kindling sessions (current study). In these studies, P showed kindling-retarding effect, especially at low doses that do not affect electrographic AD expression (Fig.1B), which is consistent with disease-modifying activity. Despite concerns on validity and generalization to other models, kindling is a very useful model for rapid identification of disease-modifying drugs.

P targets multiple molecular and cellular mechanisms relevant to epileptogenesis, including signaling cascades of inflammation, apoptosis, neurogenesis, and synaptic plasticity [41]. Therefore, P may be a natural disease-modifying agent. In the present study, P significantly suppressed the rate of development of kindled seizure activity. P's effects on kindling epileptogenesis are sensitive to blockade by finasteride, which inhibits both type I and type

II 5 α -reductase isoenzymes that convert P into neurosteroids [32–36], predominantly allopregnanolone, that may mediate P's antiepileptogenic effects. Given during kindling development, finasteride accelerated the kindling acquisition, supporting previous studies that indicate the significant role of endogenous neurosteroids in epileptogenesis in post-status epilepticus models [28,29]. Thus, it is likely that endogenous neurosteroids modulate the rate of epileptogenesis. The potential mechanisms of neurosteroid interruption of epileptogenesis in the hippocampus remain unclear. Neurosteroids such as allopregnanolone are potent positive allosteric modulators of synaptic and extrasynaptic GABA-A receptors that mediate phasic and tonic inhibition in the hippocampus [17,19]. Thus, neurosteroid augmentation of tonic inhibition in the hippocampus could inhibit the spread of the seizure discharge from the hippocampus focus and thereby suppress the development rate of behavioral kindled seizure activity without affecting the focal electrographic discharges (Fig.5). Increased tonic inhibition by allopregnanolone is shown to impair the *N*-methyl-*D*-aspartate receptor-mediated excitability in the hippocampus [42]. It is likely that such a mechanism may underlie the neurosteroid's disease-modifying effects in the kindling model.

In addition, there are other limitations of the kindling and finasteride protocol employed in this study. Although the estrous cycle has minimal effects on kindling seizures [43], there is evidence that kindling produces acyclicity and an increase in testosterone levels, seemingly due to polycystic ovaries [44]. Both conditions could affect seizures. It is possible that finasteride influences on this because it can block all 5 α -reduced neurosteroids, including testosterone-derived androstenediol [24]. However, it is highly unlikely that seizure protection is independent of an effect on allopregnanolone biosynthesis because we utilized an exogenous P treatment protocol, which results in a massive increase in allopregnanolone in the brain. Apart from blocking the conversion of P to allopregnanolone, finasteride also inhibits the production of other 5 α -reduced, anticonvulsant neurosteroids, including androstenediol and THDOC, which are derived from testosterone and deoxycorticosterone, respectively [24,25, 32,33]. The finasteride treatment protocol employed in this study offers little specificity regarding its inhibition of specific neurosteroid synthesis or the brain region involved in neurosteroidogenesis. Therefore, it is likely that the action of finasteride may be due to its ability to reduce all 5 α -reduced neurosteroids in the brain.

Neurosteroid biosynthesis is regulated by various endogenous signals. Recent evidence indicates that neurosteroids are mainly present in principal neurons in the hippocampus and neocortex [16]. The biosynthesis of neurosteroids is controlled by the translocator protein (TSPO), formerly called peripheral or mitochondrial benzodiazepine receptor [45]. Activation of TSPO by endogenous signals and ligands facilitates the intramitochondrial flux of cholesterol and thereby promotes neurosteroid synthesis. Thus, dysregulation of neurosteroid synthesis may play a role in epileptogenesis. This premise has been demonstrated in a post-status epilepticus model of epileptogenesis [28,29]. In this study, the rate of kindling was accelerated by finasteride alone, which may reflect independent effects of finasteride on epileptogenesis due to inhibition of endogenous neurosteroid synthesis. Additional studies are needed to investigate whether augmentation of endogenous neurosteroid synthesis represents a unique strategy for preventing epileptogenesis.

The ovarian cycle regulates the seizure sensitivity in females by the modulation of GABA-A receptor plasticity [46,47]. In the present study, animals were not checked for estrous cycle stages due to a chronic experimental design that precluded grouping of animals based on their ovarian cycle. Nevertheless, the results of this study suggest that the finasteride blockade of the disease-modifying effect of P is due to the inhibition of brain neurosteroid synthesis and also neurosteroids derived from the conversion of ovarian P to allopregnanolone. There is pilot evidence of gender differences in neurosteroid sensitivity [48,49]. Clearly, more needs to be learned about the impact of estrous cycle and ovarian

neurosteroids (gonadectomy) on kindling measurements using a large group size. These studies are underway, including the gender differences in P and neurosteroid's effects on kindling epileptogenesis.

In this study, finasteride has little influence on acute seizure susceptibility in fully-kindled mice at the low dosage used in the experiments. This is very interesting observation given the effect of neurosteroids on acute seizures in epilepsy models. In the absence of exogenous P treatment, it is likely that finasteride inhibition of neurosteroid biosynthesis lead to little changes in the seizure expression in fully-kindled mice exhibiting maximal stage 5 seizures. Alternately, the time course of evaluation at 1 h post finasteride administration may be another factor in this paradoxical finding. However, finasteride is able to alter the rate of epileptogenesis when given during kindling development. Finasteride inhibits 5 α -reductase enzyme that exists in two isoforms, designated as types I and II, which convert P into 5 α -dihydroprogesterone and ultimately lead to the production of allopregnanolone. The type I isoenzyme is more prominent in the brain, whereas type II exists mainly in androgen-sensitive glandular tissues such as the prostate [33–35]. In human tissues, finasteride inhibits both isoforms but is more potent on the type II isoenzyme than the type I, thus conferring prostatic selectivity. However, in the rodent models, finasteride is also a highly potent inhibitor of the type I enzyme. Because finasteride does not select between the rodent isoenzymes, our studies do not provide an answer on the relative role of the two isoenzymes in neurosteroid biosynthesis, especially in rodents.

Finasteride treatment has led to exacerbation of seizures in epilepsy rats [50] and accelerated the rate of epileptogenesis in mice (Fig.3). However, there is little evidence that finasteride causes seizures in humans who do not have epilepsy or exacerbates epileptogenesis in persons at high risk for developing epilepsy. Finasteride is used clinically for the treatment of benign prostatic hypertrophy and male pattern baldness. There is published evidence of seizure exacerbation as an adverse event of finasteride treatment [51]. Finasteride provoked seizures in a woman with epilepsy who experienced an increase in seizure frequency and severity during finasteride use. Therefore, in the clinical setting, finasteride should be used with great caution in patients with epilepsy because of its potential to exacerbate seizures or epileptogenesis in patients at risk for developing epilepsy.

In conclusion, our results demonstrate for the first time that pretreatment with finasteride blocks the protective ability of P to retard kindling-induced limbic epileptogenesis. Thus, the disease-modifying or antiepileptogenic effect of P may be mediated through a mechanism involving neurosteroids, which are emerging endogenous agents that can retard acquired epileptogenesis [2,26]. Thus, neurosteroids such as allopregnanolone may possess antiepileptogenic properties. Finasteride inhibition of neurosteroidogenesis may influence seizure susceptibility in persons with TLE or related epileptogenic risk factors.

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ABBREVIATIONS

AD	afterdischarge
AP	allopregnanolone
GABA	γ -aminobutyric acid

P	progesterone
TSPO	translocator protein

References

1. Pitkänen A, Immonen RJ, Gröhn OH, Kharatishvili I. From traumatic brain injury to posttraumatic epilepsy: what animal models tell us about the process and treatment options. *Epilepsia*. 2009; 50(Suppl 2):21–29.
2. Reddy DS, Mohan A. Development and persistence of limbic epileptogenesis are impaired in mice lacking progesterone receptors. *J Neurosci*. 2011; 31:650–658. [PubMed: 21228174]
3. Herzog AG, Fowler KM. The NIH Progesterone Trial Study Group. Sensitivity and specificity of the association between catamenial seizure patterns and ovulation. *Neurology*. 2008; 70:486–487. [PubMed: 18250294]
4. Pack AM, Reddy DS, Duncan S, Herzog A. Neuroendocrinological aspects of epilepsy: Important issues and trends in future research. *Epilepsy Behav*. 2011; 22:94–102. [PubMed: 21454133]
5. Craig CR. Anticonvulsant activity of steroids: separability of anticonvulsant from hormonal effects. *J Pharmacol Exp Ther*. 1966; 153:337–343.
6. Kokate TG, Banks MK, Magee T, Yamaguchi S, Rogawski MA. Finasteride, a 5 α -reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. *J Pharmacol Ex Ther*. 1999; 288:679–684.
7. Reddy DS, Castenada DA, O'Malley BW, Rogawski MA. Antiseizure activity of progesterone and neurosteroids in progesterone receptor knockout mice. *J Pharmacol Exp Therap*. 2004; 310:230–239. [PubMed: 14982969]
8. Bäckström T, Zetterlund B, Blom S, Romano M. Effect of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. *Acta Neurology Scandinavia*. 1984; 69:240–248.
9. Herzog AG. Progesterone therapy in women with complex partial and secondary generalized seizures. *Neurology*. 1995; 45:1600–1662.
10. Herzog AG. Progesterone therapy in women with epilepsy: a 3-year follow-up. *Neurology*. 1999; 52:1917–1918. [PubMed: 10371551]
11. Herzog AG, Fowler KM, Smithson SD. the Progesterone Trial Study Group. Progesterone versus placebo therapy for women with epilepsy: A randomized clinical trial. *Neurology*. 2012 (in press).
12. Herzog AG, Klein P, Ransil BJ. Three patterns of catamenial epilepsy. *Epilepsia*. 1997; 38:1082–1088. [PubMed: 9579954]
13. Reddy DS. The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy. *Epilepsy Res*. 2009; 85:1–30. [PubMed: 19406620]
14. Frye CA, Rhodes ME, Walf A, Harney J. Progesterone reduces pentylentetrazol-induced ictal activity of wild-type mice but not those deficient in type I 5 α -reductase. *Epilepsia*. 2002; 43 (Suppl 5):14–17. [PubMed: 12121288]
15. Lonsdale D, Burnham WM. The anticonvulsant effects of progesterone and 5 α -dihydroprogesterone on amygdala-kindled seizures in rats. *Epilepsia*. 2003; 44:1494–1499. [PubMed: 14636318]
16. Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, Tonon MC, Pelletier G, Vaudry H. Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Front Neuroendocrinol*. 2009; 30:259–301. [PubMed: 19505496]
17. Herd MB, Belelli D, Lambert JJ. Neurosteroid modulation of synaptic and extrasynaptic GABA-A receptors. *Pharmacol Ther*. 2007; 116:20–34. [PubMed: 17531325]
18. Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA-A receptors containing the delta subunit. *J Neurosci*. 2002; 22:1541–1549. [PubMed: 11880484]

19. Reddy DS. Role of anticonvulsant and antiepileptogenic neurosteroids in the pathophysiology and treatment of epilepsy. *Front Endocrinol.* 2011; 2 (article 38):1–11.
20. Belelli D, Bolger MB, Gee KW. Anticonvulsant profile of the progesterone metabolite 5 α -pregnan-3 α -ol-20-one. *Eur J Pharmacol.* 1989; 166:325–329. [PubMed: 2792198]
21. Kokate TG, Svensson BE, Rogawski MA. Anticonvulsant activity of neurosteroids: correlation with gamma-aminobutyric acid-evoked chloride current potentiation. *J Pharmacol Exp Ther.* 1994; 270:1223–1229. [PubMed: 7932175]
22. Kaminski RM, Livingood MR, Rogawski MA. Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. *Epilepsia.* 2004; 45:864–867. [PubMed: 15230714]
23. Reddy DS, Rogawski MA. Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. *J Pharmacol Exp Ther.* 2000; 295:1241–1248. [PubMed: 11082461]
24. Reddy DS. Testosterone modulation of seizure susceptibility is mediated by neurosteroids 3 α -androstenediol and 17 β -estradiol. *Neuroscience.* 2004; 129:195–207. [PubMed: 15489042]
25. Reddy DS, Rogawski MA. Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA-A receptor function and seizure susceptibility. *J Neurosci.* 2002; 22:3795–805. [PubMed: 11978855]
26. Edwards HE, Mo V, Burnham WM, MacLusky NJ. Gonadectomy unmasks an inhibitory effect of progesterone on amygdala kindling in male rats. *Brain Res.* 2001; 889:260–263. [PubMed: 11166716]
27. Reddy DS, Gangisetty O, Briyal S. Disease-modifying activity of progesterone in the hippocampus kindling model of epileptogenesis. *Neuropharmacology.* 2010; 59:573–581. [PubMed: 20804775]
28. Biagini G, Longo D, Baldelli E, Zoli M, Rogawski MA, Bertazzoni G, Avoli M. Neurosteroids and epileptogenesis in the pilocarpine model: Evidence for a relationship between P450scc induction and length of the latent period. *Epilepsia.* 2009; 50(Suppl 1):53–58. [PubMed: 19125849]
29. Biagini G, Baldelli E, Longo D, Pradelli L, Zini I, Rogawski MA, Avoli M. Endogenous neurosteroids modulate epileptogenesis in a model of temporal lobe epilepsy. *Exp Neurol.* 2006; 201:519–524. [PubMed: 16780839]
30. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog Neurobiol.* 2004; 73:1–60. [PubMed: 15193778]
31. Racine RJ. Modification of seizure activity by electric stimulation. II. Motor seizure. *Electroencephalogr. Clin Neurophysiol.* 1972; 32:281–294.
32. Azzolina B, Ellsworth K, Andersson S, Geissler W, Bull HG, Harris GS. Inhibition of rat 5 α -reductases by finasteride: evidence for isozyme differences in mechanism of inhibition. *J Steroid Biochem Mol Biol.* 1997; 61:55–64. [PubMed: 9328210]
33. Steiner JF. Finasteride: a 5 alpha-reductase inhibitor. *Clin Pharm.* 1993; 12:15–23. [PubMed: 7679063]
34. Melcangi RC, Poletti A, Cavarretta I, Celotti F, Colciago A, Magnaghi V, Motta M, Negri-Cesi P, Martini L. The 5 α -reductase in the central nervous system: expression and modes of control. *J Steroid Biochem Mol Biol.* 1998; 65:295–299. [PubMed: 9699883]
35. Finn DA, Beadles-Bohling AS, Beckley EH, Ford MM, Gililand KR, Gorin-Meyer RE, Wiren KM. A new look at the 5 α -reductase inhibitor finasteride. *CNS Drug Rev.* 2006; 12:53–76. [PubMed: 16834758]
36. Paba S, Frau R, Godar SC, Devoto P, Marrosu F, Bortolato M. Steroid 5 α -reductase as a novel therapeutic target for schizophrenia and other neuropsychiatric disorders. *Curr Pharm Des.* 2011; 17:151–167. [PubMed: 21361868]
37. Gangisetty O, Reddy DS. Neurosteroid withdrawal regulates GABA-A receptor α 4-subunit expression and seizure susceptibility by activation of progesterone receptor-independent early growth response factor-3 pathway. *Neuroscience.* 2010; 170:865–880. [PubMed: 20670676]
38. Reddy DS, Gould J, Gangisetty O. A mouse kindling model of perimenstrual catamenial epilepsy. *J Pharmacol Exp Ther.* 2012 Mar 20. [Epub ahead of print].
39. Dusková M, Hill M, Hanus M, Matoušková M, Stárka L. Finasteride treatment and neuroactive steroid formation. *Prague Med Rep.* 2009; 110:222–230.

40. Brandt C, Glien M, Gastens AM, Fedrowitz M, Bethmann K, Volk HA, Potschka H, Löscher W. Prophylactic treatment with levetiracetam after status epilepticus: lack of effect on epileptogenesis, neuronal damage, and behavioral alterations in rats. *Neuropharmacology*. 2007; 53:207–221. [PubMed: 17585956]
41. Stein DG, Sayeed I. Is progesterone worth consideration as a treatment for brain injury. *Am J Roentgenol*. 2010; 194:20–22. [PubMed: 20028900]
42. Shen H, Sabaliauskas N, Sherpa A, Fenton AA, Stelzer A, Aoki C, Smith SS. A critical role for $\alpha 4\beta 8$ GABA-A receptors in shaping learning deficits at puberty in mice. *Science*. 2010; 327(5972):1515–1518. [PubMed: 20299596]
43. Wahnschaffe U, Löscher W. Lack of changes in seizure susceptibility during the estrous cycle in kindled rats. *Epilepsy Res*. 1992; 13:199–204. [PubMed: 1493782]
44. Edwards HE, Burnham WM, Ng MM, Asa S, MacLusky NJ. Limbic seizures alter reproductive function in the female rat. *Epilepsia*. 1999; 40:1370–1377. [PubMed: 10528931]
45. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov*. 2010; 9:971–988. [PubMed: 21119734]
46. Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA-A receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci*. 2005; 8:797–804. [PubMed: 15895085]
47. Gangisetty, O.; Reddy, DS. FASEB J. 2011 Apr. Ovarian cycle-related neurosteroid regulation of GABA-A receptor subunit plasticity in the hippocampus subfields; p. lb410
48. Reddy DS. Gender differences in antiseizure sensitivity of neurosteroids in the pilocarpine model of status epilepticus. *Epilepsia*. 2009; 50(Suppl 11):126.
49. Gorin-Meyer RE, Wiren KM, Tanchuck MA, Long SL, Yoneyama N, Finn DA. Sex differences in the effect of finasteride on acute ethanol withdrawal severity in C57BL/6J and DBA/2J mice. *Neuroscience*. 2007; 146:1302–1315. [PubMed: 17428611]
50. Lawrence C, Martin BS, Sun C, Williamson J, Kapur J. Endogenous neurosteroid synthesis modulates seizure frequency. *Ann Neurol*. 2010; 67:689–693. [PubMed: 20437568]
51. Herzog AG, Frye CA. Seizure exacerbation associated with inhibition of progesterone metabolism. *Ann Neurol*. 2003; 53:390–391. [PubMed: 12601707]

HIGHLIGHTS

- In this study, we investigated a potential mechanism underlying progesterone's (P) disease-modifying activity.
- P has been shown to exert disease-modifying effects in rodent model of epileptogenesis.
- Finasteride pretreatment significantly inhibited the P-induced retardation of limbic epileptogenesis in mice.
- These studies support a role for neurosteroids in limbic epileptogenesis.

Fig. 1.

Finasteride (F) inhibition of the disease-modifying effect of progesterone (P) on kindling epileptogenesis in mice. **(A)** Mice treated with P displayed a marked retardation of kindling development, as expressed by lower mean seizure stage. **(B)** AD duration was similar between control and P groups. P (25 mg/kg, sc) was given 30-min prior to stimulation sessions, whereas finasteride (50 mg/kg, ip) was given 60-min before P injections. Values represent the mean $\pm$ SEM ($n = 6-8$ mice per group). * $p < 0.01$ vs. control; # $p < 0.01$ vs. P-treatment group (Mann-Whitney U-test or Student's t-test).

Fig. 2.

Effect of finasteride on mean number of stimulations to achieve kindling stages during kindling progression. P (25 mg/kg, sc) was given 30-min prior to stimulation sessions, whereas finasteride (50 mg/kg, i.p.) was given 60-min before P injections. Values represent the mean $\pm$ SEM of number of stimulation values derived from the experiments in Fig. 1A and B. * $p < 0.01$ vs. control; # $p < 0.01$ vs. P-treatment group (Student's t-test).

Fig. 3.

Effect of finasteride on the rate of kindling epileptogenesis in control and P-treated mice. Rate of kindling (# of stimulations required to elicit third stage 5 seizures) was significantly delayed in P-treated mice, which was reversed by finasteride. P (25 mg/kg, sc) was given 30-min prior to stimulation sessions, whereas finasteride (50 mg/kg, ip) was given 60-min before P injections as described in Fig.1. Values represent the mean $\pm$ SEM (n = 6–8 mice per group). *p<0.01 vs. control; #p<0.01 vs. P-treatment group (Student's t-test).

Fig. 4. Effect of finasteride on seizure expression (A) and AD duration (B) in mice. Animals were stimulated daily and were used when they exhibited fully-kindled (stage 5) seizures on three consecutive days. P (25 mg/kg, sc) was given 30-min prior to stimulation sessions, whereas finasteride (50 mg/kg, ip) was given 60-min before P injections in fully-kindled mice. Values represent the mean $\pm$ SEM ($n = 6-8$ mice per group).