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SHORT COMMUNICATION

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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Summary 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 but 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.

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

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and DeSantis, 2013)]. Many factors likely contribute to these controversial reports such as estrogen doses but also different criteria for behavioral seizure 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 and 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 and 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 and 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 and 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 + 4× oil, $n = 10$; OVX + 4× EB, $n = 12$; Diestrus + 4× oil, $n = 6$; Diestrus + 4× 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 h 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 °C in the aCSF for 1 h (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 (Fig. 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 (Fig. 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: