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Gonadal status-dependent effects of *in vivo* β-estradiol administration to female rats on *in vitro* epileptiform activity induced by low $[Mg^{2+}]_o$ in combined hippocampus-entorhinal cortex slices

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Abstract

There are controversial data regarding estrogen effects on neuronal excitability. We investigated whether β-estradiol (EB) administration to ovariectomized (OVX) or gonadally intact female rats alters epileptiform activity within the dentate gyrus network induced *in vitro* by removing $[Mg^{2+}]_o$ in combined hippocampus-entorhinal cortex slices. *In vivo* EB administration significantly influenced the epileptiform activity in gonadal status-dependent manner. The onset of epileptiform discharges was modestly delayed in slices from OVX rats replaced with physiologically relevant doses of EB but the number of discharges was not affected. In contrast, EB administration to gonadally intact rats had robust effects such that: EB delayed the onset of discharges and significantly increased their number within the dentate gyrus network. Our data suggest that EB in physiologically relevant concentrations does not seem to negatively affect hippocampal neuronal excitability nevertheless supraphysiological EB levels may enhance seizure severity.

Keywords

Epileptiform discharges; neuronal excitability; β-estradiol; female rats; dentate gyrus network; *in vitro* electrophysiology

Introduction

Female sex hormones modulate seizure susceptibility and neuronal excitability. While anticonvulsant effects of progesterone are well established, clinical and animal studies show conflicting results of estrogen-mediated effects on seizures and neuronal excitability [for review see (Velíšková & DeSantis, 2013)]. Many factors likely contribute to these controversial reports such as estrogen doses but also different criteria for behavioral seizure

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assessment among the laboratories. For example, several laboratories used kainic acid model to examine effects of β-estradiol (EB) on seizure susceptibility in naïve or ovariectomized rats and found either no effect, or proconvulsant as well as anticonvulsant effects (Nicoletti et al., 1985; Reibel et al., 2000; Velíšková & Velíšek, 2007; Woolley, 2000). The kainic acid model is an established model for seizures with temporal lobe origin where especially the dentate gyrus plays an important role as it serves as a gate for activity flow and thus, it regulates seizure propagation into the hippocampus proper (Danzer et al., 2008; Heinemann et al., 1992; Lothman et al., 1992). A weakening or disruption of the dentate gyrus gate has been linked to seizure development (Behr et al., 1998; Pun et al., 2012). **Previously we have established that administration of repeated doses of EB replacement to ovariectomized rats leads to genomically regulated changes of the dentate gyrus network that affect its excitability, while there were no effects of acutely circulating estradiol (Velíšková & Velíšek, 2007).**

The goal of present study was to investigate how these EB-induced changes in the dentate gyrus network excitability affect epileptiform activity within individual regions belonging to dentate gyrus network. Therefore, we investigated effects of *in vivo* EB administration in relation to gonadal status on epileptiform activity induced *in vitro* by lowering $[Mg^{2+}]_o$ within the dentate gyrus network (Amaral et al., 2007). In combined entorhinal cortex-hippocampal slices, decreased $[Mg^{2+}]_o$ levels produce spontaneous epileptiform events by activation of NMDA receptors, by reduced membrane surface charge screening, increased transmitter release while depending on the extent of neuronal interconnectivity (Mody et al., 1987; Walther et al., 1986; Anderson et al., 1986).

Material and methods

Adult female Sprague-Dawley rats (Taconic Farms; 150-200 g) were kept on a 12-h light/dark cycle (lights on at 0700). Some rats were ovariectomized (OVX) under ketamine/xylazine (50/7 mg/kg i.p.) anesthesia one week prior to hormonal replacement. Peanut oil (0.1 ml/day) or 17β-estradiol benzoate (EB, 2 μg/0.1 ml/day) were injected subcutaneously for 4 days (Velíšková & Velíšek, 2007). This low dose EB treatment in OVX rats produces estradiol plasma levels corresponding to the second day of diestrus (Neal-Perry et al., 2005; **Velíšková & DeSantis, 2013**) but leads to supraphysiological levels in gonadally intact (non-OVX) rats, a situation resembling the use of estrogen-based contraceptives in women. Oil/EB-injected non-OVX rats were used at diestrus. The following experimental groups were studied: OVX+4x oil n=10; OVX+4x EB n=12; Diestrus+4x oil n=6; Diestrus+4x EB n=5. Measurements of vaginal impedance and smears confirmed successful OVX, EB treatment, and stage of estrous cycle in non-OVX rats.

Standard electrophysiological procedures were used (Nebieridze et al., 2012; Velíšek et al., 1999). At 24 hours following the last oil or EB injection, rats were decapitated under deep CO_2 anesthesia and brains removed. Combined hippocampal-entorhinal cortex slices were cut horizontally in ice-cold aCSF (composition in mM): KCl 5, $MgSO_4$ 2, NaH_2PO_4 1.2, $CaCl_2$ 2, glucose 10, and $NaHCO_3$ 26, gassed with 95% O_2/5% CO_2, pH=7.4, transferred to interface-type recording chamber to recover at 33-34^{0}C in the aCSF for 1 hour (Nebieridze et al., 2012; Velíšek et al., 1999). Epileptiform activity was induced by perfusion with 0 Mg^{2+}-containing aCSF (Velíšek et al., 1999). Simultaneous recordings (glass recording micropipette filled with 2 M NaCl; resistance 2-5 MΩ) were performed in the entorhinal cortex layer II, layer of dentate granule cells and in the layer of CA3/4 pyramidal neurons.

We first used three-way ANOVA (factors: hippocampal structure, gonadal status and EB treatment) to analyze whether the epileptiform activity preferentially started in any of the regions studied. To analyze the onset of a first discharge and **status epilepticus-like** activity,

two-way ANOVA (factors: gonadal status and EB treatment) was used. To analyze progression of epileptiform activity, three-way ANOVA with two between factors (gonadal status and EB treatment) and one within factor (time) was used. Level of significance was preset to $p<0.05$.

Results

In the dentate gyrus and CA3 region, removal of $[Mg^{2+}]_o$ lead to short recurrent discharges (SRDs) characterized by small amplitude and irregular frequency increasing over time and eventually progressing to **status epilepticus-like** activity, which is a continuous activity with large amplitude and high frequency discharges (Heinemann et al., 1992; Velíšek et al., 1999). In the entorhinal cortex, initial seizure-like events (SLEs) consisted of significant DC shifts. The SLEs later progressed into a faster **status epilepticus-like** activity called late recurrent discharges (LRDs) **characterized by recurrent DC deflections** with short duration (Velíšek et al., 1999). We did not find any changes in the pattern of temporal relationship among the three major dentate gyrus network regions specific to hormonal or gonadal status. Three-way ANOVA did not show any effect of region or gonadal status on the onset of discharges. Because there was no effect of region and no interaction of region with other two factors, we further evaluated regions separately.

Neuronal excitability in the dentate gyrus network depends on hormonal status

Onset of low $[Mg^{2+}]_o$-induced epileptiform discharges was significantly affected by EB treatment in all studied regions (Figure 1a-c). Two-way ANOVA revealed that EB replacement significantly delayed the onset of discharges (dentate gyrus: $F_{1,29=}6.977$, $p=0.013$; CA3 region: $F_{1,29=}5.158$, $p=0.031$; entorhinal cortex: $F_{1,29=}7.669$, $p=0.010$) irrespective of the gonadal status (dentate gyrus: $F_{1,29=}0.277$; $p=0.603$; CA3 region: $F_{1,29=}1.325$, $p=0.259$; entorhinal cortex: $F_{1,29=}0.374$, $p=0.545$).

The onset of **status epilepticus-like** activity (Figure 1d-f) was also significantly delayed in EB-treated animals in all three regions (dentate gyrus: $F_{1,29=}5.707$, $p=0.024$; CA3 region: $F_{1,29=}6.039$, $p=0.020$; entorhinal cortex: $F_{1,29=}4.190$, $p=0.049$). Gonadal status had no effect on the onset of **status epilepticus-like** activity (dentate gyrus: $F_{1,29=}0.473$; $p=0.497$; CA3 region: $F_{1,29=}1.070$, $p=0.309$; entorhinal cortex: $F_{1,29=}0.040$, $p=0.843$) but in the dentate gyrus, EB delayed the onset of **status epilepticus-like** activity only in non-OVX rats (interaction $F_{1,29=}4.531$, $p=0.042$).

Progress and severity of epileptiform activity induced by low $[Mg^{2+}]_o$

Comparisons using the within factor (progress in time) in all recorded regions showed a significant progression and increasing discharge frequency over time in all experimental groups ($p<0.0001$). There were region-specific differences in number of discharges dependent on EB administration and gonadal status measured within one-minute period at one, 10, 20 and 30 minutes from the onset of a first discharge (Figure 2a-c).

In the dentate gyrus, three-way ANOVA showed that EB administration (main factor) had a significant effect on number of low $[Mg^{2+}]_o$–induced SRDs (interaction $F_{1,28=}7.258$; $p=0.012$) with distinct effects in OVX compared to non-OVX animals (interaction $F_{1,28=}4.955$; $p=0.034$). EB administration only to non-OVX rats significantly increased number of SRDs. Gonadal status had no effect on number of discharges (interaction $F_{1,28=}2.872$; $p=0.101$) but the course of epileptiform-like activity progression was affected by the factor of gonadal status (interaction $F_{3,84}=3.352$, $p=0.022$).

In the CA3 region, EB administration had a significant effect on number of SRDs ($F_{1,28=}$8.964; p=0.006). EB administration significantly increased the number of epileptiform discharges in non-OVX but not in OVX rats (interaction $F_{1,28=}$5.112; p=0.032). Gonadal status had a non-significant borderline effect on number of SRDs ($F_{1,28=}$3.929; p=0.057) as non-OVX oil-injected rats had lower number of SRDs at their onset but this difference diminished during **status epilepticus-like** activity. There were no additional interactions among the factors.

In the entorhinal cortex, comparisons between the factors did not reveal any effect of gonadal status ($F_{1,28=}$0.281; p=0.600) or EB administration ($F_{1,28=}$1.643; p=0.211) on the number of DC deflections.

Discussion

In this study, we found that EB administration significantly controls low $[Mg^{2+}]_o$-induced epileptiform activity in hormonal status-dependent manner. EB replacement in OVX rats only modestly delayed the onset but had no effect on the number of discharges. In contrast, EB administration to non-OVX rats had more robust effects: While the onset of low $[Mg^{2+}]_o$-induced discharges was delayed compared to all other groups, number of discharges in all regions were significantly increased by supraphysiological levels of EB.

Studies using *in vivo* seizure models showed that EB administration in doses leading to supraphysiological levels irrespective of gonadal status enhanced seizure severity and accelerated the onset of tonic-clonic seizures underscoring that high circulating EB levels can promote progression of seizures to more advanced stages (Nicoletti et al., 1985; Velíšková & DeSantis, 2013). Tonic-clonic seizures independent of seizure origin are highly sensitive to blockade of NMDA receptors (Velíšek et al., 1990). Our findings using the *in vitro* low $[Mg^{2+}]_o$ model, which involves NMDA receptor activation (Mody et al., 1987), are relevant to the *in vivo* data as we also found increased severity of discharges in slices from female rats with supraphysiological levels of EB. In contrast, restoring physiologically relevant EB levels in OVX rats did not accelerate or increase severity of epileptiform discharges within the dentate gyrus circuitry. Current findings in OVX rats expand on our previous data when a similar EB regimen did not accelerate low $[Mg^{2+}]_o$-induced discharges in the entorhinal cortex or in the CA1 region (Velíšek et al., 1999). Thus, both studies show that administration of EB producing physiologically relevant plasma concentrations does not increase neuronal excitability in any hippocampal region or the entorhinal cortex.

In females in diestrus with natural levels of estradiol and progesterone, neuronal excitability was not different compared to OVX rats suggesting that interactions between estradiol and progesterone may dampen any effects of each hormone on excitability. This is in accordance with our previous recordings in entorhinal cortex and CA1 region from OVX rats with combined EB and progesterone administration within physiologically relevant doses. The study found no effect of the combined hormonal treatment on low $[Mg^{2+}]_o$-induced discharges, while progesterone alone had region-specific effects: It delayed the onset of discharges in the entorhinal cortex but accelerated discharges in CA1 region (Velíšek et al., 1999).

In conclusion, the current *in vitro* study shows that hippocampal neuronal excitability is not enhanced when the brain is primed by physiologically relevant levels of EB. This is in support of recent *in vivo* studies [for review see (Velíšková & DeSantis, 2013)]. On the other hand, supraphysiological levels of EB can contribute to seizure exacerbation and enhanced severity as found in some women with epilepsy following hormonal additives (Harden et al., 2006) and also in animal studies (Velíšková & DeSantis, 2013).

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Disclosure

The authors disclose no conflict of interest. The authors confirm reading the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

Amaral DG, Scharfman HE, Lavenex P. The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Prog Brain Res. 2007; 163:3–790. [PubMed: 17765709]

Anderson WW, Lewis DV, Swartzwelder HS, Wilson WA. Magnesium-free medium activates seizure-like events in the rat hippocampal slice. Brain Res. 1986; 398:215–219. [PubMed: 3801897]

Behr J, Lyson KJ, Mody I. Enhanced propagation of epileptiform activity through the kindled dentate gyrus. J Neurophysiol. 1998; 79:1726–1732. [PubMed: 9535942]

Danzer SC, Kotloski RJ, Walter C, Hughes M, McNamara JO. Altered morphology of hippocampal dentate granule cell presynaptic and postsynaptic terminals following conditional deletion of TrkB. Hippocampus. 2008; 18:668–678. [PubMed: 18398849]

Harden CL, Herzog AG, Nikolov BG, Koppel BS, Christos PJ, Fowler K, Labar DR, Hauser WA. Hormone replacement therapy in women with epilepsy: a randomized, double-blind, placebo-controlled study. Epilepsia. 2006; 47:1447–1451. [PubMed: 16981859]

Heinemann U, Beck H, Dreier JP, Ficker E, Stabel J, Zhang CL. The dentate gyrus as a regulated gate for the propagation of epileptiform activity. Epilepsy Res Suppl. 1992; 7:273–280. [PubMed: 1334666]

Lothman EW, Stringer JL, Bertram EH. The dentate gyrus as a control point for seizures in the hippocampus and beyond. Epilepsy Res. 1992; 7(Suppl):301–313.

Mody I, Lambert JDC, Heinemann U. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. J Neurophysiol. 1987; 57:869–888. [PubMed: 3031235]

Neal-Perry GS, Zeevalk GD, Santoro NF, Etgen AM. Attenuation of preoptic area glutamate release correlates with reduced luteinizing hormone secretion in middle-aged female rats. Endocrinology. 2005; 146:4331–4339. [PubMed: 15994345]

Nebieridze N, Zhang XL, Chachua T, Velíšek L, Stanton PK, Velíšková J. β-Estradiol unmasks mGluR-mediated metaplasticity in the female dentate gyrus. Psychoneuroendocrinology. 2012; 37:1845–1854. [PubMed: 22541715]

Nicoletti F, Speciale C, Sortino MA, Summa G, Caruso G, Patti F, Canonico PL. Comparative effects of estradiol benzoate, the antiestrogen clomiphene citrate, and the progestin medroxyprogesterone acetate on kainic acid-induced seizures in male and female rats. Epilepsia. 1985; 26:252–257. [PubMed: 3159567]

Pun RY, Rolle IJ, Lasarge CL, Hosford BE, Rosen JM, Uhl JD, Schmeltzer SN, Faulkner C, Bronson SL, Murphy BL, Richards DA, Holland KD, Danzer SC. Excessive Activation of mTOR in Postnatally Generated Granule Cells Is Sufficient to Cause Epilepsy. Neuron. 2012; 75:1022–1034. [PubMed: 22998871]

Reibel S, Andre V, Chassagnon S, Andre G, Marescaux C, Nehlig A, Depaulis A. Neuroprotective effects of chronic estradiol benzoate treatment on hippocampal cell loss induced by status epilepticus in the female rat. Neuroscience Letters. 2000; 281:79–82. [PubMed: 10704747]

Velíšek L, Kusá R, Kulovaná M, Mareš P. Excitatory amino acid antagonists and pentylenetetrazol-induced seizures during ontogenesis. I. The effects of 2-amino-7-phosphonoheptanoate. Life Sciences. 1990; 46:1349–1357. [PubMed: 2161066]

Velíšek L, Velíšková 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]

Velíšková J, DeSantis KA. Sex and hormonal influences on seizures and epilepsy. Hormones and Behavior. 2013; 63:267–277. [PubMed: 22504305]

Velíšková J, Velíšek L. β-Estradiol Increases Dentate Gyrus Inhibition in Female Rats via Augmentation of Hilar Neuropeptide Y. J Neurosci. 2007; 27:6054–6063. [PubMed: 17537977]

Walther H, Lambert JDC, Jones RSG, Heinemann U, Hamon B. Epileptiform activity in combined slices of the hippocampus, subiculum and entorhinal cortex during perfusion with low magnesium. Neuroscience Letters. 1986; 69:156–161. [PubMed: 3763042]

Woolley CS. Estradiol facilitates kainic acid-induced, but not flurothyl-induced, behavioral seizure activity in adult female rats. Epilepsia. 2000; 41:510–515. [PubMed: 10802755]

Highlights

- Seizure modulating effects of estradiol in female rats depend on gonadal status.
- Estradiol delays epileptiform activity within the dentate gyrus network.
- Estradiol promotes epileptiform discharges in brain slices from intact female rats.

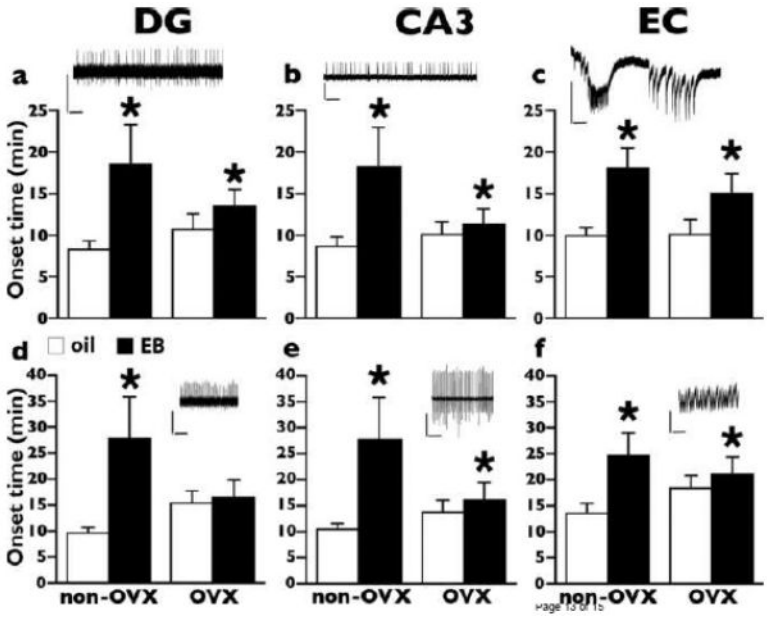

Figure 1. EB-induced changes in dentate gyrus network neuronal excitability.

a-c: In the dentate gyrus (DG), CA3 region and entorhinal cortex (EC), the onset of low $[Mg^{2+}]_o$-induced epileptiform discharges was significantly delayed by EB (β-estradiol benzoate) treatment in all studied regions (two-way ANOVA, *p<0.05; see text for detailed statistics). Time = 20 sec; calibration = 1 mV (DG), 2 mV (CA3 and EC).

d-f: Similarly, the onset of **status epilepticus-like** activity was also delayed in EB-treated animals in all three regions (two-way ANOVA, *p<0.05). Experimental groups: 1. OVX+4x oil n=10; 2. OVX+4x EB n=12; 3. Diestrus+4x oil n=6; 4. Diestrus+4x EB n=5. OVX = ovariectomy. Time = 20 sec; calibration = 1 mV (DG), 5 mV (CA3), 2 mV (EC).

Insets are representative recordings of initial burst activity from the DG and CA3 regions (a, b) showing typical short recurrent discharges (SRDs) with small amplitude and irregular frequency and seizure-like events (SLEs) in the EC with a DC deflection (c). The initial ictal activity then progressed to continuous status epilepticus-like activity in all three regions, which consisted of stable large amplitude and high frequency discharges in the DG (d) and field CA3 (e), or recurrent DC deflections with short duration in the EC (f) and lasted as long as slices were perfused with aCSF containing 0 $[Mg^{2+}]_o$. The pattern and progression of epileptiform activity within individual dentate gyrus network regions did not differ among treatment groups. All traces represent recordings from a single slice.

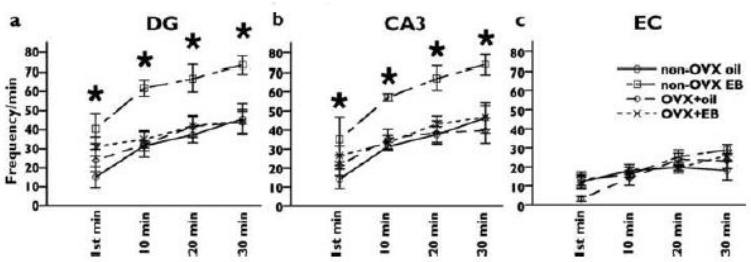

Figure 2. Severity of epileptiform activity induced by low $[Mg^{2+}]_o$ in the dentate gyrus network.
a-c: In the dentate gyrus (DG) and CA3 regions, the **number of individual epileptiform discharges measured within one-minute period at one, 10, 20 and 30 minutes (a, b)** following the onset of discharges was significantly increased in non-OVX animals treated with EB (Three-way ANOVA, *$p<0.05$; see text for detailed statistics). **However, in the entorhinal cortex (EC), the number of DC deflections per minute was not different amongst treatment groups (c).**