Brain Research Bulletin 121 (2016) 59–67

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Serotonin receptor antagonists increase fast ripple activity in rats treated with kainic acid

Carla García-Barba, Laura Medina-Ceja*,[1]

Laboratory of Neurophysiology and Neurochemistry, Department of Cellular and Molecular Biology, CUCBA, University of Guadalajara, Jalisco, Mexico

ARTICLE INFO

Article history:
Received 3 October 2015
Received in revised form
30 November 2015
Accepted 4 January 2016
Available online 6 January 2016

Keywords:
Fast ripples
Hippocampus
Kainic acid
Morphologic analysis
$5HT_{1A}$ receptor
$5HT_2$ receptor

ABSTRACT

Fast ripples (FR, 250–600 Hz) are field potentials that occur only in those areas capable of generating seizures, such as the hippocampus, and modulation of FR by serotonin has been reported. Therefore, we hypothesized that the receptor antagonists 5HT1A and 5HT2A, B, C will increase FR in rats treated with kainic acid (KA, 0.8 μg/0.5 μl). For this purpose, the intracranial EEG recordings of the hippocampus from animals treated with KA and the serotonin antagonists WAY100135 and ritanserin (dose 0.2 mg/Kg, i.p) were analyzed. In addition, morphologic parameters were analyzed after staining samples with cresyl violet, Timm stain, NeuN and GFAP and observing immunofluorescence. The results showed an increase in the number of events of FR ($p < 0.0001$) and duration of each FR event after the administration of WAY100135 ($p < 0.030$). Additionally, there was an increase in the number of events of FR ($p < 0.0001$) and amplitude of FR after ritanserin administration ($p < 0.014$). In relation to changes in unspecified cells, there was a significant decrement in the width of the CA3 pyramidal layer of the hippocampus ($p < 0.001$), and there were no significant changes in reactive glia and fiber sprouting. However, a slight gain of astrocytes marked with GFAP and larger astrocytes with more projections were observed. In conclusion, these results support the modulation of FR by serotonin with participation of the 5HT1A receptor as a possible mediator of the effect. However the exact mechanisms resulting in such effect is not known.

1. Introduction

Almost two decades ago, high frequency oscillations (HFO), particularly fast ripples (FR), were associated with epileptic activity in the hippocampus (Bragin et al., 1999). FR (250–600 Hz) are field potentials that occur only in those areas capable of generating seizures (Bragin et al., 1999; Jacobs et al., 2008). This suggests that FR are an important biomarker of epileptogenesis in temporal lobe epilepsy (TLE) (Zijlmans et al., 2012; Aibel-Weiss et al., 2015) as well as in extratemporal epilepsies (Wang et al., 2013).

Events of neuronal synchronization corresponding to the wide band of FR are markedly increased during sleep in comparison with wakefulness, particularly in slow wave sleep (Staba et al., 2004) in which serotonin levels are decreased significantly (Park et al., 1999). Additionally, serotonin has been widely studied as a potential modulator of epileptic activity, with evidence suggesting that using selective serotonin reuptake inhibitors decreases the occurrence of seizures (Bagdy et al., 2007) or inhibition of serotonin activity increases epileptiform activity (Shiha et al., 2015; Cuellar-Herrera et al., 2014).

The 5HT1A receptors are widespread through the limbic system There is also evidence suggesting that the inhibition of several serotonin receptors, such as the 5HT1 and 5HT2 families, increases the number and intensity of seizures as well as seizure-related deaths, and decreases the threshold for seizures (Watanabe et al., 2000; Clinckers et al., 2004; Gariboldi et al., 1996; Tecott et al., 1995; Brennan et al., 1997; Upton et al., 1998; Wesolowska et al., 2006; Ogren et al., 2008; Kazmierska and Konopacki, 2015). WAY100 135 is a potent 5HT1A receptor antagonist that has an IC_{50} of 15.5 nM (±4.6, SEM) (Cliffe et al., 1993) and has proven to block the antiepileptic effects of serotonin in partial and absence seizures (Salgado-Commissariat and Alkadhi, 1997; Ohno et al., 2010). Ritanserin on the other hand has an IC_{50} of 0.9 nM to the $5HT_2$ receptors and effectively blocks 5HT2A, 2C receptor activity and it has been proven to antagonize the antiepileptic effect of 2,5-dimethoxy-4-iodoamphetamine (DOI) in a model of absence seizures (Ohno et al., 2010).

Abbreviations: DG, dentate gyrus; FR, fast ripples; HFO, high frequency oscillations; KA, kainic acid; SE, *Status epilepticus*; TLE, temporal lobe epilepsy.

* Corresponding author at: Laboratorio de Neurofisiología y Neuroquímica, Departamento de Biología Celular y Molecular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Camino Ing. R. Padilla Sánchez 2100, Las Agujas, Nextipac, Zapopan, Jalisco CP 45110, Mexico. Fax: + 52 33 37771191.
E-mail address: lauramedcej@gmail.com (L. Medina-Ceja).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.brainresbull.2016.01.003
0361-9230/

In addition, successful modulation of FR by serotonin was observed in one of our previous studies in which the elevation of serotonin levels induced by citalopram reduced the occurrence of spontaneous FR (57%), the mean number of oscillation cycles per FR event (34%) and the average frequency of FR (33%) (Pardo-Peña et al., 2014).

Based on this information, our principal goal of this study was to evaluate the effects of the serotonin receptor antagonists 5HT1A and 5HT2A,B,C on FR activity in rats treated with KA.

2. Materials and methods

2.1. Intrahippocampal injection of KA

Fifteen male Wistar rats (200–250 g) were housed individually in cages that were kept in a temperature controlled room (22 ± 2 °C) with a 12-h light-dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) and access to food and water *ad libitum*. All experimental procedures were designed to minimize animal suffering and the total number of animals used. This protocol conformed to the Rules for Research in Health Matters (Mexican Official Norms NOM-062-ZOO-1999, NOM-033-ZOO-1995), and it was approved by the local Animal Care Committee.

The experimental animals were anesthetized with inhaled isoflurane in 100% O_2 and secured in a Stoelting stereotaxic frame (Stoelting Co.IL. USA) with the incisor bar positioned at −3.3 mm. Afterwards the animals were injected in the right posterior hippocampus (coordinates in relation to Bregma: AP −5.6 mm, ML −5.5, DV −7 mm), with a 10 μl syringe connected to and controlled by an infusion pump to inject a dose of 0.8 μg/0.5 μl of KA dissolved in 0.9% saline solution. After the injection, the needle was maintained in the same position for 15 minutes to avoid leaks of the solution. The perforation was sealed with dental wax and the skin was sutured with nylon surgical thread, after which the animals were returned to their cages for observation.

2.2. Observation of convulsive behavior

Animal behavior was scored using the Racine scale (Racine, 1972) by continuous observation and immediately after KA administration. Briefly, behavior was scored as follows: 1, mouth and facial movements; 2, head nodding; 3, forelimb clonus; 4, rearing with forelimb clonus; and 5 rearing, forelimb clonus and falling. *Status epilepticus* began 20 min to 50 min after KA administration and lasted from 60 to 130 min, and terminated spontaneously. After three hour surveillance, the animals were returned to their room and fed with multivitamin beverages for three days. Animals were kept in their cages for a minimum of 15 days before microelectrode implantation.

2.3. Animal surgery

Both KA and control rats were anesthetized with inhaled isoflurane in 100% O_2 and secured in a Stoelting stereotaxic frame (Stoelting Co.IL. USA) with the incisor bar positioned at −3.3 mm. An arrangement of four independent pairs of tungsten wires (60 μm in diameter), with a 500 μm vertical tip separation was mounted in a mobile device consisting in a microelectrode holder attached to a worm drive; this device was implanted along the right region of the hippocampus (RAH, AP −3.5/−5.0 mm, ML −3.0 mm, DV −2.5 mm). In addition, two stainless steel screws were driven into the bone above the bregma, which served as indifferent and ground electrodes. The mobile device with the microelectrodes were arranged on a pin connector and fastened to the skull with dental cement and protected with cardboard and parafilm. After surgery animals were returned to their cages and treated with Enrofloxacin (Enroxil, 22 mg/kg weight, oral administration) and paracetamol (700 mg/kg weight, oral administration) in order to prevent infection and pain.

2.4. Drug administration

Rats were divided into three groups and were injected with different drugs per via intraperitoneal (i.p.). The first group of animals ($n = 3$) was injected with WAY 100135 (0.2 mg/kg, i.p.; Sigma–Aldrich. St. Louis, Missouri, USA; Charrier et al., 1994; Arborelius et al., 1995; Bickerdike et al., 1995; Fornal et al., 1996), which antagonizes the activity of the 5HT1A serotonin receptor and the second group ($n = 3$) was treated with ritanserin (0.2 mg/Kg, i.p.; Sigma–Aldrich. St. Louis, Missouri, USA; Schremmer et al., 1990; Jäkälä et al., 1995) which is a 5HT2 A,B,C receptor antagonist. The third group of animals was treated with KA (0.8 μg/0.5 μl, intrahippocampal administration; Sigma–Aldrich. St. Louis, Missouri, USA), and after 15 days of KA administration, in this experimental group ($n = 6$), the first antagonist (WAY 100135, i.p.) was injected 24 h after microelectrode implantation surgery and the second antagonist (ritanserin, i.p.), was given 72 h after the first antagonist.

2.5. Intracranial EEG recordings and analysis

Intracranial EEG activity was recorded in free-moving rats. Five 4-channel MOSFET small amplifiers were attached to the cable connector to eliminate movement artifacts. Hippocampal electrical activity was recorded on a polygraph with eight amplifiers (Model 7D, Grass Technologies, RI, USA) at a bandwidth of 0.1–3 kHz with a sensitivity of 75 μV/cm per channel. The sampling rate was set at 5 kHz/channel with 12-bit precision using an iMac A1048 (Apple, USA) and MP150 software system (BIOPAC Systems, CA, USA). Once recorded, EEG traces from all recordings for a period of three hours were analyzed by choosing four periods of 5 min each hour with a total of 60 min of EEG recording analyzed per rat. The selected periods were manually examined according to previous studies (Pardo-Peña et al., 2014; Ventura-Mejía and Medina-Ceja, 2014), by setting fragments of 100 ms for a better image resolution. When encountered, FR activity was chosen according the frequency band (250–600 Hz), amplitude (minimum 200 μV) and oscillations per event of FR (a minimum of 3); the evaluation of the frequency of appearance of spontaneous FR, number of oscillations per event of FR, frequency and duration of each event of FR were also analyzed. The administration of the first antagonist was performed after 60 min of recording and the injected animals were recorded for 3 more hours, while the second antagonist was administered 72 h after the first antagonist in the same animals and were recorded for 3 more hours. Animals were recorded one hour per day (3 consecutive days) after the end of the experiment with the first antagonist in order to be sure that the electrical activity returns to basal recording (pre-drug). All the experimental animals used for this study showed FR activity.

2.6. Morphologic evaluation

After 3 days of each experiment, all the animals ($n = 12$, control and experimental groups) were anesthetized with sodium pentobarbital and perfused transcardially with 150 ml of normal saline (0.9%) in 0.12 M buffer/$CaCl_2$, followed by 300 ml of 4% paraformaldehyde in 0.12 M buffer/$CaCl_2$ (pH 7.3). The animal's brain was then removed and kept in a postfixative solution of paraformaldehyde (4% and glutaraldehyde 0.1%) for a period of 24–72 h and coronal sections (50 μm thick) were obtained for the application of different techniques and all the reagents used were supplied by Sigma.