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Allopregnanolone decreases interictal spiking and fast ripples in an animal model of mesial temporal lobe epilepsy

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ABSTRACT

The objective of this study was to characterize the impact of allopregnanolone, a neurosteroid that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors, on interictal spikes and high-frequency oscillations (ripples: 80–200 Hz, fast ripples: 250–500 Hz) in the pilocarpine model of mesial temporal lobe epilepsy. Seven out of 25 Sprague-Dawley rats experiencing 1 h of pilocarpine-induced *status epilepticus* (SE) began treatment with allopregnanolone (9.6–12.8 mg/kg/day) on the following day. On day 4 after SE, video-depth EEG recordings from the hippocampal CA3 subfield and the entorhinal cortex were initiated and continued for 12 consecutive days. We found that 66.7% (12/18) of untreated animals exhibited seizures compared to 28.6% (2/7) of allopregnanolone-treated animals. Interictal spikes occurred less frequently in the CA3 subfield of allopregnanolone-treated rats ($n = 4$) than in untreated animals presenting ($n = 4$) or not presenting ($n = 4$) with spontaneous seizures ($p < 0.05$), and were less frequent in the entorhinal cortex compared to both untreated groups ($p < 0.05$). Finally, allopregnanolone-treated rats had significantly lower rates of interictal spikes with fast ripples (250–500 Hz) compared to untreated animals but only in CA3 ($p < 0.05$). Our findings show that allopregnanolone reduces the frequency of interictal spikes and fast ripples in CA3, a structure that plays an important role in ictogenesis and epileptogenesis. Neurosteroids may therefore influence pathological network activity leading to spontaneous seizures following pilocarpine-induced SE. Recordings after termination of allopregnanolone treatment will be however required to establish whether allopregnanolone exerts disease-modifying properties.

1. Introduction

Allopregnanolone is a neurosteroid that acts as a positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors. An endogenous compound, allopregnanolone is synthesized from ovarian progesterone in the periphery and *de novo* from cholesterol in glutamatergic principal neurons in the brain (Agís-Balboa et al., 2006; Mody, 2012; Reddy, 2010; Reddy and Rogawski, 2012). Since $GABA_A$ receptors are the main mediators of fast inhibitory neurotransmission (Avoli and Krnjević, 2016), alterations in allopregnanolone and related $GABA_A$ receptor active neurosteroids can have profound effects on neural network excitability. For instance, withdrawal of allopregnanolone at menstruation predisposes women with epilepsy to catamenial seizures (Reddy and Rogawski, 2009, 2012). Exogenously administered allopregnanolone protects against seizures induced by various convulsant stimuli in seizure models using non-epileptic animals, such as in the pentylenetetrazole, 6-Hz, pilocarpine, kainic acid and NMDA seizure tests (Kaminski et al., 2004; Kokate et al., 1996). In addition, allopregnanolone inhibits the behavioural seizure stage and afterdischarge duration of seizures provoked by electrical stimulation in the amygdala kindling epilepsy model (Lonsdale and Burnham, 2007; Reddy et al., 2012). There is also evidence that neurosteroids may have antiepileptogenic properties since the duration of the latent period in the pilocarpine model of mesial temporal lobe epilepsy (MTLE) is shortened when their synthesis is blocked (Biagini et al., 2006, 2009).

In *in vitro* brain slice preparations, $GABA_A$ receptor positive modulatory neurosteroids, including allopregnanolone, cause a

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concentration-dependent suppression of the epileptiform activity induced by the K^+ channel blocker 4-aminopyridine or the $GABA_A$ receptor antagonist picrotoxin (Herrington et al., 2014; Salazar et al., 2003). In addition, the related neurosteroid allotetrahydrodeoxycorticosterone has been shown to reduce the occurrence of high-frequency oscillations (HFOs, ripples: 80–200 Hz, fast ripples: 250–500 Hz) that are associated with 4-aminopyridine-induced epileptiform discharges in brain slices *in vitro* (Herrington et al., 2014, 2015). HFOs reflect the activity of dysfunctional neuronal networks (Jefferys et al., 2012) and are considered to be biomarkers of epileptogenesis and ictogenesis in animal models of MTLE (Bragin et al., 2004; Lévesque et al., 2011, 2012) and in epileptic patients (Jacobs et al., 2009; Staba et al., 2002).

To date, no study has addressed the impact of continuous, systemic administration of a $GABA_A$ receptor positive modulatory neurosteroid on spontaneous seizures in the pilocarpine model of MTLE. Moreover, it is unclear whether any antiseizure effects of neurosteroids obtained in this situation are accompanied by changes in interictal spike or HFO occurrence. Therefore, we used depth brain EEG recordings from the hippocampal CA3 subfield and the entorhinal cortex (EC) to establish the effects induced by continuous treatment with allopregnanolone in Sprague-Dawley rats following pilocarpine-induced SE.

2. Material and methods

2.1. Ethical approval

All procedures were approved by the Canadian Council of Animal Care and the McGill University Animal Care Committee. All efforts were made to minimize the number of animals and their suffering.

2.2. Pilocarpine treatment

Male Sprague-Dawley rats (150–200 g, 45–50 days old) were purchased from Charles River Laboratories (St-Constant, Qc, Canada) and allowed to habituate for 72 h after delivery before pilocarpine treatment. On the day of SE induction, they received scopolamine methylnitrate (1 mg/kg i.p.; Sigma-Aldrich, Qc, Canada). Thirty minutes later, they were injected with a single dose of pilocarpine hydrochloride (380 mg/kg, i.p.; Sigma-Aldrich, Qc, Canada) (Lévesque et al., 2012; Salami et al., 2014). Their behavior was scored according to the Racine scale (Racine, 1972). SE was defined as continuous stage 5 seizure activity. SE was terminated after 1 h by injecting diazepam (5 mg/kg, s.c.; CDMV, Qc, Canada) and ketamine (50 mg/kg, s.c.; CDMV, Qc, Canada) (Martin and Kapur, 2008). Animals that did not experience SE were excluded from further analysis.

2.3. Treatment with allopregnanolone

On the day following *status epilepticus* (SE), a 2 ml ALZET osmotic minipump (DURECT Corporation, CA, USA) calibrated to deliver 5.0 μl/h was subcutaneously implanted on the dorsal side under light isoflurane anesthesia (2% in 100% O_2) in order to deliver allopregnanolone solution for 12 consecutive days (n = 7 animals). The entire surgical procedure lasted less than 5 min. To prepare the treatment solution, 2 g of sulfobutyl ether-β-cyclodextrin (SBEBCD; Captisol, Ligand Pharmaceuticals, CA, USA) was dissolved in 5 ml of water and 0.08 g of allopregnanolone was added to obtain a concentration of 16 μg/ml. Therefore, the dose rate (k_0) of allopregnanolone for rats weighing between 150 and 200 g would be from 9.6 mg/kg/day to 12.8 mg/kg/day. The clearance (CL) of allopregnanolone in rats has been determined to be 4.8 l/h/kg (Martinez Botella et al., 2015). The expected steady-state blood concentration can be estimated as k_0/CL, which is 83–110 ng/ml. Immediately following implantation of the pump, animals were injected with a 15 mg/kg loading dose of allopregnanolone to avoid a delay in achieving the steady-state level. Some untreated animals were treated with saline (n = 11) and others received no treatment (n = 7). Since seizure rates were not significantly different between animals treated with saline and animals that received no treatment, data obtained from these two groups were pooled together and compared with those from allopregnanolone-treated animals.

2.4. Stereotaxic surgery

On the third day after SE, rats were anaesthetized with isoflurane (3%) in 100% O_2 and fixed in a stereotaxic frame. An incision was made in the skin and four stainless steel screws (2.4 mm length) were fixed to the skull. Three small holes were also drilled to allow the implantation of bipolar electrodes (20–30 kΩ; distance between exposed tips: 500 μm). Electrodes were implanted in the CA3 subfield of the hippocampus (AP: −4.4, ML: ± 4.2, DV: 4.3) and the EC (AP: 6.6, ML: ± 4, DV: −8.8) since these regions are often seizure onset zones in this animal model of MTLE (Bortel et al., 2010; Lévesque et al., 2011, 2012; Salami et al., 2014). Screws and electrodes were fastened to the skull with dental cement. A fifth bipolar electrode was placed under the frontal bone, after the removal of the insulating material, and used as reference. After surgery, animals received topic application of chloramphenicol (Erfa, Qc, Canada) and lidocaine (5%; Odan, Qc, Canada) and were injected with ketoprofen (5 mg/kg s.c.; Merail, Qc, Canada), buprenorphine (0.01–0.05 mg/kg s.c., CDMV, Qc, Canada) and 2 ml of 0.9% saline (s.c.).

2.5. EEG recordings

After surgery, rats were housed individually in custom-made Plexiglas boxes (30 × 30 × 40 cm). The recording electrodes were connected to a multichannel cable and electrical swivel (Commutator SL 18C, HRS Scientific, Qc, Canada) and continuous EEG recordings were collected for 12 days. EEG signals were amplified via an interface kit (Mobile 36ch LTM ProAmp, Stellate, Qc, Canada); they were low-pass filtered at 500 Hz and sampled at 2 kHz per channel. EEG recordings were performed using monitoring software (Harmonie, Stellate, Qc, Canada). Throughout the recordings, animals were placed under controlled conditions (22 ± 2 °C, 12 h light/dark schedule) and were provided with food and water *ad libitum*.

2.6. Seizure analysis

All EEG and video recordings were reviewed manually in order to detect seizures. The occurrence of the first spontaneous seizure (convulsive or non-convulsive) after SE marked the end of the latent period.

2.7. Interictal spike and HFO analysis

We compared rates of interictal spikes and rates of HFOs from the untreated and the allopregnanolone-treated group. For each animal selected for analysis, one epoch of 10 min was extracted for each day of recording. Only epochs of non-REM sleep were used for analysis because of the low rates of movement artefacts and because HFOs are more prominent during this sleep stage (Bagshaw et al., 2009). Extracted epochs were exported to Matlab 7.11.0 (The Mathworks, MA, USA) using custom-built routines and analysed off-line. Interictal spikes were detected based on