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NOVEL ANTICONVULSIVE EFFECTS OF PROGESTERONE IN A MOUSE MODEL OF HIPPOCAMPAL ELECTRICAL KINDLING

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Abstract—Progesterone is a known anticonvulsant, with its inhibitory effects generally attributed to its secondary metabolite, 5α,3α-tetrahydroprogesterone (THP), and THP's enhancement of $GABA_A$ receptor activity. Accumulating evidence, however, suggests that progesterone may have non-genomic actions independent of the $GABA_A$ receptor. In this study, we explored THP/$GABA_A$-independent anticonvulsive actions of progesterone in a mouse model of hippocampal kindling and in mouse entorhinal slices *in vitro*. Specifically, we examined the effects of progesterone in kindled mice with or without pretreatments with finasteride, a *5α-reductase* inhibitor known to block the metabolism of progesterone to THP. In addition, we examined the effects of progesterone on entorhinal epileptiform potentials in the presence of a $GABA_A$ receptor antagonist picrotoxin and finasteride. Adult male mice were kindled via a daily stimulation protocol. Electroencephalographic (EEG) discharges were recorded from the hippocampus or cortex to assess ''focal'' or ''generalized'' seizure activity. Kindled mice were treated with intra-peritoneal injections of progesterone (10, 35, 100 and 160 mg/kg) with or without finasteride pretreatment (50 or 100 mg/kg), THP (1, 3.5, 10 and 30 mg/kg), midazolam (2 mg/kg) and carbamazepine (50 mg/kg). Entorhinal cortical slices were prepared from naïve young mice, and repetitive epileptiform potentials were induced by 4-aminopyridine (100 μM), picrotoxin (100 μM) and finasteride (1 μM). Pretreatment with finasteride did not abolish the anticonvulsant effects of progesterone. In finasteride-pretreated mice, progesterone at 100 and 160 mg/kg decreased cortical but not hippocampal afterdischarges (ADs). Carbamazepine mimicked the effects of progesterone with finasteride pretreatments in decreasing cortical discharges and motor seizures, whereas midazolam produced effects similar to progesterone alone or THP in decreasing hippocampal ADs and motor seizures. In brain slices, progesterone at 1 μM inhibited entorhinal epileptiform potentials in the presence of picrotoxin and finasteride. We suggest that progesterone may have THP/$GABA_A$-dependent and independent anticonvulsive actions in the hippocampal-kindled mouse model.

Key words: EEG, epilepsy, neurosteroids, seizures.

INTRODUCTION

Epilepsy is characterized by recurrent seizures. Drug-refractory seizures affect one third of adults with epilepsy, and remain a significant challenge in the pharmacotherapy of epilepsy (Theodore et al., 2006). Uncontrolled seizures have significant impacts on quality of life and adverse effects of anticonvulsant drugs can be just as damaging (Viteva, 2013). It is therefore imperative to understand the pathological processes underlying epilepsy and to develop treatments for patients who cannot achieve seizure control with the currently available medications.

In both men and women, complex neuroendocrine interactions are implicated in seizure occurrence, co-morbidities, and the etiology of epilepsy (Biagini et al., 2010; Pack et al., 2011). Progesterone and its metabolites in particular have a strong influence on seizure propensity. In catamenial epilepsy and relevant animal models, when progesterone levels are high, seizures are less likely to occur (Lawrence et al., 2010; Verrotti et al., 2010; Stevens and Harden, 2011; Pack et al., 2011). Progesterone is first metabolized to 5α-dihydroprogesterone (DHP) by the unidirectional enzyme *5α-reductase* and subsequently to 5α,3α-tetrahydroprogesterone (THP) by the bidirectional enzyme *3α,5α-hydroxysteroid oxidoreductase*. The anticonvulsant effects of progesterone are generally attributed to its metabolism to THP, a powerfully inhibitory neurosteroid that enhances $GABA_A$ receptor activity at an unknown binding site distinct from the benzodiazepine and barbiturate binding sites (Lambert et al., 2009; Reddy et al., 2010; Finocchi and Ferrari, 2011). In many animal models, seizure suppression and anti-epileptogenesis effects of progesterone are lost or weakened when the animals are pretreated with finasteride, a competitive *5α-reductase* inhibitor that blocks the metabolism of progesterone to DHP, and, therefore, to THP (Kokate et al., 1999; Herzog and

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Abbreviations: ACSF, artificial cerebrospinal fluid; AD, afterdischarge; DHP, 5α-dihydroprogesterone; EEG, electroencephalographic; THP, 5α,3α-tetrahydroprogesterone.

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Frye, 2003; Finn et al., 2006; Mukai et al., 2008; Chaudhary and Turner, 2010; Reddy and Ramanathan, 2012). Several lines of evidence, however, suggest that progesterone may regulate brain function independent of its metabolite THP (Zhu et al., 2008; Hwang et al., 2009; Zheng, 2009; Johannessen et al., 2011; Luoma et al., 2011). In this study, we explored whether progesterone offers THP/$GABA_A$-independent anticonvulsive or inhibitory actions in hippocampal-kindled mice.

EXPERIMENTAL PROCEDURES

Animals

Male C57 black mice (Charles River Laboratory, Quebec, Canada), between 6 and 10 months of age, were used in the present study. Male animals were used to avoid complications from the cyclic fluctuation of progesterone and its metabolites. Animals were housed in a vivarium that was maintained at 22 °C with a 12-h light/dark cycle (lights on at 6:00 am). Food and water were available *ad libitum*. All experiments were conducted between 10 am and 5 pm. All experimental procedures were reviewed and approved by the Animal Care Committee of the University Health Network, in accordance with the guidelines of the Canadian Council on Animal Care.

Electrode implantation

Surgical procedures were modified from those previously described (Wu et al., 2008; El-Hayek et al., 2011). A modified version of a screw-free, glue-based method was used to secure implanted electrodes (Jeffrey et al., 2013). Briefly, the animal was anaesthetized with 2% isofluorane and then placed in a stereotaxic frame. After a skin incision and exposure of the skull surface, a thin plastic base (~200 μm thick, cut from plastic weighing boats) was glued onto the skull surface with cyanocrylate glue (Insta-cure +, Canadian Hobbycraft, Concord, Ontario, Canada). After the glue had set, three small holes (< 0.5 mm in diameter) were drilled through the plastic base and the skull in order to insert the stimulation and recording electrodes. The inserted electrodes were then cemented onto the skull with dental acrylic.

Two bipolar electrodes were inserted into the right and left hippocampal CA3 areas, with coordinates relative to bregma: 2.5 mm posterior, 1.3 mm lateral and 3.0 mm down from the brain surface. A reference electrode was positioned, relative to bregma, at: 1.0 mm anterior, 2.0 mm lateral, and 0.5 mm down from the brain surface. In some experiments, a bipolar electrode was implanted in CA3 on one side, and two monopolar recording electrodes were implanted contralaterally, one in CA3 (coordinates as above) and the other in the neocortex. The coordinates for cortical implantation, relative to bregma, were: 0.6 mm posterior, 1.5 mm lateral and 1.0 mm down from the brain surface. All electrodes were made of polyamide-insulated stainless steel wires (outer diameter 125 μm; Plastics One, Ranoake, VA).

Hippocampal kindling

Unilateral CA3 stimulation was conducted in all experiments. Constant unipolar square-wave current pulses (duration of 0.5 ms, intensities of 10–150 μA base to peak) were generated by a Grass stimulator and delivered through a stimulus isolation unit (model S88H, Grass Medical Instruments, Warwick RI, USA). A standard kindling protocol (Albright and Burnham, 1980; Reddy and Rogawski, 2010) was used in the present experiments. Initially, daily stimuli (60 Hz for 2 s) were applied at an intensity of 150 μA until an afterdischarge (AD) event of ⩾5 s was elicited, as > 90% of the cohort did not elicit evident AD on the first two days when stimulated with intensities from 10 to 150 μA. An ascending stimulation series was then used to determine each animal's individual AD threshold. In the ascending series, stimulation was increased from 10 to 150 μA with 10-μA increments, spaced 5 min apart, until an AD event of ⩾5 s was observed. The stimulation intensity at which an AD event of ⩾5 s was elicited was considered the AD threshold. The mice were stimulated daily at 125% of their AD threshold and were considered fully kindled when three consecutive stage 5 events (see below) were elicited. All animals were kindled within a month after receiving their first stimulation. The animals were stimulated at 125% of their AD threshold on all non-trial days to ensure the stability of ADs (< 10% fluctuation in duration) and consistent stage 5 motor seizures during the course of drug testing.

Electroencephalographic (EEG) recordings and data analysis

EEG signal was recorded from the CA3 site contralateral to the CA3 stimulation site. Local differential recordings – between the adjacent tips of the contralateral recording electrode – were used in order to reduce artifacts and common-ground EEG signals. Monopolar recordings were made if the local differential recordings were unsuccessful, presumably due to contaminated electrode tips of the twisted bipolar electrodes. In some experiments, two monopolar electrodes were implanted in the contralateral CA3 and neocortical areas for simultaneous recordings of hippocampal and neocortical EEG.

Signals were recorded via a 2-channel microelectrode AC amplifier (model 1800, A-M Systems, Carlsborg, WA, USA), with the input frequency band set in the range of 0.1–5000 Hz, and the amplification gain at 1000×. The signals were digitized at 5000 Hz (Digidata 1440A, Axon Instruments/Molecular Devices, Union City, CA, USA). PClamp software (Version 10; Axon Instruments/Molecular Devices) was used for data acquisition, storage and analyses (El-Hayek et al., 2011). EEG ADs were recognized as repetitive single-spike and poly-spike waveforms with amplitudes 2 times of baseline signals and durations of ⩾5 s. For measurements of AD durations, original EEG signals were treated with a 0.5-Hz high-pass (Bessel) filter to minimize slow drifts. In some experiments, standard deviations of cortical EEG