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RESEARCH ARTICLE

Progesterone receptor activation regulates seizure susceptibility

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Abstract

Objective: Progesterone is a potent neuromodulator that exerts effects on the brain through neurosteroids, progesterone receptors (PRs), and other molecules. Whether PR activation regulates seizures is not known. We determined whether PR activation increased seizure susceptibility. **Methods**: Adult female rats that developed epilepsy following lithium-pilocarpine-induced status epilepticus (SE) were used. Seizures were recorded by continuous-video EEG and read by an individual blinded to the treatment of the animals. The animals were treated for a week with progesterone (50 mg/kg per day), and the effect of progesterone withdrawal on seizure frequency was assessed during the subsequent week. During the week of progesterone treatment, the animals were treated with PR antagonist RU-486 (10 mg/kg per day) or a vehicle control, which was administered 30 min before progesterone. In another set of animals, we determined the effect of the PR agonist Nestorone (3 mg/kg per day) on seizure frequency. The animals were treated with Nestorone or vehicle for a week, and seizure frequencies at baseline and during the treatment week were compared. **Results**: Progesterone withdrawal induced twofold increase in seizures in 57% of animals (n = 14). RU-486 treatment in combination with progesterone, prevented this increase, and a smaller fraction of animals (17%) experienced withdrawal seizures (n = 13). The specific activation of PRs by Nestorone also increased the seizure frequency. Forty-six percent (n = 14) of Nestorone-treated animals experienced at least a 50% increase in seizures compared to only 9% of the vehicle-treated animals (n = 11). **Interpretation**: PR activation increased seizure frequency in epileptic animals. Thus, PRs may be novel targets for treating catamenial epilepsy.

Introduction

Approximately thirty percent of women with epilepsy who are of reproductive age experience menstrual cycle-linked seizure exacerbations called catamenial epilepsy.[1,2] These seizures are linked to fluctuations in the levels of estrogen and progesterone during the menstrual cycle; the levels of these hormones are low during the perimenstrual period, a time that is associated with increased seizure frequency in a majority of women with catamenial epilepsy. Catamenial seizures represent the most prevalent drug-refractory seizures in women, and currently, there are no effective strategies that have been tested in phase III clinical trials to treat these seizures.[3] In the NIH progesterone clinical trial, progesterone treatment did not differ from placebo treatment in seizure reduction in either the catamenial or noncatamenial patients, except in women with severe perimenstrual seizure exacerbation.[3] A prespecified binary logistic regression analysis identified C1 and C2 patterns as predictors of progesterone responders; however, the secondary analysis requires formal confirmation. Thus, prevailing hypothesis needs further analysis and testing.

We found previously that RU-486 treatment reduced neurosteroid withdrawal-induced seizures in an animal model.[4] Since RU-486 is a potent progesterone receptor

(PR) antagonist, this previous study raised the possibility that PR activation regulates seizure frequency. We also found that progesterone upregulated AMPA receptor expression via PR activation, which may increase excitability. These findings advocate for an in-depth investigation of progesterone-activated cellular signaling separate from neurosteroids, which may also regulate seizures. Our previous studies suggest that PR-mediated effects counteract the anticonvulsant effects mediated through allopregnanolone. However, PR regulation of seizure susceptibility has not been studied in depth.[5,6]

PRs are classic, nuclear-hormone receptors that mediate the genomic effects of progesterone.[7] There are two isoforms of PR, PR-A, and PR-B, which are widely expressed in the brain, including areas involved in generating and propagating seizures, such as the hippocampus, amygdala, and neocortex.[8–10] Upon binding with progesterone, PRs dissociate from heat shock proteins, translocate to the nucleus, and trigger the expression of target genes that contain a progesterone response element in their promoter region.[11,12].

The existing animal model of catamenial epilepsy, which we used in our prior study, involved treating animals with the Pregnant Mare Serum Gonadotropin (PMSG) and human chorionic gonadotropin (β-HCG) hormones to increase serum progesterone levels.[4] However, these hormones, particularly β-HCG, may exert additional effects due to their structural similarity to luteinizing hormone.[13] Seizure exacerbation was also triggered by finasteride treatment, which causes neurosteroid withdrawal rather than progesterone withdrawal.[4] Since finasteride treatment increases progesterone levels, it may also exert additional effects. To overcome these limitations, we developed a model of progesterone-withdrawal-induced seizures in which animals were treated with progesterone for a week and the effect of withdrawal was assessed in the subsequent week.

We posit that PR activation increases seizure susceptibility. However, testing this hypothesis has been hampered because the most commonly used anti-progestin, RU-486, also blocks glucocorticoid receptors (GRs).[14] A specific, high-affinity PR agonist, Nestorone, is available. Nestorone has a 10-fold higher affinity for PRs than progesterone, and it neither activates nor blocks GR activity.[15] Furthermore, metabolites of Nestorone do not affect GABAR function.[16] We determined whether the direct activation of PRs by Nestorone increased seizure frequency.

Materials and Methods

All animals were handled according to a protocol approved by the University of Virginia Animal Care and Use Committee, and efforts were made to minimize animal stress and discomfort. Progesterone, RU-486, Nestorone, and β-hydroxy-cyclodextrin were purchased from Sigma-Aldrich. All the experiments were performed in a blinded manner, and the researcher who read the EEG was blinded to the treatment received by the animals.

Induction of TLE and EEG recordings

Status epilepticus (SE) was induced in adult Sprague-Dawley female rats (200–220 g) using the lithium-pilocarpine method as described previously.[4,17] Thirty days after SE, animals were implanted with bilateral subdural cortical electrodes, a bipolar left hippocampal electrode, and a cerebellar reference electrode.[4] Following seven days of recovery from surgery, continuous video-EEG monitoring was started and performed as described in our earlier studies.[4] Seizures are characterized by temporally evolving high frequency, high amplitude rhythmic discharges followed by a brief suppression of EEG and various criteria have been used to define seizures in the past.[18,19] We have consolidated these criteria and define electrographic seizures as high frequency (>2 Hz) rhythmic spike wave discharges with amplitudes at least three times that of the baseline EEG and lasting 15 sec or longer. The seizures were manually identified. The duration of the seizures was measured from the initial high amplitude spike to the last spike that was at least twice the baseline EEG amplitude and was not followed by another spike for at least 2 sec. The behavior associated with electrographic seizures was scored on a modified Racine scale.[20]

Progesterone treatment and withdrawal in epileptic animals

The animals were monitored for 14 days to determine their basal seizure frequency and divided into two groups (vehicle and RU-486 treated groups, see below), such that the seizure frequencies were balanced.[4] One group of animals received injections of PR antagonist RU-486 (10 mg/ kg per day, i.p.), and the other group was treated with the vehicle (20% β-hydroxy-cyclodextrin, i.p.). Progesterone (50 mg/ kg per day, i. p.) was administered to all animals 30 min after RU-486 or vehicle injection. The doses of RU-486 and progesterone were identical to those used in our previous study.[4] These treatments were repeated daily for one week between 10 AM and 11 AM (lights on 6 AM). Animal monitoring was continued for another week without progesterone or RU-486 treatment to determine the effect of progesterone withdrawal. A doubling of seizures during particular phases of the menstrual cycle compared to other phases is used as a criterion for diagnosing catamenial epilepsy.[21,22] We used a similar criterion to distinguish animals which experienced seizure exacerbation during the withdrawal week from those that did not.

Nestorone treatment of epileptic animals

After monitoring animals for 14 days to determine the baseline seizure frequency, the animals were divided into two groups; the first group of animals received daily injections of Nestorone (3 mg/ kg in 20% β-hydroxy-cyclodextrin, subcutaneous) for one week, and the other group received vehicle injections. The drugs were administered as described above. The dose of Nestorone was selected based on its blood and brain pharmacokinetics reported in previous studies.[15,16,23] Since Nestorone treatment causes PR activation but not neurosteroid withdrawal, a lower threshold of a 50% increase in seizure frequency during the week of treatment was set to define seizure exacerbation.

Detection of hormone levels

Sera samples were collected via tail vein before the start of treatment and after a week of daily hormone treatment. The levels of progesterone, estrogen, and testosterone were measured using ELISA assays at University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core as described before.[4] For the progesterone assay, an ELISA kit (Immunobiological Laboratories Inc. #IB79105) with an assay range of 0.3–40 ng/mL and sensitivity of 0.045 ng/mL was used. Similarly, an ELISA kit with a detection range of 0.2 to 16 ng/mL and sensitivity of 0.083 ng/mL (Immunobiological Laboratories Inc. #IBL IB79106) was used for measuring testosterone levels. Estradiol levels were measured using an ELISA kit from Calbiotech (#ES180S-100), which has an assay range of 3–300 pg/mL and a sensitivity of 3 pg/mL.

Statistical analysis

The fraction of animals that experienced seizure exacerbation between the drug and vehicle treatment groups were compared using the Fisher's exact test. The seizure frequencies had non-normal distribution and were compared using one-sided Wilcoxon matched-pairs signed rank test. The values were considered significant at $P < 0.05$.

Results

RU-486 blocked the progesterone withdrawal-induced increase in seizure frequency.

We treated epileptic animals with progesterone and RU 486 (P + RU486, 50 mg/ kg per day and 10 mg/ kg per day, respectively, i. p.) or progesterone and vehicle for one week as illustrated in Fig. 1A. A cohort of 27 epileptic animals was monitored for 2 weeks to determine the baseline seizure frequency; then, they were divided into 2 groups, with 14 animals in the progesterone + vehicle treatment group and 13 animals in the progesterone + RU486 treatment group, such that the seizure frequency was balanced. All recurrent spontaneous seizures that were recorded from hippocampal electrodes, irrespective of the associated behavior, were counted. Because there were variations in the daily seizure frequency, we summed the seizures in each animal during the baseline week. The average weekly seizure frequency in the animals assigned to P + vehicle was 30 ± 9 seizures (n = 14) and that in the animals assigned to P + RU-486 group was 41 ± 16 ($n = 13$, $P = 0.9$, Mann-Whitney test). Seizure frequency did not change in either group during the week of progesterone treatment (data not shown). However, during the week of withdrawal, the two groups appeared to differ. Based on the number of seizures experienced by these animals during the withdrawal week compared to that during the baseline week, the animals were divided into two groups: those that experienced seizure exacerbation (at least twice as many seizures during the withdrawal week as the baseline week) and those that did not experience seizure exacerbation. Many P + vehicle-treated animals experienced seizure exacerbation compared to the animals treated with P + RU-486 (Fig. 1C, Table 1). Fifty-seven percent of the P + vehicle-treated animals experienced at least a doubling of seizures during the withdrawal week compared to only 17% of animals in the P + RU-486 treatment group ($P = 0.046$, Fisher's exact test, Fig. 1D).

We also compared the seizures experienced by animals in each group during the baseline week and withdrawal week (Fig. 1E). In the P + vehicle treatment group, the weekly seizure frequency increased from 30 ± 9 during baseline to 57 ± 16 during withdrawal ($n = 14$, $P = 0.030$, one-tailed Wilcoxon matched-pairs signed rank test). In contrast, the seizure frequency in the P + RU-486 treatment group remained unchanged throughout the experiment (Fig. 1E, withdrawal: 43 ± 13 and baseline 41 ± 16, $n = 13$, $P = 0.39$, one-tailed Wilcoxon matched-pairs signed rank test). Thus, RU-486 treatment reduced progesterone withdrawal-induced seizures.

The worsening of seizures following progesterone withdrawal could also manifest as prolongation of seizure duration and/or expression of intense behavioral seizures. However, progeserone withdrawal in neither RU-486-treated nor vehicle-treated animals affected the duration of electrographic seizures (Fig. 1F). Furthermore, seizure-associated behavior was scored on a Racine scale and the highest score for individual seizure was determined. Progesterone withdrawal did not cause intense behavioral seizures in either of the treatment groups (data not shown).

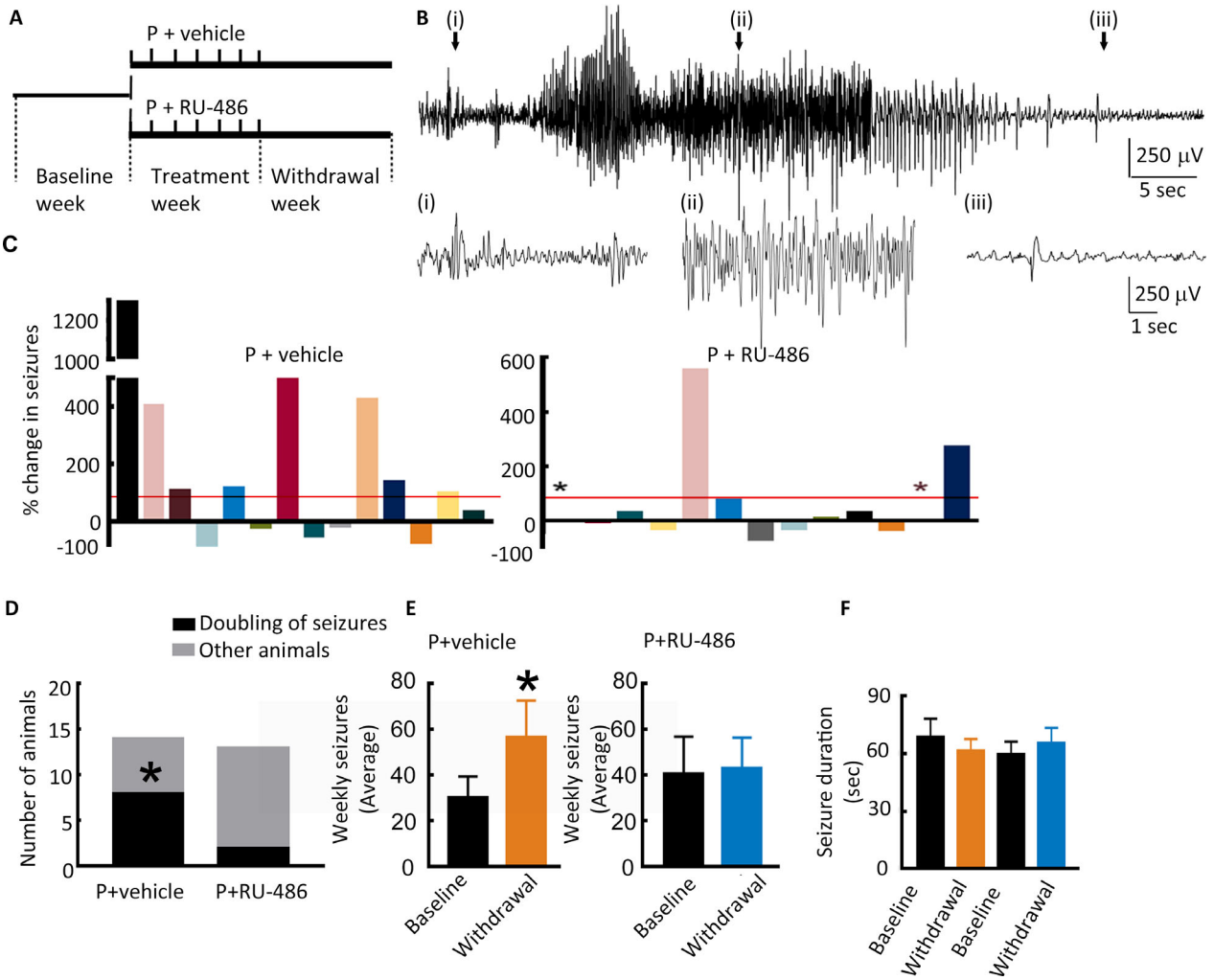

Figure 1. RU-486 treatment prevents progesterone withdrawal-induced seizures. (A) Schematic of the experiment. The three weeks of the experiment—baseline, treatment, and withdrawal—are illustrated. During the treatment week, one group of animals received daily injections of RU-486 (10 mg/ kg, i. p.) 30 min before progesterone treatment (50 mg/ kg, i. p.); the other group of animals received injections of the vehicle (20% β-hydroxy-cyclodextrin) followed by progesterone treatment (P). (B) A representative recurrent spontaneous seizure. Traces (i), (ii), and (iii) illustrate magnified areas marked by arrows. (C) Seizures recorded during the week of withdrawal are expressed as the percentage of change over the baseline seizure frequency in individual animals. Each column represents one animal in that group. The dotted line represents a 100% increase in seizure frequency. Please note that in one animal, the seizures increased from 1 during the baseline week to 14 during the week of withdrawal, which was a 1300% increase over the baseline. The columns marked by stars represent animals in which the change in seizure frequency was close to 0%. (D) The percentage of animals that experienced at least a doubling of seizures (100% increase) in seizures during the withdrawal week compared to the baseline week (n = 14 for progesterone and 13 for the P + RU-486 group, *P = 0.046, Fisher's exact test). (E) Weekly seizure frequency in the two groups of animals during baseline and withdrawal weeks (n = 14 in the P + vehicle group and n = 13 in the P + RU-486 treatment group, *P = 030, one-tailed Wilcoxon matched-pairs signed rank test). (F) Duration of the seizures recorded during the baseline and withdrawal weeks.

Thus, progesterone withdrawal increased seizure frequency but it did not cause prolongation of seizures or intensify associated behavior.

Specific activation of PRs increased seizure frequency

Since RU-486 also blocks glucocorticoid receptors (GRs), the suppression of seizures in RU-486-treated animals could have involved the antagonism of PRs and/or GRs. To assess the role of PRs, we used a specific synthetic progestin, Nestorone. Epilepsy was induced in the animals, and baseline seizure frequency was monitored; then, the animals were divided into two groups, such that their basal seizure frequency was balanced. The animals in the Nestorone treatment group experienced 31 ± 9 seizures during the baseline week (n = 14),

Table 1. Weekly seizure frequency of progesterone and vehicle or RU-486-treated animals.

Treatment→Animals ↓	P + vehicle Baseline week	P + vehicle Withdrawal week	P + RU-486 Baseline week	P + RU-486 Withdrawal week
Animal 1	1	14*	2	2
Animal 2	2	15*	75	72
Animal 3	71	160*	9	9
Animal 4	25	4	70	48
Animal 5	33	73*	11	72*
Animal 6	119	120	4	7
Animal 7	28	171*	8	2
Animal 8	4	2	190	136
Animal 9	72	63	120	130
Animal 10	12	63*	21	27
Animal 11	38	91*	9	6
Animal 12	7	2	1	1
Animal 13	4	8*	14	52*
Animal 14	6	8		

Total seizures experienced by each animal during the baseline week and withdrawal week. Animals in which seizure exacerbation (100% or more increase in seizures over the baseline frequency) was observed during the withdrawal week are marked by stars.

whereas those assigned to the vehicle treatment group experienced 40 ± 17 (n = 11) seizures during the baseline week (P = 0.76, Mann-Whitney test). The animals in one group were treated daily with Nestorone (3 mg/kg), and those in the other group received vehicle (Fig. 2A). We compared whether the seizure frequency changed during the week of treatment.

Many Nestorone-treated animals had more seizures during the treatment week (Fig. 2B, Table 2). Since Nestorone treatment is expected to activate PRs but not cause neurosteroid withdrawal, a lower threshold (50%) was set to differentiate animals that experienced seizure exacerbation from those that did not. Half of the animals in the Nestorone treatment group experienced at least a 50% increase in seizure frequency. In contrast, only 9% of vehicle-treated animals experienced more seizures (Fig. 2C, P = 0.042, Fisher's exact test). There was substantial variability in the seizure frequency in these animals; the increase in seizure frequency due to Nestorone treatment ranged from 10% to 1100% (Fig. 2B). However, a pair-wise comparison of weekly seizures in each animal revealed that Nestorone treated animals experienced more seizures compared to seizure frequency at baseline (Fig. 2D). The average weekly seizure frequency before Nestorone treatment was 31 ± 9, which increased to 42 ± 12 after treatment (n = 14, P = 0.025, one-tailed Wilcoxon matched-pairs signed rank test). In contrast, the weekly seizure frequency before the start of vehicle treatment was 40 ± 17, and it was 35 ± 19 during the week of vehicle treatment (n = 11, P = 0.31, one-tailed Wilcoxon matched-pairs signed rank test). Nestorone treatment did not affect the duration of seizures (Fig. 2E).

Estrous cyclicity and hormone levels in the epileptic animals

Recurrent spontaneous seizures are known to affect estrous cycles in epileptic animals.[4,24] In agreement with the prior studies, animals used in these experiments also had irregular cycles. We monitored a cohort of 10 epileptic animals for estrous cycle. The length of the estrous cycle ranges from 4 to 5 days in naïve animals; in contrast, 60% of epileptic animals in these studies stayed in a persistent diestrus stage. In 3 animals, the vaginal cytology consisted of cornified cells and leukocytes, and this stage persisted for 4 to 5 days before transitioning to the subsequent stage; a normal transition of vaginal cytology was observed in only one animal. Thus, the estrous cycles were affected in a majority of epileptic animals, which is similar to observations from our prior study.[4]

Since progesterone and Nestorone treatment may alter the levels of circulating reproductive hormones, which may affect seizure frequency, we measured the hormone levels before the start of treatment and at the end of 7 days of treatment. Progesterone treatment did not affect circulating estrogen levels (Fig. 3B); however, testosterone levels were elevated in these animals (Fig. 3C), likely due to enzymatic conversion of progesterone to testosterone. In contrast, Nestorone treatment did not affect the levels of progesterone, estrogen, or testosterone (Fig. 3A–C).

Discussion

The major findings of this study are that RU-486 suppresses progesterone withdrawal-induced seizures, and specific activation of PRs using Nestorone increases

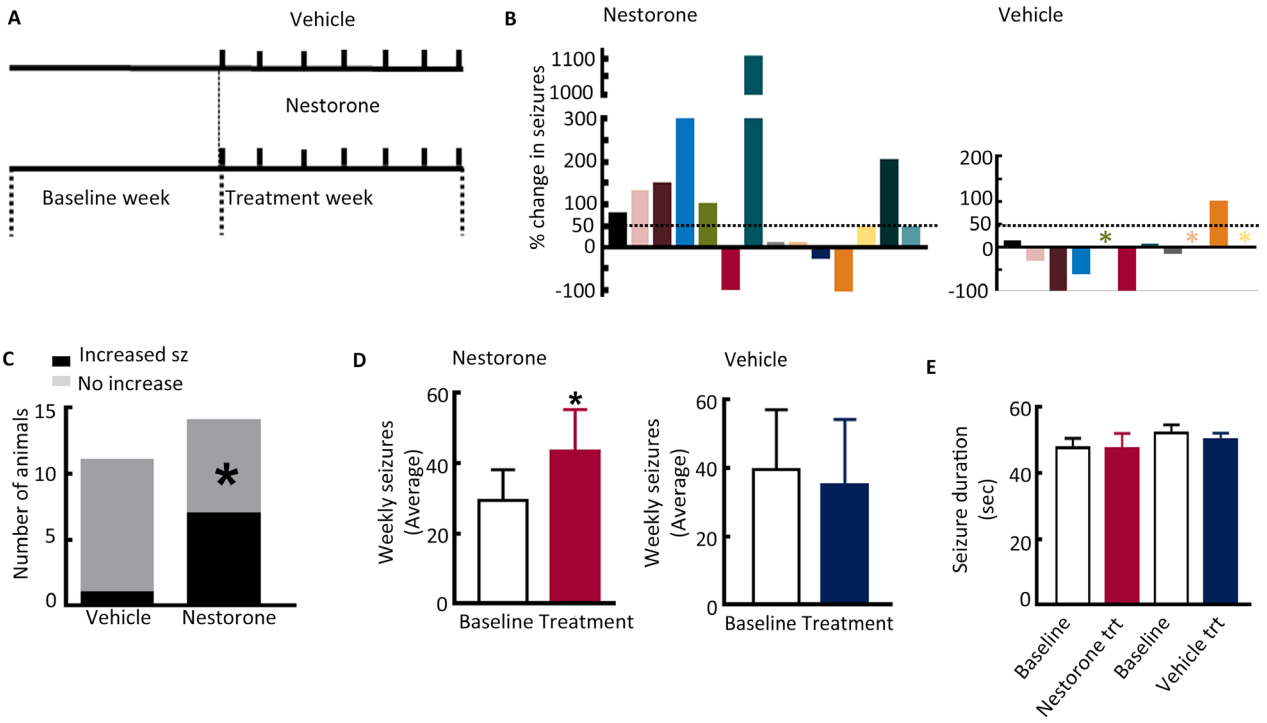

Figure 2. Specific activation of PRs by Nestorone increased seizure frequency. (A) The experimental schematic showing recording during the baseline week and the week of Nestorone (3 mg/kg per day, subcutaneous) or vehicle treatment (20% β-hydroxy-cyclodextrin). (B) Change in seizure frequency in each animal over baseline seizure frequency. Each column represents individual animal in that group. The dotted line represents a 50% increase in seizure frequency. The green, orange, and yellow stars in the vehicle treatment group indicate animals in which the increase in seizure frequency was close to 0. Please note that in one animal in the Nestorone group, seizures increased from 1 during the baseline week to 12 during the treatment week, which was a 1100% increase. (C) The percentage of animals that experienced at least a 50% increase in weekly seizures in the Nestorone and vehicle treatment groups; the number of replicates is the same as that given in B (*P = 0.042, Fisher's exact test). (D) Increase in seizure frequency over the baseline during the treatment week in the two groups (n = 14 Nestorone-treated animals and n = 11 in vehicle-treated animals, *P = 0.025, one-tailed Wilcoxon matched-pairs signed rank test). (E) Duration of seizures recorded during the baseline and treatment weeks from the Nestorone and vehicle-treated animals shown in panel B.

seizure frequency in epileptic animals. We propose that PRs modulate seizure susceptibility.

This study revealed a novel role of PRs in regulating seizure susceptibility. Catamenial seizures are common in women with temporal lobe foci,[25] and progesterone had limited efficacy in suppressing these seizures in a phase III clinical trial.[3] We have found that PR activation increases the AMPAR-mediated glutamatergic transmission on CA1 pyramidal neurons and upregulates AMPAR expression in the hippocampus.[4] Synaptic strength is directly correlated with synchronization;[26,27] thus, the increased AMPAR-mediated neurotransmission could boost excitability and contribute to the increase in seizure frequency observed in Nestorone-treated animals. These findings also identify PRs as novel potential therapeutic targets in catamenial epilepsy.

We have developed a novel model of catamenial seizure exacerbation; this model depends on progesterone treatment in contrast to previous model of catamenial epilepsy in which activation of luteinizing hormone (LH) receptors with gonadotropins was used to increase progesterone levels.[4,17,28] However, the use of gonadotropins to elevate progesterone levels could have additional effects. β-HCG is a potent and long-acting agonist of LH receptors, which are widely expressed in the brain.[29] LH receptor activation stimulates protein kinase A, Akt, MAP kinases, and mTOR signaling, which could alter neuronal activity.[30,31] Thus, treatment with gonadotropins could have influenced excitability via effects mediated through mechanisms independent of progesterone. In addition, β-HCG also increases testosterone production by the ovaries [13] and may activate androgen receptors, which are also expressed in the female brain.[32,33] Furthermore, the earlier model also involved treatment with finasteride, which blocks the activity of enzyme 5α-reductase and causes allopregnanolone depletion coupled with increase in progesterone levels.[17,28]

This model does not fully replicate perimenstrual withdrawal which occurs endogenously in women. The daily intraperitoneal progesterone administration is likely to

Table 2. Weekly seizure frequency of Nestorone and vehicle-treated animals.

Treatment→ Animals ↓	Nestorone Baseline week	Nestorone treatment week	Vehicle Baseline week	Vehicle treatment week
Animal 1	40	71*	116	131
Animal 2	11	25*	25	18
Animal 3	26	64*	1	0
Animal 4	15	64*	43	18
Animal 5	4	8*	7	7
Animal 6	42	2	45	0
Animal 7	1	12*	180	189
Animal 8	19	21	8	7
Animal 9	136	149	6	6
Animal 10	20	15	3	8*
Animal 11	9	0	2	2
Animal 12	65	95		
Animal 13	3	9*		
Animal 14	42	61		

Total seizures experienced by each animal during the baseline and the treatment week. Animals in which seizure exacerbation (50% or more increase in seizures over the baseline frequency) was observed during the treatment week are marked by stars.

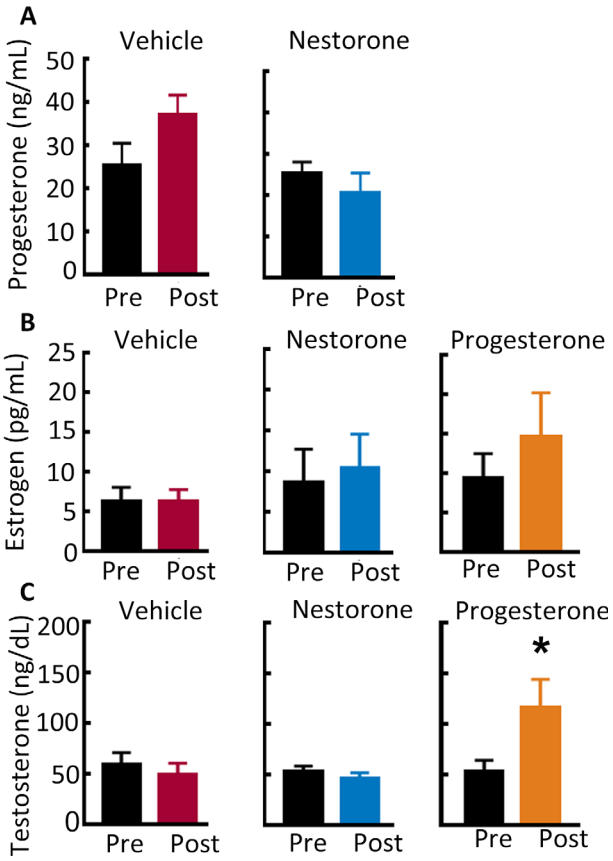

Figure 3. Hormone treatment did not have a major impact on circulating steroid hormones. (A) Levels of circulating progesterone in epileptic animals before and after a week of Nestorone treatment (3 mg/kg per day) for a week (n = 5 in each group). (B) Circulating estrogen levels in the animals treated with vehicle, Nestorone, or progesterone (50 mg/kg) daily for a week (n = same as in panel A). (C) Serum testosterone levels in the vehicle and hormone treated animals (n = same as in panel A, *P = 0.034 paired t-test)

cause cyclic acute elevation in its levels compared to a slow rise, stable high progesterone levels maintained for a few days followed by slow decline in its levels in women. Furthermore, the rise in progesterone levels follows that in estrogen levels during endogenous cycles. The exogenous administration of progesterone did not replicate this association between estrogen and progesterone. Thus, the estrogen priming, which can occur endogenously, was absent in this model.

A key challenge in understanding drug effects on seizure frequency is the natural variability of seizures. The seizure frequency varied between animals; some animals experienced many seizures every day, some experienced few seizures every day, whereas others experienced seizures in clusters. Currently, it is unclear whether endogenous hormonal fluctuations contributed to this variability. Furthermore, since seizures could alter gene expression, the variability in seizure frequency between animals could be a confounder. To minimize the effect of this variability, pre and post comparisons were done within each animal and animals encompassing all three patterns of seizure occurrence were included in each treatment group.

Progesterone, either alone or in combination with estrogen, is commonly used in contraceptives for women. Since PR activation increases seizure frequency, a further investigation of safety of progestin-only contraceptives is needed for women with epilepsy. Indeed, an epidemiological survey of women with epilepsy revealed that progestin-only contraceptives increase the risk of seizures in women with epilepsy.[34,35] The excitatory effects of PR activation may be linked to the increased risk of seizures in these women.

Previous studies in PR-knockout animals complement the discovery that PR activation increases seizures. The PR-knockout mice kindled more slowly than the wild-type mice, and they also had shorter seizures.[5] Furthermore, the rate of kindling increased in wild-type mice following the abrupt discontinuation of chronic progesterone treatment, and this increase was not observed in PR-knockout mice.[6] Thus, the absence of PR signaling reduced the detrimental effects of progesterone withdrawal. Additionally, in the former study, the PR-knockout animals were hypersensitive to the anticonvulsant effect of progesterone. Kindled PR-knockout and wild-type mice were tested 30 min after

progesterone injection; the precise timing of the triggered seizures may have enabled the investigators to record an enhanced anticonvulsant effect of progesterone in PR-knockout mice.

It is currently unclear whether the seizure suppression observed following progesterone withdrawal in RU-486-treated animals also involves the antagonism of GRs. Stress, which is the most common self-reported seizure-precipitating factor in epilepsy patients,[36,37] increases glucocorticoid levels, which can activate GRs.[14] GRs are abundantly expressed in the cortex and hippocampal tissue of epilepsy patients,[38] and their expression appears to be upregulated in the hippocampi of epilepsy patients.[37] Hence, the potential anti-GR effect of RU-486 can also be utilized if RU-486, which is already in clinical use, is used as a novel drug to treat seizures.

In contrast to the putative proconvulsant effects of PR activation observed here, RU-486 treatment appeared to block the inhibitory effects of progesterone on tetanization-induced ictal-like discharges in hippocampal slices prepared from estrogen-primed ovariectomized female rats.[39] However, these studies were performed *in vitro* and the drugs were acutely applied. RU-486 treatment also seemed to increase the susceptibility of animals in the diestrus stage to kainate-induced status epilepticus;[40] it also seemed to block the anticonvulsant action of progesterone against PTZ-evoked seizures.[41] These studies were performed in non-epileptic animals and it is possible that epilepsy-associated alterations in the neurotransmitter receptor expression, cellular signaling, and neuronal networking contributed to the differences.

In conclusion, this study revealed that RU-486 treatment protects against progesterone withdrawal seizures, and PR activation increases seizure susceptibility. These findings may provide novel avenues of therapeutic intervention for catamenial epilepsy.

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Conflict of Interest

The Authors have no conflict of interest.

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