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Estrogen-priming can enhance progesterone's anti-seizure effects in part by increasing hippocampal levels of allopregnanolone

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Abstract

Estrogen can be proconvulsant, while progesterone and its metabolite allopregnanolone typically have anti-seizure effects. We investigated whether estrogen-priming also has anti-seizure effects by altering progesterone's metabolism to allopregnanolone, or levels of brain-derived neurotrophic factor (BDNF), in the hippocampus. Two experiments investigated effects of different estrogen-priming regimen (Experiment 1—10 μg; Experiment 2—2 μg) on pentylenetetrazole (PTZ)-induced seizures and levels of estrogen, progesterone and allopregnanolone in plasma and hippocampus. In Experiment 1, ovariectomized (ovx) rats were administered sesame oil vehicle or 10 μg 17β-estrogen at hour 0. Forty-four hours later, progesterone (500 μg; SC) or vehicle was administered. At hour 47, PTZ (70 mg/kg IP) was administered. For Experiment 2, a similar protocol was used except that ovx rats were administered vehicle or 2 μg 17β-estradiol at hours 0 and 24. Progesterone, alone or in conjunction with either 10 or 2 μg estrogen-priming, tended to increase the latency to, and significantly reduced the number of, tonic seizures and elevated levels of progestins in hippocampus and plasma. Two, but not 10, micrograms of estrogen alone had anti-seizure effects and increased levels of allopregnanolone in the hippocampus. BDNF levels in the hippocampus were increased by estrogen-priming, but reduced by progesterone administration. Thus, estrogen may have anti-seizure effects by enhancing formation of allopregnanolone.

Keywords: Estradiol; Neurosteroid; Non-genomic; Ictal activity

Steroid hormones influence brain function from gestation throughout life and may thereby affect seizures by altering neuronal excitability (Morrell, 1992). In general, it is thought that estrogen enhances neuronal excitability and increases the risk of seizures, whereas progesterone diminishes neuronal excitability and has an inhibitory effect on seizures. However, there is also evidence that estrogen either does not have proconvulsant effects or that it may even have protective effects.

Seizure frequency and severity can change at various life stages that are associated with alterations in hormones (Morrell, 2002). At puberty and during adolescence, patterns of specific epilepsy syndromes change. Benign rolandic epilepsy often remits, while idiopathic generalized epilepsies, such as juvenile myoclonic epilepsy and photosensitive epilepsy, may arise (Morrell, 1992; Wheless and Kim, 2002). Little is known about the effect of menarche on seizures, albeit a relationship between seizures and the menstrual cycle has been observed for many years (Almqvist, 1955; Bandler et al., 1957; Dickerson, 1941; Duncan et al., 1993; Laidlaw, 1956; Newmark and Penry, 1980; Tauboll et al., 1984). Catamenial epilepsy, or menstrual cycle-related changes in seizure disorder, may affect up to 70% of women with epilepsy; however, incidence varies with criteria for changes in seizures (Foldvary-Schaefer et al., 2004). Three distinct patterns of catamenial epilepsy have been described with increases in

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seizures typically occurring during the perimenstrum, around ovulation, and/or during an inadequate luteal phase (Herzog et al., 1997). Studies directly associating seizures with changes in hormone concentrations have suggested that the ratio of estrogen to progesterone influences epileptiform activity (Backstrom, 1976; Herzog, 1991), such that greater levels of estrogen may be predisposing to hyperexcitability and progesterone may have anti-epileptiform effects. These findings are reinforced by observations that seizures often remit during pregnancy, when the balance of progesterone to estrogen is greater (Morrell, 2002; Pimentel, 2000). As well, progesterone administration can be effective in management of seizures (Backstrom et al., 1984; Herzog, 1986, 1995; Taly et al., 1985).

Not all clinical studies support a positive relationship between estrogen and seizures. For example, in a study of 15 institutionalized women with primary generalized epilepsy, fewer seizures occurred in midcycle, when estrogen levels were elevated (and calcium levels were decreased), than at other stages of the menstrual cycle (Jacono and Robertson, 1987). Similarly, although the perimenopause is a transition during which many women with catamenial epilepsy are at risk for increased seizure frequency, the use of estrogen-based hormone therapy can benefit some women (Peebles et al., 2000); however, it increases seizure occurrence in some women (Harden, 2003).

In animal models, administration of estrogen has been reported to have either no effects on, increase, or decrease seizures. For example, estrogen administration to adult male mice or ovariectomized (ovx) rats attenuates neither kainic-acid nor pentylenetetrazole (PTZ)-induced seizures (Budziszewska et al., 2001; Hoffman et al., 2003; Reibel et al., 2000; Perez et al., 1988). Although estrogen administration to young rats did not alter the rate of kindling (Schultz-Krohn et al., 1986), acute estrogen treatment (30 or 50, but not 10 μg) to adult male amygdaloid-kindled rats, leads to an intensification of seizures (Saberi et al., 2001). Estrogen administration to ovx rats decreases the latency to onset of kainic-acid induced seizures but increases the latency to NMDA-induced seizures (Slamberova and Vathy, 2000). Anti-seizure effects of estrogen include that estradiol replacement blocks the ovx-induced increase in the susceptibility to cyclosporin A-induced convulsions (Tominaga et al., 2001) and that estrogen administration reduces the total number of seizures produced by infusions of NMDA (Kalkbrenner and Standley, 2003).

In part due to the variable effects of estrogen on seizures, it has been proposed that estrogen may have independent effects on seizure behavior and to protect the hippocampus from damage that seizures produce (Hoffman et al., 2003; Lason, 2000). Although, estrogen administration to ovx rats reduces the total number of seizures and the hippocampal damage produced by infusions of NMDA or kainic-acid (Kalkbrenner and Standley, 2003; Veliskova et al., 2000), estrogen's effects on seizures may not underlie its effects on neuronal integrity. For example, ovx rats administered estrogen had no fewer kainic-acid induced seizures but did have less damage in the dentate hilus and CA3 pyramidal layer (Hoffman et al., 2003; Reibel et al., 2000).

Another possibility to consider in explanation for some of the inconsistent effects of estrogen on seizures is that estrogen can modify synthesis of progestins (Cheng and Karavolas, 1973; Micevych et al., 2003; Vongher and Frye, 1999). Progesterone may reduce seizures in part through actions of its metabolite allopregnanolone, which enhances $GABA_A$ receptor function and thereby inhibits neuronal excitability (Harrison et al., 1987; Herzog and Frye, 2003; Lambert et al., 2001; Majewska et al., 1986, Reddy, 2004; Reddy et al., 2004). Effects of progestins are generally anticonvulsant (Lonsdale and Burnham, 2003); however, whether anticonvulsant or proconvulsant effects are observed depends upon concentrations of allopregnanolone produced and the duration of exposure (Frye and Bayon, 1998, 1999a,b; Hoffman et al., 2003; Moran and Smith, 1998).

Another factor that may underlie some of estrogen's and/or progesterone's effects on neuronal excitability are effects on brain-derived neurotrophic factor (BDNF). There is an estrogen-response element on the *BDNF* gene (Scharfman et al., 2003). Furthermore, estrogen can enhance levels of BDNF in the hippocampus and may thereby mitigate hyperexcitability in the hippocampus (Scharfman et al., 2003; Gresack and Frick, 2004; Fernandez and Frick, 2004). In addition, progesterone can attenuate (Bimonte-Nelson et al., 2004) or amplify the effects of estrogen on BDNF (Gibbs, 1998). Notably, BDNF has been implicated in the pathogenesis of epileptic seizures. Data from several reports suggest that BDNF is involved in seizure thresholds and epileptiform activity in the hippocampus (Binder et al., 1994; Kokaia et al., 1995).

The purpose of the present experiments was to test the hypotheses that estrogen may alter seizure thresholds in part by increasing production of allopregnanolone and/or BDNF levels in the hippocampus.

1. Methods

These methods were pre-approved by the Institutional Animal Care and Use Committee at SUNY-Albany.

1.1. Animals and housing

Female Long–Evans rats ($N=102$), approximately 55 days of age, were obtained from the breeding colony at SUNY-Albany. All rats were group housed (4 per cage) in a temperature-controlled room (21±1 °C) in the Laboratory Animal Care Facility. Rats were maintained on a 12/12 h reversed light cycle (lights off 8:00 am) with ad lib access to Purina Rat Chow and tap water in their home cages.

1.2. Hormone regimens

All rats were ovx, under Rompun (12 mg/kg) and Ketaset (80 mg/kg) anesthesia at least 7 days prior to testing, to control endogenous levels of hormones. Ovx rats were administered steroid hormones obtained from Sigma (St. Louis, MO) and dissolved in sesame oil to yield a subcutaneous (SC) injection volume of 0.2 cc. Rats were administered 17β-estradiol or vehicle. In Experiment 1 ($n=10$/group), each rat was administered 10 μg estradiol or vehicle at hour 0. This regimen has been used to produce levels of estradiol similar to that observed during behavioral estrus (Vongher and Frye, 1999). For Experiment 2, rats ($n=5-7$/group) were administered 2 μg estradiol or vehicle at hours 0 and 24, which produces estrogen levels akin to those of diestrous rats (Veliskova et al., 2000). Forty-four hours after the initial administration of estrogen or vehicle, all rats were administered progesterone (500 μg) or vehicle. Three hours following progesterone or vehicle administration, all rats were tested as described below for seizures. This progesterone regimen produces physiological levels of progestins (Frye et al., 2000; Vongher and Frye, 1999; Rhodes and Frye, 2004, 2005). For this experiment, there were 5 rats in the vehicle condition, and 7 in the estrogen only, progesterone only, and estrogen in conjunction with progesterone groups. NB: separate rats were used for steroid measurement and seizure activity in Experiment 1, whereas, in Experiment 2, the same rats were used for both endpoints. However, the hormone levels produced by each of the regimen were commensurate with levels seen previously in our lab.

1.3. Behavioral testing

Immediately prior to testing, rats were weighed to ensure accurate dosing with PTZ. Rats were then placed in a plexiglass arena ($50\times30\times25$ cm) for 5 min to habituate. Rats were then administered PTZ (70 mg/kg, IP) and ictal behaviors were recorded for 10 min. Within seconds of PTZ administration, myoclonus is initiated. This is followed by facial and forelimb clonus, and then tonic seizures, which were characterized by loss of righting reflex and tonic forelimb flexion/extension, followed by whole-body clonus. As hormones effects on tonic seizures are the most robust, these are the effects reported.

1.4. Biochemical measures

1.4.1. Procedures

For Experiment 1, tissues of additional rats in analogous conditions to those described above were collected in the same time frame following estrogen and/or progesterone administration as those rats assessed for ictal activity. This enabled plasma and hippocampal estrogen, progesterone, and allopregnanolone levels produced by this regimen to be determined. For this, there were tissues from 12 rats in the vehicle condition, 7 in the estrogen only, 10 in the progesterone only, and 9 in the estrogen in conjunction with progesterone groups.

For Experiment 2, tissues were collected from rats that had seizures in order to examine effects of hormone regimen on estrogen, progesterone, and allopregnanolone levels in plasma and hippocampus, as well as BDNF levels in the hippocampus.

1.4.2. Tissue collection

Rats in each condition were decapitated and brains were rapidly removed, the hippocampus was dissected bilaterally, placed on dry ice, and stored at −70 °C until radioimmunoassay. Trunk blood was collected and remained on ice until refrigerated centrifugation (4 °C at 3000 $\times g$ for 8 min). Serum was aliquoted and stored at −70 °C until radioimmunoassay.

1.4.3. Extraction of steroids

Estrogen and progestins were extracted from plasma samples using diethyl ether. Estrogen was also extracted from hippocampal tissues with diethyl ether following homogenization with a glass/Teflon homogenizer in distilled water. Progestins were extracted from hippocampus following homogenization with a glass/glass homogenizer in 5 ml 50% MeOH, 1% acetic acid. Tissues were centrifuged at 3000 $\times g$ and the supernatant was chromatographed using Sepak-cartridges and increasing concentrations of 5 ml MeOH (50% MeOH, 1% acetic acid, 50% MeOH, 100% MeOH). All solvents were removed using a speed drier, and samples were reconstituted in assay buffer (pH=7.4).

1.4.4. Radioimmunoassays

The levels of estrogen, progesterone, and allopregnanolone in the hippocampus and plasma were measured by radioimmunoassay (RIA) according to previously published methods (Frye et al., 2000).

1.4.5. Radioactive probes

Tritiated estradiol [^{3}H] E_2 (NET-317, 51.3 Ci/mmol), progesterone (NET-208: specific activity=47.5 Ci/mmol), and allopregnanolone (NET-1047: specific activity=65.0 Ci/mmol), used for RIAs, were purchased from New England Nuclear (Boston, MA).

1.4.6. Antibodies

The estradiol antibody, (E#244, Dr. G.D. Niswender, Colorado State University, Fort Collins, CO) was used in a 1:40,000 dilution and bound between 40% and 60% of [^{3}H] estradiol. The progesterone antibody (P#337 from Dr. G.D. Niswender, Colorado State University) was used in a 1:30,000 dilution and bound between 30% and 50% of [^{3}H] progesterone. The allopregnanolone antibody (purchased from Dr. Robert Purdy, Veterans Medical Affairs, La Jolla, CA), was used in a 1:5000 dilution, which binds between 40% and 60% of [^{3}H] allopregnanolone.

1.4.7. Set-up and incubation of RIAs

Standard curves were prepared in duplicate to give a range of nine concentrations. For estrogen, the range for the standard curve was 12.5–1000 pg. For progestins, the range of standard curves was from 50 to 4000 pg. The standards were added to BSA assay buffer, followed by addition of the appropriate antibody and [^{3}H] steroid. The total assay volumes for estrogen, progesterone, and allopregnanolone were 800, 950, and 1200 μl, respectively. Estrogen was incubated at room temperature for 50 min, progestins were incubated overnight at 4 °C.

1.4.8. Termination of binding

Separation of bound and free steroid was accomplished by the rapid addition of dextran-coated charcoal. Following incubation with charcoal, samples were centrifuged at 3000 $\times g$ and the supernatant was pipetted into a glass scintillation vial with scintillation cocktail. Sample tube concentrations were calculated using the logit–log method of Rodbard and Hutt (1974), interpolation of the standards, and correction for recovery. The intra-assay and inter-assay coefficients of variance for each assay were: estrogen 0.09 and 0.10, progesterone 0.12 and 0.13, allopregnanolone 0.13 and 0.15.

1.4.9. ELISA for BDNF

BDNF levels in the hippocampus were measured according to previously published methods using the Promega BDNF E_{max} Immunoassay System (Promega, Madison, WI; Frye et al., 2005). Briefly, 200 μl Promega lysis buffer was added to 100 μl aliquots of previously prepared samples. Samples were sonicated with a microtip at power level 4 for 15 s. Samples were then centrifuged at 16,000 $\times g$ for 30 min. Twenty-five microliter aliquots of the resulting supernatant were removed and diluted with 100 μl of DPBS buffer. A 100 μl anti-BDNF monoclonal antibody (mAB) diluted 1:1000 in carbonate coating buffer was applied to a 96-well polystyrene plate and incubated overnight at 4 °C. Unabsorbed mAB was removed and plates were washed once with TBST wash buffer. Plates were blocked using 200 μl Promega 1X Block and Sample buffer followed by incubation, without shaking, for 1 h at room temperature. Plates were then washed once using TBST wash buffer. Two hundred microliters of each standard (0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 pg/ml) were added in duplicate to plates. One hundred microliters of samples were added in duplicate to plates. Plates were incubated for 2 h with shaking at room temperature. Plates were then washed five times with TBST wash buffer. Anti-human BDNF polyclonal antibody (pAB; 100 μl diluted 1:500 in 1X Block and Sample buffer) was added to each well and plates were incubated for 2 h with shaking at room temperature. Plates were washed five times with TBST wash buffer. Anti-Ig Y horseradish peroxidase conjugate (100 μl diluted 1:200 in 1X Block and Sample buffer) was then added to each well and plates were incubated for 1 h with shaking at room temperature. Plates were emptied again and washed five times with TBST wash buffer. Finally, plates were developed using 100 μl Promega TMB One Solution and the reaction was stopped 10 min using 100 μl 1 N HCl. Protein was measured using Bradford's method (Bradford, 1976). Absorbance was measured at 450 nm. BDNF levels are reported as ng/mg tissue.

1.4.10. Statistical analyses

The latency to, and incidence of, tonic seizures and endocrine measures following estrogen and/or progesterone administration were compared across groups using one-way analyses of variance (ANOVA). Where appropriate, ANOVAs were followed by Fisher's Least Square Difference post hoc tests. The alpha level for significance was considered $P \leq 0.05$, trends were considered when the alpha level for significance was $P \leq 0.10$.

2. Results

2.1. Experiment 1: effects of 10 μg estrogen-priming on PTZ-induced seizures and hormone levels

There was a tendency for latencies to ($F(3,36)=2.654$, $P<0.06$; Fig. 1, top), and significant effects in the numbers of ($F(3,36)=3.591$, $P<0.02$), tonic seizures to be different across the groups. Mean (±SEM) number of tonic seizures of the groups were: vehicle=1.4±0.3, estrogen=1.1±0.3, progesterone=0.5±0.2, estrogen+progesterone=0.5±0.2 (presented as percent change from vehicle in Fig. 1, bottom).

Concentrations of estrogen ($F(3,34)=16.813$, $P<0.0001$), progesterone ($F(3,34)=19.958$, $P<0.0001$), and allopregnanolone ($F(3,34)=38.599$, $P<0.0001$) in the hippocampus were significantly different across groups. Post hoc tests indicated that levels of estrogen produced in the hippocampus were greater for those animals administered estrogen compared to vehicle (vehicle=1.9±0.3, estrogen=7.1±2.5, progesterone=1.4±0.3, estrogen+progesterone=7.2±2.5; Fig. 2, top presented as percent change from vehicle). Hippocampal concentrations of progesterone (vehicle=1.2±0.4, estrogen=0.7±0.2, progesterone=9.8±1.9, estrogen+progesterone=9.4±2.4; Fig. 2, middle) and allopregnanolone (vehicle=0.5±0.9, estrogen=3.3±1.5, progesterone=6.4±0.3, estrogen+progesterone=7.7±0.3; Fig. 2, bottom) were also significantly greater among the groups administered progesterone as compared to vehicle. Levels of estrogen, progesterone, and allopregnanolone produced by estrogen and/or progesterone administration were commensurate with those previously observed for naturally cycling rats in behavioral estrus (Frye and Bayon, 1999a,b).

As expected, circulating concentrations of estrogen ($F(3,34)=47.105$, $P<0.0001$), progesterone ($F(3,34)=73.410$, $P<0.0001$), and allopregnanolone ($F(3,34)=$

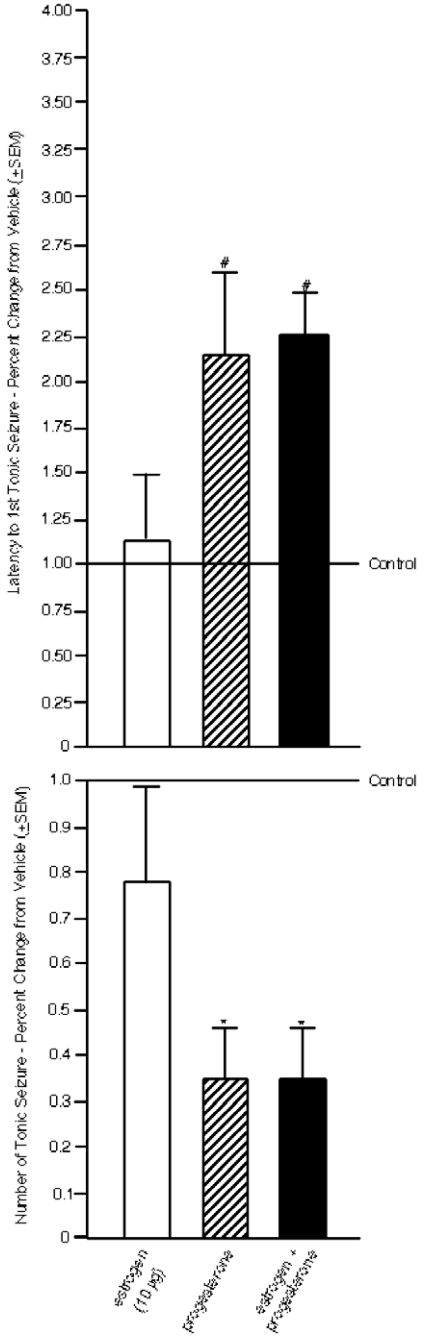

Fig. 1. Represents percent change from vehicle ($n=10$) of latencies to (±SEM; top panel), and number of (±SEM; bottom panel), PTZ-induced tonic seizures of ovx rats administered estrogen (10 μg; white bars; $n=10$), progesterone (striped bars; $n=10$), or estrogen+progesterone (black bars; $n=10$). # indicates tendency for difference from vehicle group. *Indicates significant difference from vehicle control group.

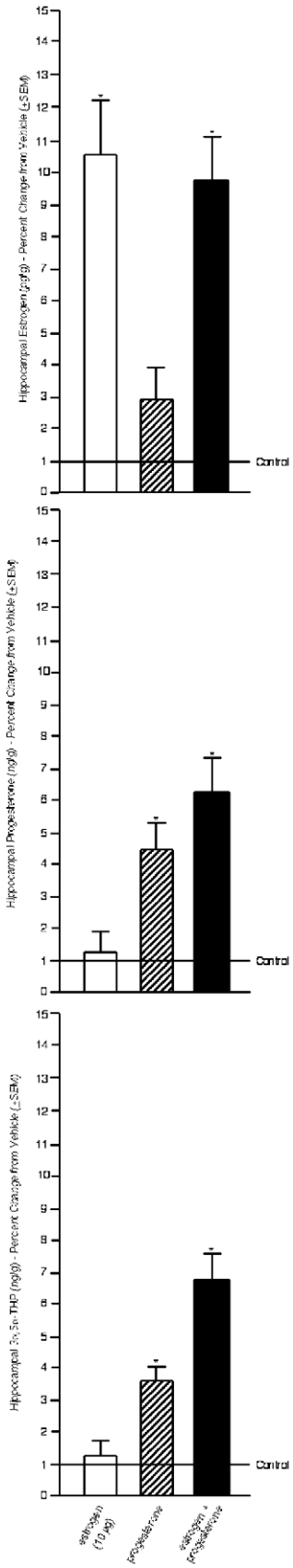

Fig. 2. Percent change from vehicle ($n=12$) in levels of hippocampal estrogen (±SEM; top panel), progesterone (±SEM; middle panel), and 3α,5α-THP (±SEM; bottom panel) of ovx rats administered estrogen (10 μg; white bars; $n=7$), progesterone (striped bars; $n=10$), or estrogen+progesterone (black; $n=9$). *Indicates significant difference from all other groups.

27.901, $P<0.0001$) were significantly greater for rats administered estrogen and/or progesterone compared to vehicle (Table 1). Plasma levels were also similar to those previously reported as being produced by this regimen and/or rats in behavioral estrus (Frye and Bayon, 1999a,b).

2.2. Experiment 2: effects of 2 μg estrogen-priming on PTZ-induced seizures and endocrine measures

There was a tendency for latencies to tonic seizures to be different among groups. ($F(3,22)=2.633$, $P<0.07$; Fig. 3, top). The number of tonic seizures was significantly different between groups ($F(3,22)=5.442$, $P<0.001$). Rats administered progesterone, with or without estrogen-priming (2 μg), had fewer numbers of tonic seizures (vehicle = 1.6 ± 0.2, estrogen = 0.7 ± 0.3, progesterone = 0.4 ± 0.2, estrogen + progesterone = 0.3 ± 0.2, Fig. 3, bottom presented as percent change from vehicle).

Hippocampal concentrations of estrogen ($F(3,24)=3.788$, $P<0.02$), progesterone ($F(3,24)=10.088$, $P<0.001$), and allopregnanolone ($F(3,24)=25.278$, $P<0.0001$) were significantly different across groups. Levels of estrogen produced in the hippocampus were greater for those animals administered estrogen compared to vehicle (vehicle = 1.9 ± 0.3, estrogen = 7.1 ± 2.5, progesterone = 1.4 ± 0.3, estrogen ± progesterone = 7.2 ± 2.5, Fig. 4, top). Concentrations of progesterone (vehicle = 1.2 ± 0.4, estrogen = 0.7 ± 0.2, progesterone = 9.8 ± 1.9, estrogen + progesterone = 9.4 ± 2.4, Fig. 4, middle) and allopregnanolone (vehicle = 0.5 ± 0.1, estrogen = 3.3 ± 1.5, progesterone = 6.4 ± 0.3, estrogen + progesterone = 7.7 ± 0.3, Fig. 4, bottom) in the hippocampus were also significantly greater for rats administered progesterone versus vehicle. Hippocampal levels of estrogen, progesterone, and allopregnanolone produced by this estrogen and/or progesterone administration were commensurate with those previously observed for naturally cycling rats in diestrus that have not had seizures (Frye and Bayon, 1999a,b).

Similarly, plasma concentrations of estrogen ($F(3,24)=7.057$, $P<0.001$), progesterone ($F(3,24)=21.963$, $P<0.0001$), and allopregnanolone ($F(3,24)=63.727$, $P<0.0001$) were significantly greater for rats administered estrogen and/or progesterone than their respective vehicles

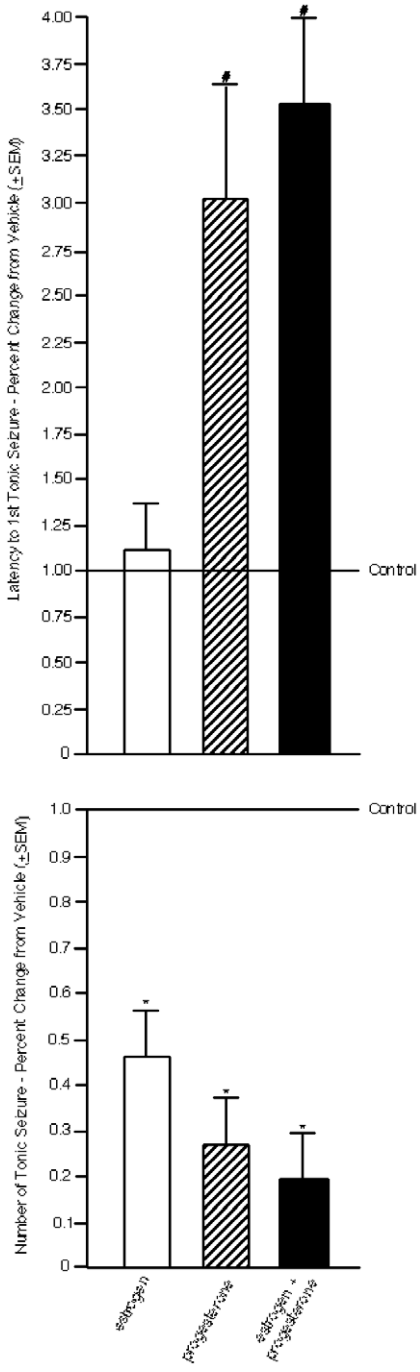

Fig. 3. Represents percent change from vehicle ($n=5$) of latencies to (±SEM; top panel), and number of (±SEM; bottom panel), PTZ-induced tonic seizures of ovx rats administered estrogen (2 μg; white bars; $n=7$), progesterone (striped bars; $n=7$), or estrogen + progesterone (black bars; $n=7$). #Indicates tendency for difference from vehicle control group. *Indicates significant difference from vehicle control group.

Table 1
Percent change from ovx control of plasma levels of estrogen, progesterone, and 3α,5α-THP following administration of estrogen (10 μg), progesterone, or both

Condition	Plasma hormone levels		
	Estradiol (pg/ml)	Progesterone (ng/ml)	3α,5α-THP (ng/ml)
Estrogen (10 μg)	88 ± 13.7*	0.9 ± 0.2	0.7 ± 0.2
Progesterone	1.3 ± 0.4	27.8 ± 2.9*	24.6 ± 3.8*
Estrogen and progesterone	87 ± 10.9*	36.3 ± 3.1*	22.1 ± 3.0*

*Indicates significantly ($P<0.001$) different from ovx controls.

(Table 2). Circulating levels of these hormones were also similar to those previously reported for naturally cycling rats in diestrus (Frye and Bayon, 1999a,b).

There was a tendency for levels of BDNF in the hippocampus to vary across the groups ($F(3,22)=2.562$, $P<0.08$; Table 3). Rats administered estrogen alone had

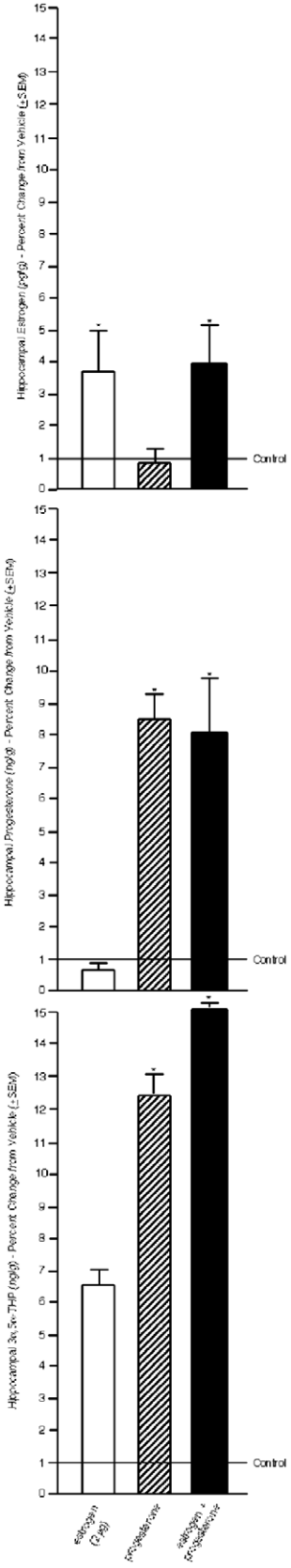

Table 2
Percent change from ovx control of plasma levels of estrogen, progesterone, and 3α,5α-THP following administration of estrogen (2 μg), progesterone, or both

Condition	Plasma hormone levels		
	Estradiol (pg/ml)	Progesterone (ng/ml)	3α,5α-THP (ng/ml)
Estrogen (2 μg)	12.1±3.8*	1.1±0.2	1.4±0.3
Progesterone	1.3±0.2	10.8±2.1*	13.4±1.4*
Estrogen and progesterone	10.6±3.0*	11.3±1.4*	13.3±1.0*

*Indicates significantly ($P<0.001$) different from ovx controls.

apparent increases in BDNF in the hippocampus compared to all other groups.

3. Discussion

The primary hypothesis that estrogen-priming had effects on seizures and allopregnanolone concentrations was partially supported. Estrogen-priming with 2, but not 10, μg estrogen increased the latencies to, and decreased the number of, PTZ-induced seizures and elevated concentrations of allopregnanolone in the hippocampus compared to vehicle. Similarly, 2 but not 10 μg of estrogen, had apparent effects to amplify the effects of progesterone, such that somewhat greater anti-seizure effects were observed with 2 μg estrogen and progesterone (albeit, not significantly different than 2 μg estrogen alone). These results confirm previous findings that estrogen in conjunction with progesterone can have anti-seizure effects (Frye and Bayon, 1998, 1999a,b) and extend them to further suggest that perhaps some of estrogen's protective effects may involve formation of allopregnanolone. Indeed, it has long been known that estrogen can enhance actions of the enzymes that convert progesterone to allopregnanolone (Cheng and Karavolas, 1973). More recent research has focused on how estrogen can enhance de novo production of progesterone in the brain (Micevych et al., 2003). Results from animal models and clinical case reports have substantiated that blocking formation of allopregnanolone can attenuate progesterone's anti-seizure effects (Frye et al., 1998, 2002; Frye and Scalise, 2000; Herzog and Frye, 2003; Kokate et al., 1999). Notably, this is the first report that estrogen-priming can amplify progesterone's anti-seizure effects in part due to enhancing formation of progestins. As such, these results suggest some of the variability in the reports of estrogen's effects on seizures may be associated with the extent to

Fig. 4. Percent change from vehicle ($n=10$) of hippocampal estrogen (±SEM; top panel), progesterone (±SEM; middle panel), and 3α,5α-THP (±SEM; bottom panel) of ovx rats estrogen (2 μg; white bars; $n=10$), progesterone (striped bars; $n=10$), or estrogen+progesterone (black bars; $n=10$). *Indicates significant difference from vehicle control group. **Indicates significant from all other groups.

Table 3
Percent change of hippocampal BDNF levels compared to ovx controls

Condition	Hippocampal BDNF (ng/mg)
Estrogen (2 μg)	4.7±1.90#
Progesterone	1.4±0.21
Estrogen and progesterone	1.9±0.40

#Indicates tendency ($P=0.08$) to be different from ovx control group.

which different estrogen regimen can enhance production of allopregnanolone.

The secondary hypothesis that estrogen's effects on seizures were related to concentrations of BDNF in the hippocampus was not entirely supported. The 2 μg estrogen-priming regimen utilized significantly increased seizure thresholds and tended to increase levels of BDNF in the hippocampus compared to vehicle administration. However, administration of 2 μg estrogen and progesterone together increased seizure thresholds, but did not increase BDNF in the hippocampus. Although these findings confirm that estrogen can enhance BDNF levels in the hippocampus and progesterone can abrogate these effects (Scharfman et al., 2003; Bimonte-Nelson et al., 2004), the pattern of results of the BDNF levels in the hippocampus cannot account for all of the effects on seizures across the hormone groups investigated. Yet some of the effects of estrogen alone could be due to BDNF. Levels of hippocampal BDNF immunoreactivity and population spikes in CA3 (evoked by repetitive hilar stimuli) are greater during behavioral estrus compared to diestrus or for ovx rats. The hyperexcitability in CA3 observed during behavioral estrus could be blocked by exposure to the high-affinity neurotrophin receptor antagonist K252 (Scharfman et al., 2003). In order to address whether actions at BDNF alone may account for estrogen's anti-seizure effects in this paradigm, further studies would be required involving co-administration of estrogen and an antagonist, such as K252.

The present findings that some regimen of estrogen can have anti-seizure effects that may involve formation of allopregnanolone in the hippocampus, do not preclude other mechanisms underlying estrogen's diverse effects on seizures. Estrogen can have actions via nuclear estrogen receptors (ERs); however, in the hippocampus, there are few intracellular ERs (McEwen, 2001). Notably, effects of estrogen to attenuate duration of slow spike-and-wave discharges, are not blocked by the non-specific ER antagonist, tamoxifen (Persad et al., 2004). Alternative mechanisms through which estrogen in the hippocampus may influence seizures may occur via actions at non-nuclear ERs in dendrites, presynaptic terminals, and/or glial cells. These non-nuclear ERs may couple to second messenger systems to regulate cellular functioning and/or to signal transcriptional regulators, such as CREB (McEwen, 2001). Thus, the multiplicity of estrogen's possible actions may account for some of its variable effects on seizures.

The results of the present study that reveal a modest anti-seizure effect of estrogen with one priming regimen (2 μg), but not another (10 μg), are interesting but must be interpreted with caution. Findings from both animal and clinical studies reveal a complex interrelationship between seizures and endocrine processes. In animal models, seizures disrupt normal ovarian cyclicity. Seizures can delay the onset of puberty, cause acyclicity, induce pseudopregnancy, or the development of polycystic ovaries (Bhanot and Wilkinson, 1982; Edwards et al., 1999, 2000). Seizures also disrupt the anterior pituitary response to gonadotrophin releasing hormone and compromise ovulatory-type surge of LH or FSH produced by progesterone injection to estrogen-primed rats (Wilkinson et al., 1982). Indeed, we have previously demonstrated that levels of expression of the enzymes that convert progesterone to allopregnanolone can be altered in the hippocampus by seizures (Rhodes et al., 2004). As well, it is possible that seizure experience may upregulate the expression of developmentally regulated substrates, such as ER-X to recapitulate some effects of estrogen on developmental processes (Toran-Allerand, 2004).

Similarly, the incidence of reproductive endocrine disorders is inordinately high among women with epilepsy (Herzog et al., 1986; Herzog, 1993). Changes in luteinizing hormone (LH) pulse frequency and increased prolactin levels have been reported following the occurrence of generalized or partial seizures (Bauer, 2001). As a consequence, menstrual cycles may be disturbed. Indeed, levels of estrogen and progestins have been reported to be much lower for some women with epilepsy (Bunter and Rosciszewska, 1985) and may be related to their patterns of seizures over the menstrual cycle (Rosciszewska, 1980). As well, the age at menopause may be reduced in women with poorly controlled seizures, perhaps due to the effect of seizures on endocrine (dys)function (Harden, 2003). In clinical studies, it is a challenge to separate the effects of seizures on hormones from the effects of anti-epileptic drugs, which may enhance the metabolism of hormones due to their effects on the hepatic cytochrome *P*450 enzyme system (Rosciszewska et al., 1986). The notion that low levels of estrogen can protect against seizures through an allopregnanolone-related mechanism has important implications for treatment of hormonally modulated seizure disorders, such as catamenial epilepsy. Given that an estimated 1.2 million women of childbearing age in the US alone have epilepsy, it is important to parse out any contributing effects that may influence anti-epileptic effects of existing AEDs or that can enhance existing alternative therapies for management of epilepsy, such as progesterone (Herzog, 1991).

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