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Seizure Facilitating Activity of the Oral Contraceptive Ethinyl Estradiol

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Abstract

Contraceptive management is critical in women with epilepsy. Although oral contraceptives (OCs) are widely used by many women with epilepsy, little is known about their impact on epileptic seizures and epileptogenesis. Ethinyl estradiol (EE) is the primary component of OC pills. In this study, we investigated the pharmacological effect of EE on epileptogenesis and kindled seizures in female mice using the hippocampus kindling model. Animals were stimulated daily with or without EE until generalized stage 5 seizures were elicited. EE treatment significantly accelerated the rate of epileptogenesis. In acute studies, EE caused a significant decrease in the afterdischarge threshold and increased the incidence and severity of seizures in fully-kindled mice. In chronic studies, EE treatment caused a greater susceptibility to kindled seizures. Collectively, these results are consistent with moderate proconvulsant-like activity of EE. Such excitatory effects may affect seizure risk in women with epilepsy taking OC pills.

Keywords

Oral contraceptive; Pregnancy; Kindling; Ethinyl estradiol; Epileptogenesis

1. Introduction

Contraceptive management in women with epilepsy is critical owing to the potential maternal and fetal risks if contraception or seizure management fails. A wide range of hormonal contraceptive methods are available for women, including injectable progestogens and oral contraceptive (OC) pills. The combination OC pills are composed of low-dose synthetic estrogen and progestogen and are usually taken for 21 days with a 7 day gap. Ethinyl estradiol (EE) is a major estrogen constituent in OCs including monophasic, biphasic, triphasic and extended-cycle regimens (Reddy, 2010). There are many factors to consider in the selection of contraception since some antiepileptic drugs (AED) may affect

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the efficacy of OCs owing to pharmacokinetic interaction (Crawford et al., 1990). These interactions between AEDs and OCs can influence drug efficacy and seizure control. Although it is known that steroid hormones and neurosteroids can affect seizure susceptibility, there is limited information on the potential impact of OCs on seizures in women with epilepsy (Reddy, 2014). A recent study suggests that OCs may exacerbate seizures (Herzog, 2015); however, previous reports mostly attest lack of evidence to support this premise (Crawford et al., 1986; Harden and Leppik, 2006). Emerging data from 750 women within the Epilepsy Birth Control Registry revealed a significantly greater (6-fold) frequency of seizure exacerbation with hormonal than non-hormonal contraception (Herzog, 2015). There is little basic data to suggest that EE may have neuroexcitatory properties similar to estradiol (Scharfman and MacLusky, 2006). Therefore, this study was undertaken to investigate the pharmacological effect of EE on epileptogenesis and kindled seizure activity in female mice using the hippocampus kindling model.

2. Material and Methods

2.1. Animals

Adult female C57BL/6 mice (25 to 30 g) were used in this study. The mice were housed in an environmentally controlled animal facility with a 12 h light/dark cycle. The animals were cared for in strict compliance with the guidelines outlined in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Animals were randomized into groups without subdividing them according to the estrous cycle phases. All animal procedures were performed in a protocol approved by the university's Institutional Animal Care and Use Committee.

2.2. Hippocampus kindling model

To study the seizure modulating activity of EE, we used the hippocampus kindling model of complex partial seizures. Electrode implantation and stimulation procedures for mouse hippocampus kindling were performed as described previously (Reddy and Mohan, 2011; Reddy et al., 2012). Mice were anesthetized by an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). A twisted bipolar stainless-steel wire electrode (model MS303/1; Plastic Products, Roanoke, VA) was stereotactically implanted in the right hippocampus (2.9 mm posterior and 3.0 mm lateral to bregma and 3.0 mm below the dorsal surface of the skull) using the Franklin and Paxinos atlas and anchored with dental acrylic to four jeweler's screws placed in the skull. A period of 10 days was allowed for recovery. The stimulation paradigm consisted of 1-ms duration, bipolar, square current pulses delivered at 60 Hz for 1s using a kindling stimulator (A-M Systems, Sequim, WA). The afterdischarge (AD) threshold was determined by stimulating at 5-min intervals beginning with an intensity of 25 μ A. Stimulation on consecutive days used a stimulation intensity of 125% AD threshold value. Seizure activity after each stimulation was rated according to the Racine scale as modified for the mouse: stage 0, no response or behavior arrest; stage 1, chewing or head nodding; stage 2, chewing and head nodding; stage 3, forelimb clonus; stage 4, bilateral forelimb clonus and rearing; and stage 5, falling. Kindling stimulation was performed daily until stage 5 seizures were elicited on 3 consecutive days, which is considered the "fully kindled" state.

2.3. Test drugs and treatments

Stock solutions of EE (Sigma-Aldrich, St. Louis, MO) for injections were made in 0.1% solutol solution (Polyoxyethylated 12-hydroxystearic acid; Sigma-Aldrich, St. Louis, MO) and additional dilutions were made using normal saline. Drug solutions were administered in a volume equaling 1% of the animal's body weight. To examine the ability of EE to modulate the expression of seizures, fully kindled mice were injected subcutaneously with EE (10–100 µg/g body weight) fifteen minutes prior to stimulation. In kindling acquisition study, EE was given 30 min prior to stimulation. Vehicle was given to control groups. The EE dosage was selected based on previous reports and also to be comparable to clinically relevant levels during OC therapy (Budziszewska et al., 2001; Reddy, 2004; Reddy, 2010; Herzog, 2015).

2.4. Data analysis

Data was expressed as the mean $\pm$ standard error of the mean (SEM). Differences in kindling seizure stages between groups were compared with the nonparametric Kruskal-Wallis test followed by the Mann-Whitney U test. Comparison of means of the seizure duration, AD threshold and AD duration between groups was made with a one-way analysis of variance, followed by Student's t-test. In all statistical tests, the criterion for statistical significance was $P < 0.05$.

3. Results

3.1. Acute effect of EE on seizure activity in fully-kindled mice

To determine whether the acute EE treatment is associated with heightened seizure susceptibility, we analyzed the stimulation-evoked seizure activity in fully-kindled animals treated with various doses of EE. Four parameters were assessed as indices of seizure propensity: (a) AD threshold (ADT) current for generalized seizures; (b) stimulation-induced electrographic AD duration, (c) behavioral seizure intensity measured as per the Racine scale, and (d) duration of generalized seizures. Consistent with seizure exacerbation, there was a marked decrease in the ADT current required to induce generalized seizures after EE treatment (Fig. 1A). The mean duration of the individual generalized seizures was longer in EE-treated mice than in control animals (Fig. 1B). The total duration of AD was significantly higher in EE-treated animals (Fig. 1C). The electrographic events are illustrated in Fig. 1E. EE-treated animals showed continuous bursts of spikes that progressively increased in amplitude and duration, indicating heightened epileptiform activity. The number of animals exhibiting generalized seizures at 50% ADT current was significantly higher in EE-treated than in the control group (Fig. 1D), which indicates the potential for seizure exacerbation following EE treatment.

3.2. Chronic effect of EE on seizure activity in fully-kindled mice

To simulate the chronic daily use of OCs, EE was evaluated following a chronic treatment in fully-kindled mice. Effect of sub-effective doses of EE was tested on seizure activity in mice that have been treated daily with EE (25 µg/g, sc) for 21 days. The seizure susceptibility of EE-treated animals was assessed with EE challenge (0, 10 and 25 µg/g, sc) doses. Mice displayed a significant reduction in ADTs in response to EE challenge (25 µg/g, sc) one day

after the chronic treatment as compared to control group (Fig. 1F). In addition, mice chronically treated with EE (25 µg/g, sc) displayed significant increase in AD duration (Fig. 2H) without significant change in generalized seizure intensity (Fig. 1G). There was a significant increase in the number of EE-treated animals exhibiting generalized seizures at subthreshold ADT current than vehicle controls (Fig. 1I). Seizure facilitating effect was clearly apparent upon EE challenge only (Fig. 1FH). These responses suggest that EE can moderately facilitate epileptic seizures upon chronic treatment.

3.3. Effect of EE on the development of kindling epileptogenesis in female mice

To determine the effect of EE on epileptogenesis, we studied the development of hippocampus kindling in female mice. To check the lowest EE dose that does not affect behavioral or EEG seizures, we did a dose-response relationship and selected EE (25 µg/kg) for the kindling development experiment. This dose did not affect the severity or occurrence of seizure activity in fully-kindled mice (Fig. 1AD). The progression of electrographic AD activity, behavioral seizures, and the rate of kindling were recorded as main indices of epileptogenesis (Fig. 2). Mice treated with EE (25 µg/g, sc) showed a significant increase in susceptibility to the development of kindling epileptogenesis, as evident in the decreased number of stimulations required to elicit behavioral seizures compared with vehicle controls (Fig. 2A). Measures of evoked electrographic characteristics revealed a significant increase in AD duration in EE-treated mice (Fig. 2B,E). The duration of the initial AD was similar in control and EE-treated animals (Fig. 2E). Despite a relatively higher AD following EE treatment, there was no corresponding acceleration in seizure activity until 6 stimulations (Fig. 2A). The rate of kindling, expressed as the mean seizure stage per stimulation through stage 5 seizures, was significantly lower in EE-treated mice compared with vehicle controls (Fig. 2C). The mean number of stimulations required to achieve progressively higher seizure stages was markedly reduced in EE-treated mice (Fig. 2D), which are relatively more prone to hippocampus epileptogenesis.

4. Discussion

The present study shows that EE therapy is associated with significant impact on seizure activity in epileptic female mice. Our results provide moderate evidence that EE affects seizure expression, seizure incidence and AD duration in fully-kindled mice. These electrographic effects are evident from a dose-dependent reduction in thresholds required to elicit generalized stage 5 seizures. Such proconvulsant-like facilitating activity is noted in both acute and chronic settings. Moreover, daily EE treatment in naïve mice significantly shortened the rate for induction of epileptogenesis for stage 5 seizures. These excitatory effects of EE in the hippocampus kindling model are consistent with observations in previous studies with estradiol (Buterbaugh, 1989; Edwards et al., 1999; Reddy, 2004). However, these results apply to the kindling seizure paradigm utilized in the study and the extent that it resemble complex partial seizures. Nevertheless, these results demonstrate that EE, like estradiol, increases epileptogenesis and facilitates seizure susceptibility in female animals.

This study confirms recent findings that hormonal contraception has a relative risk for seizures that is 6 times greater than non-hormonal contraception (Herzog, 2015). In addition

to direct excitatory impact of EE, it is likely that levels of AEDs may change due to pharmacokinetic interactions (Reddy, 2010). OCs may enhance the metabolism of certain AEDs that may lead to an enhanced risk for seizures in this cohort (Herzog et al., 2009). This is likely because non-hormonal contraceptives, which do not affect AED metabolism as much as OCs, are not associated with such risk of seizures.

There are many underlying mechanisms whereby EE affects seizures susceptibility. Animals chronically exposed to estradiol have shown increased number and density of dendritic spines and excitatory synapses on hippocampal neurons (Murphy et al., 1998; Woolley et al., 1997; Ruddick and Woolley, 2001). This mechanism increases the synchronization of synaptically driven neuronal firing in the hippocampus and could be relevant to EE's proconvulsant effects in animal models. EE may also increase excitability through modulation of neuropeptides and increased levels of brain-derived neurotrophic factor in the hippocampus (Scharfman and MacLusky, 2006). In addition to nuclear estrogen receptors, the effects of EE could arise due to interaction with membrane estrogen receptors. The rapid onset of EE may be due to its direct interactions at the membrane level or through a post-membrane secondary messenger cascades that affect neuronal excitability (Zadran et al., 2009; Roepke et al., 2011).

In conclusion, this study demonstrates that EE has neuroexcitatory effects that can accelerate epileptogenesis and exacerbate seizure activity in epileptic animals. The central effects of EE in the well-established kindling model provide evidence that EE may show similar pro-epileptic effects upon long-term administration, such as that present in OC pills. These results provide insights on the emerging reports of increased seizure frequencies in women taking OC pills. It is likely that such excitatory effects may affect seizure risk in women with epilepsy taking EE-containing OC pills.

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Highlights

- Although oral contraceptives (OCs) are widely used by many women with epilepsy, little is known about their impact on epileptic seizures.
- Here, we report a facilitating effect of ethinyl estradiol (EE), a primary component of OC pills, on epileptogenesis and kindled seizures in female mice.
- EE has proconvulsant-like effects and can be linked to heightened seizure risk in women with epilepsy taking OC pills.

Figure 1. Effects of acute and chronic EE treatment on seizure activity in fully-kindled female mice

(ABCDE) The acute effects of EE (10–100 µg/g, sc) on kindled seizures. (A) Intensity of ADT current for eliciting generalized (stage 4/5) seizures after EE treatment. (B) Duration of behavior (stage 4/5) seizures after EE treatment. (C) Duration of AD after EE treatment. (D) Percent animals exhibiting generalized seizures at 50% ADT current. (E) Representative traces illustrate EE exacerbation of electrographic seizure activity in a fully-kindled mouse. Control trace was obtained without EE treatment. **(FGHI) Effects of chronic EE treatment on kindled seizures.** Fully-kindled mice were treated with EE (25 µg/g, sc) for 21 days and then seizure activity measured on day 22 following EE challenge dose (0, 10 and 25 µg/g, sc). (F) Mean ADT current for eliciting generalized (stage 4/5) seizures after EE treatment. (G) Duration of behavior (stage 4/5) seizures after EE treatment. (H) Mean AD duration after EE treatment. (I) Percent animals exhibiting generalized seizures at subthreshold ADT current. Control group represents vehicle-treated, fully-kindled mice that were not exposed to EE therapy. All other groups represent fully-kindled mice chronically-treated with EE and then challenged with an EE dose (0, 10 and 25 µg/g, sc). Values represent the mean ± SEM (n = 6–8 mice per group). *p<0.05 versus control group.

Figure 2. Effect of EE on the development of kindling epileptogenesis in female mice

(A) EE-treated mice displayed enhanced kindling development as expressed by a higher mean seizure stage at the corresponding stimulation session. (B) AD duration was markedly higher in EE-treated mice than in vehicle controls. (C) Rate of kindling development (mean seizure stage per stimulation through stage 5 kindling) was significantly increased in EE-treated mice. (D) Mean number of stimulations to achieve hippocampus kindling stages in control and EE-treated (25 μ g/g, sc) mice. (E) Sample traces of electrographic AD activity in EE-treated mice during hippocampus kindling development. The traces show depth recordings from a right hippocampus stimulating-recording electrode after 1st and 10th stimulations. Behavioral seizure stages are indicated on the trace. Values represent the mean $\pm$ SEM (n = 6–8 mice per group). *p<0.05 versus control group.