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A novel therapeutic approach for treatment of catamenial epilepsy

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ABSTRACT

Many women with epilepsy experience perimenstrual seizure exacerbation, referred to as catamenial epilepsy. There is no effective treatment for this condition, proposed to result from withdrawal of neurosteroid-mediated effects of progesterone. A double-blind, multicenter, phase III, clinical trial of catamenial epilepsy has failed to find a beneficial effect of progesterone. The neurosteroid-mediated effects of progesterone have been extensively studied in relation to catamenial epilepsy; however, the effects mediated by progesterone receptor activation have been overlooked. We determined whether progesterone increased excitatory transmission in the hippocampus via activation of progesterone receptors, which may play a role in regulating catamenial seizure exacerbation. In a double-blind study using a rat model of catamenial epilepsy, we found that treatment with RU-486, which blocks progesterone and glucocorticoid receptors, significantly attenuated neurosteroid withdrawal-induced seizures. Furthermore, progesterone treatment as well as endogenous rise in progesterone during estrous cycle increased the expression of GluA1 and GluA2 subunits of AMPA receptors in the hippocampi, and enhanced the AMPA receptor-mediated synaptic transmission of CA1 pyramidal neurons. The progesterone-induced plasticity of AMPA receptors was blocked by RU-486 treatment and progesterone also failed to increase AMPA receptor expression in progesterone receptor knockout mice. These studies demonstrate that progesterone receptor activation regulates AMPA receptor expression and may play a role in catamenial seizure exacerbation.

1. Introduction

Approximately 30% of women of reproductive age with epilepsy experience cyclical exacerbation of seizures related to periodic changes in the serum progesterone and estrogen levels during the menstrual cycle (catamenial epilepsy) and its predominant form is perimenstrual seizure exacerbation (Herzog et al., 2015; Frye, 2008). Currently there are no scientifically-tested effective treatments of catamenial exacerbation. This period of seizure exacerbation coincides with a decline in progesterone levels at the end of the cycle following the mid-luteal peak levels.

Progesterone is a sedative and exerts an anticonvulsant action via conversion to the neurosteroid allopregnanolone, which binds to γ-amino-butyric acid (GABA) type A ($GABA_A$) receptors, and enhances GABA action on the receptor (Reddy and Rogawski, 2012; Joshi et al., 2013). It has long been proposed that perimenstrual seizure exacerbation is related to neurosteroid withdrawal, which would impair GABAergic inhibition. Based on the premise that maintaining high progesterone and allopregnanolone levels during the perimenstrual period would alleviate seizure exacerbation, a large-scale phase 3 clinical trial was conducted. However, in this trial, progesterone replacement therapy was not different than placebo to protect against this exacerbation (Herzog, 2015).

Progesterone also activates the progesterone receptors, isoforms A and B, which are ligand-activated transcription factors encoded by a single gene (Conneely et al., 1987). Upon activation, progesterone receptors localize to the nucleus and regulate gene expression (Mani and Oyola, 2012; Singh and Su, 2013). The mRNA and protein of progesterone receptors are present in the principal neurons of the hippocampus, which is involved in seizure generation and propagation (Guerra-Araiza et al., 2003; Guerra-Araiza et al., 2000). The immunoreactivity of progesterone receptors is present over the soma, axon terminals, and dendritic spines of hippocampal principal neurons (Mitterling et al., 2010). While the allopregnanolone-mediated effects

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; GABAR, γ-aminobutyric acid type A receptor; PMSG, Pregnant meyer's serum gonadotropin; β-HCG, β subunit of human chorionic gonadotropin; TLE, temporal lobe epilepsy

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of progesterone have been extensively characterized, whether progesterone receptor activation also regulates seizures is not known. The mid-luteal rise in progesterone could activate progesterone receptors and increase excitatory neurotransmission. This would be counterbalanced as long as high allopregnanolone levels maintain the GABAergic neurotransmission. However, the excitation/inhibition balance would be affected during perimenstrual period due to the decline in allopregnanolone levels and result in seizure exacerbation.

We tested the hypothesis that progesterone has two effects: an anticonvulsant action through neurosteroids and a slower excitatory action that occurs following the activation of progesterone receptors and worsens seizures. This hypothesis is informed by the homeostatic scaling theory, which states that there is a precise tuning of neuronal excitability and synaptic strength to maintain a neuron's target firing rate (Marder and Goaillard, 2006; Turrigiano, 2008). We tested whether excitatory action contributes to the seizure exacerbation observed during progesterone withdrawal after prolonged treatment (perimenstrual exacerbation).

2. 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. A majority of the experiments were performed on adult Sprague-Dawley female rats (200–220 g) with intact ovaries. In addition, adult female mice (20–25 g) lacking progesterone receptor expression ($PR^{-/-}$) (Hashimoto-Partyka et al., 2006), and C57Bl/6 and wild-type littermates ($PR^{+/+}$) were also used.

2.1. Materials

PMSG, β-HCG, finasteride, nestorone, as well as all the common chemicals were purchased from Sigma-Aldrich. Mouse monoclonal anti-GluA1 subunit (1:1000, clone RH95, Millipore), anti-GluA2 subunit (1:1000, clone 6C4, Millipore), and anti-β-actin antibody (1:5000, clone AC74, Sigma-Aldrich) were used. The specificity of these antibodies was confirmed using JCN criteria (Saper, 2005). HRP-tagged anti-mouse antibody (1:5000) was obtained from BioRad. Hybond-P PVDF membranes were obtained from GE Healthcare.

2.2. Induction of TLE

SE was induced using lithium-pilocarpine method as described before using lithium-pilocarpine (Lawrence et al., 2010). Two weeks after SE, animals were implanted with cortical and hippocampal electrodes and EEG recording was performed as described previously (Lawrence et al., 2010). The animals were monitored by continuous video-EEG recording following a week of recovery and were designated as epileptic after they had experienced at least 2 spontaneous seizures. Electrographic seizures irrespective of the associated behavior were counted to determine seizure frequency.

2.3. Treatment of epileptic animals

The animals were monitored for 14 days to determine the basal seizure frequency. The animals were divided into two groups (vehicle and RU-486 treated groups, see below) such that each group included animals with high frequency, low frequency, and clustered seizures, and they were then treated with PMSG (20 IU in saline, intraperitoneal, ip), followed 48 h later by β-HCG (10 IU in saline, ip) as described previously (Lawrence et al., 2010). All treatments were performed between 9 and 11 AM. During the course of PMSG and β-HCG treatment, progesterone receptors were blocked by a daily injection of RU-486 (10 mg/kg, ip) in half of the animals; the remaining animals were treated daily with vehicle (10% β-cyclodextrin). Neurosteroid (allopregnanolone and tetrahydrodeoxycorticosterone in females and allopregnanolone, tetrahydrodeoxycorticosterone, and androstanediol in males) withdrawal was induced by the administration of finasteride (100 mg/kg in 30% cyclodextrin, ip) on the 8th day of β-HCG injection (Lawrence et al., 2010).

Serum progesterone levels were determined using a Progesterone ELISA kit (Immunobiological Laboratories Inc., USA, #IB79105) (Xiao et al., 2017) at the Reproductive Core Facility of the University of Virginia as described previously (Lawrence et al., 2010). The detection range of the kit was 0.3 to 40 ng/ml and the sensitivity was 0.045 ng/ml. Progesterone standards at different concentrations were run with the experimental samples to confirm that the concentration in the sample is within a detectable range. The samples with progesterone concentrations higher than the detectable range were diluted and re-assayed.

2.4. Treatment of non-epileptic animals

Non-epileptic female animals were also treated with PMSG and β-HCG as above, saline-treated animals were used as controls. In a separate cohort of animals, a single dose of progesterone (50 mg/kg, ip) or vehicle (20% cyclodextrin, ip) was administered, and the experiments were performed 2 days later. Progesterone receptors were blocked by the administration of RU-486 (30 mg/kg). RU-486 or vehicle were administered 30 min before progesterone and one dose the next day. Progesterone receptors were also activated by treatment with a synthetic agonist nestorone (3 mg/kg in 10% β-cyclodextrin, subcutaneous). The animals were administered a single dose of nestorone and the experiments were performed 2 days later; animals treated with vehicle were used as controls.

2.5. Mice lacking progesterone receptor expression

PR floxed mice were kindly provided by Dr. Iruela-Arispe (UCLA, Los Angeles, California) (Hashimoto-Partyka et al., 2006; Stephens et al., 2015; Janzen et al., 2013). These mice were bred with CMV-Cre mice (B6.C-Tg(CMV-cre)1Cgn/J, Jackson laboratories) to generate progesterone receptor knockout mice ($PR^{-/-}$). C57Bl/6 and wild-type littermates were used as controls ($PR^{+/+}$). Adult female mice were monitored for estrous cycle; progesterone (100 mg/kg, ip) or vehicle (20% cyclodextrin) was administered to mice in diestrus and AMPAR expression was determined 24 h later.

2.6. Brain slicing

Acutely isolated hippocampal slices were prepared as described before (Rajasekaran et al., 2012). The animals were anesthetized with halothane and decapitated. The brain was immersed in oxygenated (95% O_2/5% CO_2) ice-cold (2–4 °C) slicing buffer (in mM, 65.5 NaCl, 2 KCl, 5 $MgSO_4$, 25 $NaHCO_3$, 1.1 KH_2PO_4, 1 $CaCl_2$, 10 glucose, and 113 sucrose; 300 mOsm), and horizontal 350-μm slices were prepared using a Vibratome (Leica VT1200S, Germany). The slices were collected in oxygenated artificial CSF (aCSF, containing in mM, 127 NaCl, 2 KCl, 1.5 $MgSO_4$, 25.7 $NaHCO_3$, 10 dextrose, and 1.5 $CaCl_2$; 300 mOsm). The slices were then used for biochemistry or electrophysiology.

2.7. Electrophysiology

AMPAR-mediated currents from CA1 pyramidal neurons were recorded using a whole-cell patch clamp technique as described previously (Sun and Kapur, 2012). The slices were perfused with oxygenated aCSF containing DL-AP5 (50 μM) and picrotoxin (100 μM), to block NMDA and $GABA_A$ receptors, respectively, at a rate of 2–3 ml/min. The patch electrode was filled with an internal solution containing in mM, cesium methane sulfonate 115, cesium chloride 20, KCl 10, HEPES 10, sodium-EGTA 0.5, MgCl2 2.5, Mg-ATP 5 and lidocaine 5,