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Short communication

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.

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 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 (sixfold) 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 (25–30 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

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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 stereotaxically 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 1 s 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 μA. 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 three 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) 15 min 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, 2010; Herzog, 2015).

2.4. Data analysis

Data were expressed as the mean ± 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.

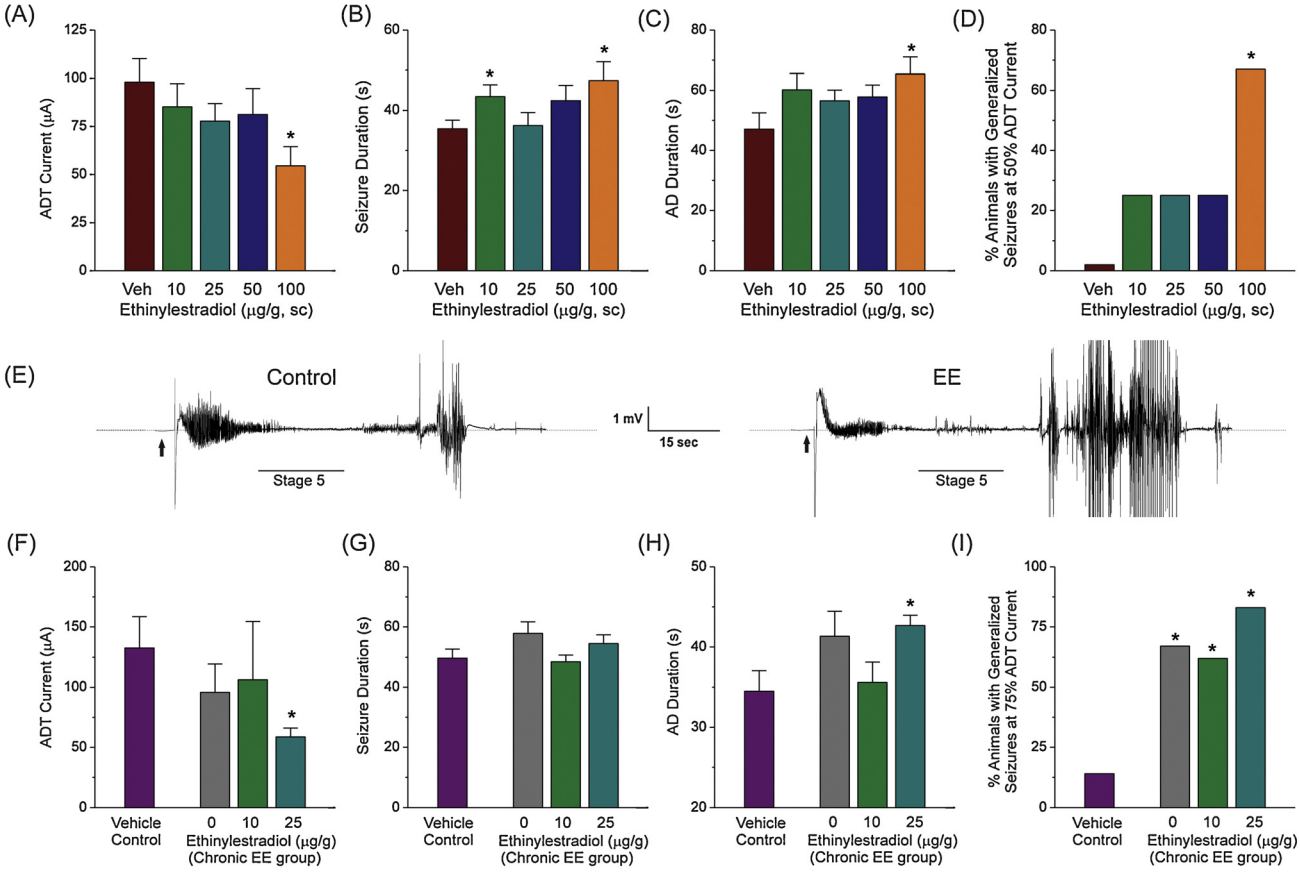

Fig. 1. Effects of acute and chronic EE treatment on seizure activity in fully-kindled female mice. (A–E) The acute effects of EE (10–100 μg/g, sc) on kindled seizures. (A) Intensity of ADT current for eliciting generalized (stage 4/5) seizures after EE treatment. (B) Duration of behavior (stage 4/5) 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. (F–I) 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.