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Laboratory Research

Estradiol Facilitates Kainic Acid–Induced, but not Flurothyl-Induced, Behavioral Seizure Activity in Adult Female Rats

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Summary: *Purpose:* This study was designed to determine whether previously demonstrated increases in hippocampal axospinous synapse density and NMDA receptor function induced by estradiol are paralleled by increased susceptibility to limbic (kainic acid induced) or generalized (flurothyl induced) behavioral seizures.

Methods: Kainic acid was injected systemically to ovariectomized adult female rats treated with either estradiol or oil vehicle. The latencies to each of five stages of seizure-related behaviors (staring, wet-dog shakes, head waving and chewing, forelimb clonus, rearing, and falling) were recorded for each animal. Flurothyl was administered by inhalation to ovariectomized adult female rats treated with estradiol alone, estradiol followed by short-term progesterone, or oil vehicle. The latencies to each of three stages of seizure-related behaviors (first myoclonic jerk, forelimb clonus, wild running and bouncing) were recorded for each animal.

Results: Estradiol treatment decreased the latency to seizure-related behaviors induced by kainic acid, but neither estradiol alone nor estradiol followed by progesterone had any effect on flurothyl-induced seizure-related behaviors.

Conclusions: The same estradiol treatment paradigm known to induce structural and functional changes in the excitatory circuitry of the hippocampus facilitates the progression of kainic acid–induced seizures, which are known to involve the hippocampus, but has no effect on flurothyl-induced seizures. The lack of an effect of estradiol alone or estradiol followed by progesterone on flurothyl-induced seizures indicates that estradiol's effects on seizure susceptibility do not result from increased neuronal excitability throughout the brain, but rather involve action within the limbic system. The data suggest that structural and functional changes in hippocampal circuitry induced by estradiol may contribute to increased susceptibility to limbic seizure activity. **Key Words:** Catamenial epilepsy—Limbic seizures—Hippocampus—CA1 pyramidal cells—Dendritic spines.

Numerous clinical and experimental studies have demonstrated that estrogen can be proconvulsant. Clinically, an association between seizure frequency and the menstrual cycle in women with epilepsy (termed catamenial epilepsy) has been recognized for over 100 years (1). Although the prevalence of catamenial epilepsy has been controversial, many studies have documented a significant relationship between seizure occurrence and menses (reviewed in 2, 3). For example, a study of 184 women with intractable partial complex seizures reported that approximately one third exhibited at least a doubling of average daily seizure frequency during one of three phases of the menstrual cycle: perimenstrual, preovulatory, or second half of cycles with an inadequate luteal phase (3). Each of these periods is characterized by a high estradiol-to-progesterone ratio (3,4), suggesting that estradiol is proconvulsant, whereas progesterone is anticonvulsant. The proconvulsant effect of estradiol is particularly clear during cycles with an inadequate luteal phase, in which seizure frequency increases when estradiol levels are elevated, but progesterone levels remain low (3).

Animal studies confirm that estradiol can facilitate seizure activity. Estradiol has been shown to decrease localized hippocampal and medial amygdala electrographic seizure threshold (5) and facilitate electroshock convulsions (6) as well as seizures kindled from several brain areas (7–9) or induced chemically (7,10). Some studies, however, have failed to show an effect of ovarian steroids on seizure activity (11, during the estrous cycle, 12), and at least one study has shown a protective

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effect of estradiol against seizures induced with picrotoxin (13).

The cellular mechanism(s) responsible for estradiol's effects on seizures are currently unknown. Estradiol has at least two distinct types of effects on neuronal circuitry that could contribute to seizure susceptibility: (a) rapid (seconds to minutes) changes in neuronal excitability and the efficacy of synaptic transmission that are dependent on the presence of the hormone (reviewed in 14), and (b) longer-term (hours to days) changes in structural synaptic connectivity that persist after removal of hormone (reviewed in 15).

Because the hippocampus is a key brain region in the generation and propagation of limbic seizure activity (16), excitatory effects of estradiol on hippocampal circuitry might increase the likelihood of seizures. My colleagues and I have previously shown that estradiol induces an increase in the density of excitatory synaptic input to CA1 pyramidal cells (17), which are major output neurons from the hippocampus. This structural change is paralleled by increased glutamate binding to the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor (18,19), increased NMDA-receptor immunofluorescence (20), and increased efficacy of NMDA receptor–mediated synaptic input (19). These structural and functional effects of estradiol might contribute to the progression of limbic seizure susceptibility by predisposing hippocampal circuitry to epileptiform activity.

Kainic acid (KA)-induced seizures are a good experimental model in which to test whether estradiol's effects on hippocampal circuitry are associated with increased seizure susceptibility, because seizures induced by KA are known to involve the hippocampus (21–27). To determine whether the structural effects of estradiol on hippocampal circuitry could be involved in estradiol facilitation of seizure activity, I tested KA seizure susceptibility using the same estradiol treatment regimen known to enhance synaptic density and NMDA receptor–mediated synaptic function in the hippocampus (19). As a control for generalized effects of estradiol on nonlimbic seizures, the effects of estradiol (with and without progesterone) on susceptibility to flurothyl-induced seizures also was evaluated.

METHODS

Animal treatments

Adult female Sprague–Dawley rats (Harlan, 220–250 g, ~70 days old) were used. Animals were group-housed in a university-operated vivarium on a 12-h light/12-h dark schedule with constant access to food and water. At least 1 week after arrival, animals were ovariectomized under methoxyflurane anesthesia using aseptic surgical technique. For KA-induced seizure testing, animals were divided into two groups (n = 22 each). One group received injections (s.c.) of 10 μg 17β-estradiol benzoate in 100 μl sesame oil on the mornings of days 3 and 4 after surgery (OVX + E). The other group received 100 μl sesame oil at the same times (OVX + O). Seizure testing was performed on day 6 after ovariectomy. For flurothyl seizure testing, animals were divided into three groups (n = 15 each). One group was treated as the OVX + E, except that the animals also received an injection of 500 μg progesterone 5 h before seizure testing (OVX + EP). The two other groups were OVX + E and OVX + O, as earlier, except that they received an injection of 100 μl sesame oil 5 h before testing. Seizure testing was done on day 6 after surgery (Fig. 1).

Kainic acid seizure testing

On the morning of day 6 after ovariectomy, animals were coded before any further procedure; the code was not broken until all analysis was complete. KA was dissolved in sterile saline (5 mg/ml) on the morning of each testing day, and the unused portion was discarded at the end of each day. Each animal was weighed, injected (i.p.) with 15 mg/kg KA (10), placed individually in a clean plexiglass cage, and observed for seizure-related behaviors for a period of 2 h. Either two or four animals were observed in a single session with two to three sessions per day. Each testing session included an equal number of OVX + O and OVX + E animals to equalize the effect of any possible diurnal variation in sensitivity to KA. The observer recorded latencies to each of five seizure-related behaviors: (a) periods of staring and immobility (staring); (b) wet-dog shakes (WDS), at least two within 1 min; (c) head waving and chewing (HW/Ch); (d) forelimb clonus (FLC); (e) rearing and falling (R/F). The observer also noted whether rats experienced full tonic extension or died during the observation period. At various intervals after the 2 h observation period, each animal was deeply anesthetized with 80 mg/kg pentobarbital (Nembutal) and transcardially perfused to gather pilot data for another study. Differences in the proportion of animals experiencing each seizure stage were analyzed statistically using the χ^2 test. Latencies to each seizure stage were analyzed statistically using repeated measures analysis of variance (ANOVA) followed by Fisher's LSD post hoc comparisons.

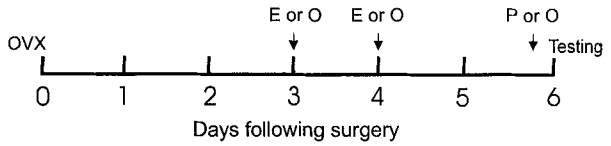

FIG. 1. Schematic of hormone-treatment schedule. All animals were ovariectomized (OVX). For the kainic acid seizure experiment, animals received injections of 10 μg estradiol benzoate (E) or oil vehicle (O) on days 3 and 4 after OVX. For the flurothyl seizure experiment, animals received E or O as above and an injection of 500 μg progesterone (P) or oil vehicle (O) 5 h before seizure testing. In each experiment, seizure testing was done on day 6 after ovariectomy.

Flurothyl seizure testing

On the morning of day 6 after ovariectomy, animals were coded before any further procedure; the code was not broken until all analysis was complete. Animals were weighed and then transferred individually into a 13 × 9 × 7-inch plexiglass testing chamber. Liquid flurothyl was pumped at a constant rate of 20 μl/min onto a filter pad suspended at the top of the chamber. Flurothyl vaporized from the filter pad to fill the testing chamber. An observer recorded the latencies to each of three stages of seizure-related behaviors: (a) first myoclonic jerk (MJ); (b) forelimb clonus (FLC); and (c) wild running and bouncing (R/B). Latencies to each seizure stage were analyzed statistically by using repeated measures ANOVA followed by Fisher's LSD post hoc comparisons.

RESULTS

Kainic acid–induced seizures

Of the 44 animals injected with KA, the majority developed stereotypical behavioral seizures. Eighteen of 22 OVX + O and 15 of 22 OVX + E animals showed at least stage 2 (WDS) or 3 (HW/Ch) behaviors. Of those animals that did show behavioral seizure activity, a slightly greater proportion of OVX + E animals (53%) than OVX + O (44%) experienced full tonic extension, and only OVX + E animals (14%) died as a result of their seizures. Analysis using the χ^2 test indicated that none of these differences was statistically significant.

Treatment of ovariectomized rats with estradiol significantly decreased latencies to KA-induced behavioral seizure activity (Fig. 2). Statistical analysis of latencies

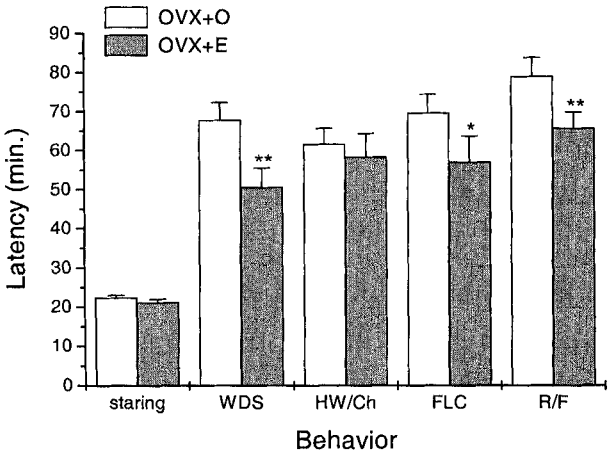

FIG. 2. Estradiol decreases latencies to kainic acid–induced behaviors. Bar graphs depict mean latencies for each of five behaviors in ovariectomized animals treated with oil vehicle (OVX + O; white bars) or estradiol benzoate (OVX + E; light gray bars). Behaviors were as follows: staring, wet-dog shakes (WDS), head waving and chewing (HW/Ch), forelimb clonus (FLC), and rearing and falling (R/F). Latencies to WDS, FLC, and R/F were significantly reduced in OVX + E compared with OVX + O animals. *p < 0.05; **p < 0.01.

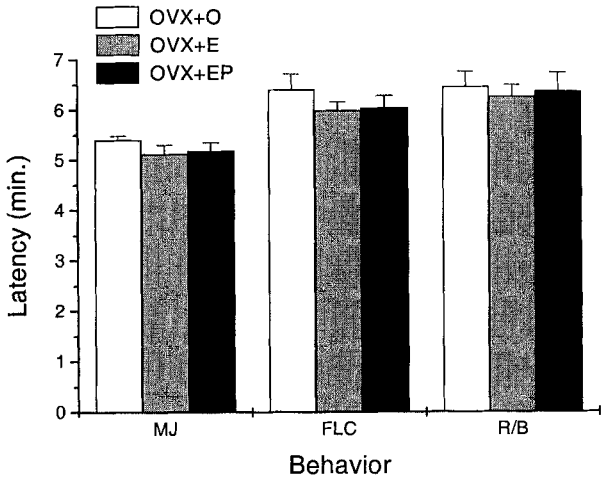

FIG. 3. Neither estradiol nor estradiol plus progesterone had any effect on latencies to flurothyl-induced behaviors. Bar graphs depict mean latencies for each of three behaviors in ovariectomized animals treated with oil vehicle (OVX + O; white bars), estradiol benzoate alone (OVX + E; light gray bars), or estradiol benzoate followed by progesterone (OVX + EP; dark gray bars). Behaviors were first myoclonic jerk (MJ), forelimb clonus (FLC), and running and bouncing (R/B).

to seizure-related behaviors using repeated measures ANOVA showed that, among animals that experienced stage 2 (WDS) or 3 (HW/Ch), there was a significant overall effect of estradiol treatment ($F_{1, 32} = 7.82$, $p < 0.01$). Post hoc analyses demonstrated that estrogen treatment significantly decreased the latency to stage 2 (WDS) by 25% ($p < 0.01$), to stage 4 (FLC) by 18% ($p < 0.05$), and to stage 5 (R/F) by 17% ($p < 0.01$). Mean latencies to stage 1 (staring) and stage 3 (HW/Ch) were essentially unchanged by estrogen treatment ($p > 0.05$).

Flurothyl-induced seizures

All the animals exposed to flurothyl developed stereotypical behavioral seizures. However, in contrast to KA-induced seizures, flurothyl-induced seizures in ovariectomized rats were unaffected by treatment with estradiol or estradiol followed by progesterone (Fig. 3). Statistical analysis of latencies to seizure-related behaviors using repeated measures ANOVA indicated no significant effect of treatment with estradiol or estradiol followed by progesterone ($F_{2, 50} = 0.59$, $p > 0.05$).

DISCUSSION

The results of this study demonstrate that treatment of adult ovariectomized female rats with the same estradiol treatment regimen previously shown to enhance NMDA receptor–mediated synaptic function in the hippocampus facilitates KA-induced behavioral seizures, but has no effect on behavioral seizures induced by flurothyl. The specificity for an estradiol effect on KA seizures suggests that estradiol facilitation of seizure susceptibility is not a result of a general increase in neuronal excitability

throughout the brain, but rather reflects seizure-promoting changes within the limbic system. These data set the stage for understanding how hormone-induced changes in the excitatory synaptic connectivity of limbic structures facilitates behavioral seizure activity.

Animal studies of estradiol and seizure susceptibility

A number of animal studies have examined the effects of estradiol on susceptibility to seizure. Several studies report seizure-facilitating effects of estradiol (5–10), others report no effect (11, during the estrous cycle, 12), and another reports a protective effect against seizure (13). Undoubtedly, the majority of these differences arise because various experimental seizure models involve various brain systems that are differentially sensitive to hormonal modulation. As such, the variations between studies may be instructive in understanding how ovarian hormones regulate seizure susceptibility.

Additionally, however, different studies also have used various routes of hormone administration, doses, durations of treatment, and delays between treatment and seizure testing (5,7–10). For example, Nicoletti et al. (10) reported that estradiol increases susceptibility to KA-induced seizures. However, comparison of this finding with estradiol-induced changes in hippocampal excitatory connectivity is not possible since Nicoletti et al. treated gonadally intact rats with hormone for 10 days and tested seizure susceptibility only 12 h after the last treatment. Thus, in this and other (e.g., 9) cases, seizure testing was performed at a time when estradiol levels were likely to be elevated, making it difficult to distinguish between effects of estradiol that depend on presence of hormone and those that persist following hormone removal.

Kainate versus flurothyl induced seizures

Systemic administration of KA produces a series of stereotyped behaviors that are characteristic of seizures with limbic origin (24). Electrographic, metabolic, and gene-expression studies have each implicated the hippocampus as a limbic region that is key in the early stages of KA-induced seizure activity. The first stages of KA-induced behavior consist of episodes of staring and immobility followed by wet-dog shakes and head waving. These behaviors are mirrored by localized paroxysmal discharge in the hippocampus (21,22) as well as increased activation of hippocampal neurons, as determined by 2-deoxyglucose mapping (22) and induction of c-Fos immunoreactivity (25–27). Subsequent seizure behaviors include forelimb clonus and rearing/falling, which are characteristic of seizures produced by electrical stimulation of limbic areas (24,28). These behaviors are paralleled by abnormal electrographic activity initially in limbic areas beyond the hippocampus (e.g., amygdala) and later spreading to the limbic cortex (21,24). Metabolic and gene-expression changes generally concur with the results of electrographic analyses in that a more widespread pattern of labeling is associated with later seizure stages (21–23,25–27).

The pattern of behavioral seizure activity that results from inhalation of flurothyl is quite different from that produced by KA. Exposure to flurothyl results in generalized motor seizures that begin with a series of myoclonic jerks followed by a tonic–clonic seizure that progresses to wild running and bouncing. The clonic phase of flurothyl-induced seizures is characteristic of "forebrain seizures," whereas the tonic phase is characteristic of "brainstem seizures." These broad classes of seizure activity are defined primarily by the types of seizure behavior that can be elicited after a precollicular transection, which separates the forebrain from the brainstem (29,30). Studies of seizure-induced c-Fos expression have shown distinct patterns of neuronal activation after clonic (i.e., forebrain) versus tonic (i.e., brainstem) flurothyl seizures (31). Expression of c-Fos indicates that the hippocampus is minimally activated by a single brief flurothyl exposure, as was used in this study (32).

Estradiol treatment differentially affects KA- versus flurothyl-induced seizure activity. Estradiol does not affect early KA-induced staring and immobility, but does facilitate progression into subsequent wet-dog shakes, and later, into forelimb clonus and rearing/falling. Interestingly, however, estradiol did not affect the latency to head waving/chewing. Wet-dog shakes are an indicator of hippocampal activity in that they can be produced by hippocampal stimulation (e.g., 33) and occur in association with hippocampal afterdischarge (21,34). Thus the decreased latency to wet-dog shakes in estradiol-treated animals may reflect changes in the hippocampus. However, the observation that estradiol did not affect the latency to head waving and chewing, which are characteristic of limbic seizures (23,24), shows that at least one measure of limbic seizure susceptibility is not affected by estradiol. The development of forelimb clonus and rearing/falling indicates the progression of KA-induced seizure activity to cortical areas (21,24). The latencies to both of these seizure stages were shortened in estradiol-treated animals, suggesting that estradiol facilitates the generalization of seizure activity from limbic to other brain areas.

In contrast to its effects on KA-induced seizure activity, estradiol has no effect on latencies to any phases of flurothyl-induced seizure behavior (present study, 12). Together, the data presented here suggest that the seizure-facilitating effects of estradiol do not result from a generalized upregulation of neuronal excitability throughout the brain, but rather reflect a more specific effect on circuitry activated by KA.

The magnitude of the effects of estradiol on latencies to KA-induced seizure-related behaviors shown here is modest (17–25%). However, it should be noted that these

are effects that persist ≥48 h after brief estradiol treatment. Previous studies of estradiol-induced seizure facilitation have used longer estradiol exposure and continued treatment up to the point of seizure testing. Such studies reported changes of only slightly greater magnitude (e.g., 27% in ref. 9) than were observed here.

Effects of estradiol on hippocampal circuitry

Estradiol has a number of effects on hippocampal circuitry that could participate in facilitation of KA-induced seizure activity. The principal excitatory neurons in the hippocampus are densely covered with dendritic spines, which are the sites of >90% of excitatory synapses on these cells (33). My colleagues and I have previously shown that the same estradiol treatment paradigm here shown to increase KA seizure susceptibility also increases the number and density of dendritic spines (19,34) and axospinous synapses (17) on hippocampal CA1 pyramidal cells, a major output cell of the hippocampus. Serial electron-microscopic studies (35) show that estrogen treatment also alters the configuration of excitatory synaptic input to CA1 pyramidal cells. These data indicate that estradiol treatment increases the number of postsynaptic spines that are synaptically coupled to single presynaptic boutons, a change that might facilitate synchronization of synaptically driven CA1 pyramidal cell activity.

Structural changes in hippocampal synaptic connectivity induced by estradiol are paralleled by physiological changes in glutamatergic synaptic function. Estradiol treatment increases both receptor binding (18,19) and immunofluorescence (20) for the NMDA subtype of glutamate receptor, whereas non-NMDA receptors appear not to be affected (18,19). Electrophysiological studies show that estradiol treatment increases sensitivity of CA1 pyramidal cells to NMDA, but not non-NMDA receptor–mediated synaptic input (19), as well as prolongs excitatory postsynaptic potentials and increases repetitive firing in these cells (36). Because the development of KA-induced seizures involves NMDA-receptor activity (37,38), it is possible that enhanced NMDA-receptor function contributes to estrogen's facilatatory effect on KA-induced seizures.

Estradiol and human epilepsy

One of the most puzzling aspects of epilepsy is the unpredictable occurrence of seizures. Several studies have suggested that seizures do not occur randomly but tend to cluster in time (e.g., 39). One hypothesis to explain nonrandom occurrence of seizure clusters is that certain naturally occurring factors predispose brain circuitry to hyperexcitability and abnormal synchronous activity associated with seizure. For the approximately one third of women with epilepsy who have a catamenial seizure pattern, one such factor may be fluctuation in levels of estradiol and progesterone during the menstrual cycle. Given the pivotal role of the hippocampus in the generation and propagation of seizure activity, one might expect such seizure-predisposing factors to affect hippocampal circuitry. Previous studies have shown that estradiol increases the sensitivity of hippocampal neurons to excitatory synaptic input and might therefore promote the propagation of epileptiform activity through the hippocampus and out to other brain areas. Although it is currently unknown to what extent estradiol's effects on the hippocampus may facilitate limbic seizure activity, the findings reported here set the stage for exploring the possibility of a causal relationship between estrogen-induced structural and functional changes in hippocampal circuitry and susceptibility to seizure.

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