Published in final edited form as:

Epilepsy Behav. 2009 November ; 16(3): 411–414. doi:10.1016/j.yebeh.2009.08.016.

Estrogen increases latencies to seizures and levels of 5 α -pregnan-3 α -ol-20-one in hippocampus of wild-type, but not 5 α -reductase knockout, mice

Danielle M. Osborne^a and Cheryl A. Frye^{a,b,c,d,*}

^aDepartment of Psychology, University at Albany—SUNY, Albany, NY, USA

^bDepartment of Biological Sciences, University at Albany—SUNY, Albany, NY, USA

^cCenter for Life Sciences, University at Albany—SUNY, Albany, NY, USA

^dCenter for Neuroscience Research, University at Albany—SUNY, Albany, NY, USA

Abstract

Sex steroids can influence seizures. Estrogen (E₂), progesterone (P₄), and its metabolite, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), in particular, have received much attention for exerting these effects. Typically, it is thought that E₂ precipitates seizures, and progestogens, such as P₄ and 3 α ,5 α -THP, attenuate seizures. However, E₂ may also have antiseizure effects, perhaps in part through its enhancement of the formation of 3 α ,5 α -THP, which has GABA_A/benzodiazepine receptor agonist-like actions. To test this hypothesis, male and female, castrated or ovariectomized, wild-type and 5 α -reductase knockout mice were implanted with Silastic capsules of E₂ or vehicle and then administered pentylenetetrazol (85 mg/kg, ip). Wild-type, but not 5 α -reductase knockout, mice administered E₂ had significantly longer latencies to myoclonus and increased levels of 3 α ,5 α -THP in the hippocampus. Thus, some of the anticonvulsive effects of E₂ may involve formation of 3 α ,5 α -THP in the hippocampus.

Keywords

Estrogen; Progesterone; 5 α -Pregnan-3 α -ol-20-one; Epilepsy; Pentylenetetrazol; 5 α -Reductase; Hippocampus; Cortex

1. Introduction

Epilepsy is a type of seizure disorder characterized by abnormal synchronization of neuronal activity. Although there can be many causes, a nonrandom clustering effect is often observed [1]. In 29% of men and 35% of women with epilepsy there is a temporal cyclicity that appears every 3–6 weeks [1], and in some women this cyclicity can align itself with the menstrual cycle [2]. Seizure frequency and intensity can co-vary with changes in hormonal states, a disorder referred to as *catamenial epilepsy* [1, 2]. This menstrual alignment implies that reproductive hormones likely play a role in mediating seizure activity.

The two primary female reproductive hormones, estrogen (E₂) and progesterone (P₄), have traditionally been considered to exert opposing effects on seizure activity. E₂ can increase

dendritic branching and glutamatergic function in the prefrontal cortex and hippocampus [3]. During female reproductive periods marked by higher E₂ levels (ovulation, hormone replacement therapy) women may experience increased seizure frequency, whereas during periods of decreased E₂ (follicular phase, menopause), they may experience a reduction in ictal activity [1], all lending to the idea that E₂ is a proconvulsant [4]. However, recent research has also demonstrated that E₂ may not always exacerbate seizures and can have antiseizure effects. Hormone-based contraceptives have no ability to increase seizure activity in epileptic women [5]. E₂ pretreatment of ovariectomized rats with kainic acid-induced seizures produced both longer latency to seizure onset and decreased seizure mortality rate [6, 7]. E₂ priming increased the anticonvulsive effects of P₄ [8]. In vitro injection of E₂ into CA1, or entorhinal cortex, slices did not produce an increase in seizure-like neuronal firing [9]. Rather, E₂ injection to the dentate gyrus prevented seizure-induced uptake of 2-deoxyglucose in animals with kainic acid-induced seizures [10]. In fact, the neuroprotective effects of E₂ on the hippocampus may counteract some effects of convulsants [11–14]. Thus, E₂ has a complex, seizurogenic role in the hippocampus.

Unlike E₂, progestogens have a better established role as anticonvulsants [15, 16]. P₄ can be converted by 5 α -reductase into dihydroprogesterone, which can be further metabolized into 5 α pregnan-3 α -ol-20-one (3 α ,5 α -THP) by the 3 α -hydroxysteroid dehydrogenase enzyme [17]. P₄ and, more specifically, its metabolite 3 α ,5 α -THP consistently have salient antiseizure effects. These effects are brought about by the positive modulation by 3 α ,5 α -THP of GABA_A receptors, which can produce effects more powerful than those of benzodiazepines and, thus, can inhibit neuronal excitability [2]. When animals are administered finasteride, a 5 α -reductase inhibitor, the anticonvulsive effects of P₄ are attenuated [18–20]. In further support, in animals pretreated with 3 α ,5 α -THP, compared with vehicle, duration of seizure activity was reduced [21]. Thus, 3 α ,5 α -THP may be the mediator of the antiseizure effects of progestin.

Of interest is the ability of E₂ to increase synthesis of 3 α ,5 α -THP. Moderate regimens of E₂ have increased hippocampal levels of 3 α ,5 α -THP in rats [8, 22]. E₂ can increase hypothalamic 3 β hydroxysteroid dehydrogenase (3 β -HSD), the enzyme necessary for conversion of pregnanolone to P₄; additionally, through its own receptors, E₂ can readily increase cytoplasmic calcium, which also increases P₄ synthesis [reviewed in 23]. Once de novo P₄ has been produced, it can be readily reduced into 3 α ,5 α -THP [8], whereas E₂ increases the activity of enzymes necessary for this conversion to occur [17]. Although E₂ is largely excitatory, it can increase levels of the anticonvulsant 3 α ,5 α -THP.

Within the realm of seizure disorders, very little research has been done to investigate this interaction between E₂, P₄, and 3 α ,5 α -THP levels. Whether chronic administration of E₂ to 5 α -reductase knockout and wild-type mice will elevate 3 α ,5 α -THP levels, and thus decrease pentylenetetrazol-induced seizure activity, is of interest. The use of 5 α -reductase knockout mice serves a dual purpose in this experiment. These mice have a very low capacity to form 3 α ,5 α -THP, and they also have abnormally high levels of E₂ [24]. In wild-type animals, E₂ administration should increase 3 α ,5 α -THP levels and, subsequently, decrease seizure activity, whereas 5 α -reductase knockout mice, because they lack the essential enzyme, will not manifest increases in 3 α ,5 α -THP and antiseizure effects of E₂, despite the high levels of E₂.

2. Methods

These methods were preapproved by the Institutional Animal Care and Use Committee at the University at Albany—SUNY.

Male and female wild-type and 5 α -reductase knockout mice ($N = 28$), approximately 55 days of age, were derived from breeder pairs purchased from Jackson Laboratories (Bar Harbor, ME, USA) on a C57BL/6 background. Mice were group-housed (four or five per cage) in polycarbonate cages ($45 \times 24 \times 21$ cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility. Mice were maintained on a 12/12-h reversed light cycle (lights off at 8:00 AM) with continuous access to Purina Mice Chow and tap water in their home cages. Genomic DNA was isolated from tails and analyzed by polymerase chain reaction in our laboratory and/or the Molecular Core Facility at SUNY—Albany to determine the genotype of our experimental mice. Young adult mice were gonadectomized or ovariectomized under sodium pentobarbital (80 mg/kg or to effect; Fort Dodge Animal Health, Fort Dodge, IA, USA) at least 2 weeks before behavioral testing.

Two days prior to seizure testing, mice were given a single Silastic implant (1.02-mm inner diameter, 2.16-mm outer diameter, 1 mm/animal; measurements slightly modified from [25]) containing 10% crystalline E₂ (wild-type $n = 7$, knockout $n = 7$) or vehicle (wild-type $n = 7$, knockout $n = 7$) to provide a sustained amount of E₂. The implants provided a net increase in central E₂ levels ranging from 2–28 pg/g in E₂-treated mice and from 3 to 18 pg/g in vehicle-treated mice across all brain regions. Peripheral levels were between 35 and 57 pg/ml in E₂-treated mice compared with 27–55 pg/ml in vehicle-treated mice.

All mice were administered pentylenetetrazol (PTZ, 85 mg/kg, ip) and placed in a Plexiglas chamber ($23.5 \times 20.5 \times 19.5$ cm). Immediately after PTZ injection, the latencies to onset of myoclonus, forelimb clonus, tonic-clonic seizures, barrel rolling, hindlimb clonus, and death were recorded. If death did not occur within 10 minutes, mice were immediately killed by cerebral subluxation followed by rapid decapitation, and tissues and blood were collected. Brains were rapidly frozen on ice. Trunk blood was centrifuged at 3000 rpm and serum was stored with brains at -80 °C until radioimmunoassay was performed to determine the levels of E₂ and 3 α ,5 α -THP in hippocampus, hypothalamus, prefrontal cortex, midbrain, and blood plasma.

The latencies to myoclonus, forelimb clonus, tonic-clonic seizures, barrel rolling, hindlimb clonus, and death were compared across groups using one-way analysis of variance (ANOVA) with hormone condition (vehicle, E₂) and genotype (WT, $-/-$) as between-subject variables. Only the myoclonus data are reported because the pattern of effects was similar across measures. ANOVAs analyzed E₂ and 3 α ,5 α -THP levels, with additional simple regression used for latencies to myoclonus. The α level for statistical significance was $P = 0.05$ and a trend was considered at $P = 0.1$.

3. Results

3.1. Wild-type mice and seizures

Wild-type mice administered E₂ had significantly longer latencies to myoclonus ($F(1,12) = 5.85$, $P < 0.05$) (Fig. 1, top) than did wild-type animals administered vehicle. Additionally, hippocampal 3 α ,5 α -THP levels tended to be higher ($F(1,12) = 3.28$, $P < 0.10$), with E₂ producing a fourfold increase in 3 α ,5 α -THP levels compared with vehicle. Hippocampal E₂ levels did not significantly differ between E₂- and vehicle-treated wild-type mice.

3.2. 5 α -Reductase mice and seizures

There was no significant difference in latency to myoclonus between 5 α -reductase knockout mice administered E₂ and those administered vehicle ($F(1,12) = 0.4$, $P > 0.05$) (Fig. 1, bottom) for latency to myoclonus. Although there were differences in hippocampal 3 α ,5 α -THP levels between 5 α -reductase mice administered E₂ and those given vehicle ($F(1,12) = 12.37$, $P < 0.05$) (Fig. 1, bottom), the levels were negligible and all were in the range of that

seen among wild-type mice administered vehicle. There were no differences in hippocampal E_2 levels ($F(1,12) = 0.02$, $P > 0.05$).

3.3. $3\alpha,5\alpha$ -THP and estrogen levels

Simple regression revealed that hippocampal $3\alpha,5\alpha$ -THP levels tended to predict latencies to myoclonus ($r = 0.35$, $P = 0.07$) (Fig. 2, top), but hippocampal E_2 levels did not ($r = 0.07$, $P > 0.05$) (Fig. 2, bottom).

4. Discussion

Our hypothesis that the anticonvulsant effects of E_2 may be due in part to its ability to enhance $3\alpha,5\alpha$ -THP was supported. Wild-type mice with E_2 implants had significantly longer latencies to myoclonus and tended to have greater hippocampal $3\alpha,5\alpha$ -THP levels than did wild-type mice with vehicle implants or 5α -reductase knockout mice in either hormone condition. This is consistent with previous research demonstrating that E_2 has anticonvulsant effects [8, 10, 25]. Additionally, in the hippocampus, there was a tendency for levels of $3\alpha,5\alpha$ -THP, but not E_2 , to account for the longer latencies to myoclonus. These results suggest that one way in which E_2 may exert anticonvulsant effects is by elevating $3\alpha,5\alpha$ -THP levels in the hippocampus.

These results are consistent with previous research showing that E_2 is capable of inducing an increase in $3\alpha,5\alpha$ -THP synthesis. E_2 can stimulate de novo P_4 synthesis by increasing the 3β -HSD enzyme necessary for its production [17, 23, 26, 27]. By doing so, E_2 can also significantly reduce seizure frequency by elevating levels of $3\alpha,5\alpha$ -THP in the hippocampus [8]. We analyzed all brain regions, but we observed the most salient differences, and area of greatest interest, in the hippocampus. The hippocampus has very high levels of metabolic enzymes and appears to be an important site for E_2 -induced neurosteroidogenesis [8, 22], which may influence ictogenesis.

Our findings support others' research indicating that E_2 is not exclusively proconvulsant. In seizure-prone mice, with Silastic capsules of E_2 providing physiological dosing, seizure frequency was reduced more than it was after vehicle administration [25]. The opposite was observed when supraphysiological levels of E_2 were administered to mice: 5α -reductase activity was attenuated; P_4 , DHP, $3\alpha,5\alpha$ -THP levels were lower [28], and ictal activity was greater [1, 12, 29]. This evidence suggests that there may exist a finite window for the antiseizure effects of E_2 , whereby supraphysiological levels of E_2 raise the $E_2:P_4$ ratio to exacerbate seizure activity, and more normative, physiological levels increase $P_4/3\alpha,5\alpha$ -THP production without increasing excitability and maintaining neuroprotection.

Although these results confirm previous work demonstrating the ability of E_2 to increase $3\alpha,5\alpha$ -THP levels and not be proconvulsant, they must be interpreted cautiously. There were no sex differences. This may be due to the small sample size. However, even though there were seven mice per group, there was adequate power, and significance, to reveal that enhancement by E_2 of $3\alpha,5\alpha$ -THP levels in the hippocampus of wild-type but not 5α -reductase mice could alter seizure activity.

Given that some men and women fail to respond to antiepileptic drugs, and can experience reproductive endocrine dysfunction, exploration into the precise hormonal mechanisms that influence seizure activity may provide insight into the etiology and/or a potential novel therapeutic treatment of seizures.

Acknowledgments

Funding from the National Institute of Mental Health (MH06769801) and the National Science Foundation (IBN 03-16083) supported this research. This research represents the completion of the first author's Masters Thesis-equivalent project.

References

1. Herzog AG. Catamenial epilepsy: definition, prevalence, pathophysiology and treatment. *Seizure*. 2008; 17:151–159. [PubMed: 18164632]
2. Reddy DS. The role of neurosteroids in the pathophysiology and treatment of catamenial epilepsy. *Epilepsy Res*. 2009; 85:1–30. [PubMed: 19406620]
3. Morrison JH, Brinton RD, Schmidt PJ, Gore AC. Estrogen, menopause, and the aging brain: how basic neuroscience can inform hormone therapy in women. *J Neurosci*. 2006; 26:10332–10348. [PubMed: 17035515]
4. Scharfman HE, MacLusky NJ. The influence of gonadal hormones on neuronal excitability, seizures, and epilepsy in the female. *Epilepsia*. 2006; 47:1423–1440. [PubMed: 16981857]
5. Dutton C, Foldvary-Schaefer N. Contraception in women with epilepsy: pharmacokinetic interactions, contraceptive options, and management. *Int Rev Neurobiol*. 2008; 83:113–134. [PubMed: 18929078]
6. Velísková J, Velísek L, Galanopoulou AS, Sperber EF. Neuroprotective effects of estrogens on hippocampal cells in adult female rats after status epilepticus. *Epilepsia*. 2000; 41(Suppl. 6):S30–S35. [PubMed: 10999516]
7. Slamberova R, Vathy I. Estrogen differentially alters NMDA- and kainite-induced seizures in prenatally morphine- and saline-exposed adult female rats. *Pharmacol Biochem Behav*. 2000; 67:501–505. [PubMed: 11164079]
8. Frye CA, Rhodes ME. Estrogen-priming can enhance progesterone's antiseizure effects in part by increasing hippocampal levels of allopregnanolone. *Pharmacol Biochem Behav*. 2005; 81:907–916. [PubMed: 16085296]
9. Velísek L, Velísková J, Etgen AM, Stanton PK, Moshé SL. Region-specific modulation of limbic seizure susceptibility by ovarian steroids. *Brain Res*. 1999; 842:132–138. [PubMed: 10526103]
10. Veliskova J. Estrogens and epilepsy: why are we so excited? *Neuroscientist*. 2007; 13:77–88. [PubMed: 17229977]
11. Azcoitia I, Sierra A, Garcia-Segura LM. Estradiol prevents kainic acid-induced neuronal loss in the rat dentate gyrus. *NeuroReport*. 1998; 9:3075–3079. [PubMed: 9804319]
12. Hoffman GE, Moore N, Fiskum G, Murphy AZ. Ovarian steroid modulation of seizure severity and hippocampal cell death after kainic acid treatment. *Exp Neurol*. 2003; 182:124–134. [PubMed: 12821382]
13. Velísek L, Velísková J. Estrogen treatment protects GABA(B) inhibition in the dentate gyrus of female rats after kainic acid-induced status epilepticus. *Epilepsia*. 2002; 43(Suppl. 5):146–151. [PubMed: 12121310]
14. Velísková J, Velísek L. Beta-estradiol increases dentate gyrus inhibition in female rats via augmentation of hilar neuropeptide Y. *J Neurosci*. 2007; 27:6054–6063. [PubMed: 17537977]
15. Frye CA, Scalise TJ. Anti-seizure effects of progesterone and 3alpha, 5alpha-THP in kainic acid and perforant pathway models of epilepsy. *Psychoneuroendocrinology*. 2000; 25:407–420. [PubMed: 10725616]
16. Lonsdale D, Burnham WM. The anticonvulsant effects of progesterone and 5alpha-dihydroprogesterone on amygdala-kindled seizures in rats. *Epilepsia*. 2003; 44:1494–1499. [PubMed: 14636318]
17. Cheng YJ, Karavolas HJ. Conversion of progesterone to 5a-pregnane-3,20-dione and 3a-hydroxy-5a-pregnan-20-one by rat medial basal hypothalamus and the effects of estradiol and stage of estrous cycle on the conversion. *Endocrinology*. 1973; 93:1157–1162. [PubMed: 4591653]

18. Rhodes ME, Frye CA. Attenuating 5 α -pregnane-3 α -ol-20-one formation in the hippocampus of female rats increases pentylenetetrazol-induced seizures. *Epilepsy Behav.* 2005; 6:140–146. [PubMed: 15710296]
19. Herzog AG, Frye CA. Seizure exacerbation associated with inhibition of progesterone metabolism. *Ann Neurol.* 2003; 53:288–291. [PubMed: 12601696]
20. Frye CA, Scalise TJ, Bayon LE. Finasteride blocks the reduction in ictal activity produced by exogenous estrous cyclicity. *J Neuroendocrinol.* 1998; 10:291–296. [PubMed: 9630399]
21. Frye CA. The neurosteroid 3 α -5 α -THP has antiseizure and possible neuroprotective effects in an animal model of epilepsy. *Brain Res.* 1995; 696:113–120. [PubMed: 8574658]
22. Walf AA, Koonce CJ, Frye CA. Estradiol or diarylpropionitrile administration to wild type, but not estrogen receptor beta knockout, mice enhances performance in the object recognition and object placement tasks. *Neurobiol Learn Mem.* 2008; 89:513–521. [PubMed: 18313947]
23. Micevych P, Sinchak K. Estradiol regulation of progesterone synthesis in the brain. *Mol Cell Endocrinol.* 2008; 290:44–50. [PubMed: 18572304]
24. Mahendroo MS, Cala KM, Landrum DP, Russell DW. Fetal death in mice lacking 5 α -reductase type 1 caused by estrogen excess. *Mol Endocrinol.* 1997; 11:917–927. [PubMed: 9178751]
25. Hom AC, Leppik IE, Rask CA. Effects of estradiol and progesterone on seizure sensitivity in oophorectomized DBA/2J mice and C57/EL hybrid mice. *Neurology.* 1993; 43:198–204. [PubMed: 8423885]
26. Soma KK, Sinchak K, Lakhter A, Schlenger BA, Micevych PE. Neurosteroids and female reproduction: estrogen increases 3 β -HSD mRNA and activity in rat hypothalamus. *Endocrinology.* 2005; 146:4386–4390. [PubMed: 16020475]
27. Micevych PE, Chaban V, Ogi J, Dewing P, Lu JK, Sinchak K. Estradiol stimulates progesterone synthesis in hypothalamic astrocyte cultures. *Endocrinology.* 2007; 148:782–789. [PubMed: 17095591]
28. Mayaan R, Fischb B, Galdora M, et al. Influence of 17 β -estradiol on the synthesis of reduced neurosteroids in the brain (in vivo) and in glioma cells (in vitro): possible relevance to mental disorders in women. *Brain Res.* 2004; 1020:167–172. [PubMed: 15312799]
29. Bäckström T. Epileptic seizures in women related to plasma estrogen and progesterone during the menstrual cycle. *Acta Neurol Scand.* 1976; 54:321–347. [PubMed: 973554]

Fig. 1.

Average latency to myoclonus (left) and hippocampal 3α,5α-THP (middle, indicated by gray circles) and hippocampal estrogen (right) levels. Results from experiment 1 (top) demonstrate that wild-type mice administered estrogen had significantly increased latencies to myoclonus, increased hippocampal 3α,5α-THP levels, and no increases in hippocampal estrogen. Results from experiment 2 (bottom) show that knockout mice administered estrogen had no significant differences in latencies to myoclonus and had 3α,5α-THP levels equal to or less than those of vehicle-treated wild-type mice. *Significant, $P < 0.05$. #Trend, $P < 0.1$.

Fig. 2. Regression analyses of hippocampal 3 α ,5 α -THP (top) and estrogen (bottom) levels with the latency to myoclonus. 3 α ,5 α -THP levels tended to positively correlate with latency to myoclonus; however, there were no correlations between hippocampal estrogen levels and latency to myoclonus.