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Research report

Altered taste preference and loss of limbic-projecting serotonergic neurons in the dorsal raphe nucleus of chronically epileptic rats

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HIGHLIGHTS

- Kainate-induced epilepsy in rats is associated with anhedonia.
- Epileptic rats show 5-HT cells loss in interfascicular part of the dorsal raphe.
- Depression in epilepsy may be related to loss of 5-HT neurons in the dorsal raphe.

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ABSTRACT

Mood disorders and major depression are frequently comorbid with epilepsy. While the nature of this comorbidity is not fully understood, multiple lines of evidence suggest that changes in serotonin (5-HT) neurotransmission may be an underlying mechanism. In this study, we tested the hypothesis that chronic epilepsy in rats can be associated with loss of 5-HT neurons in the dorsal raphe (DR) nuclear complex, the main source of 5-HT projections to the cerebral cortex, which would help to explain respective behavioral deficits. Epilepsy was induced using the kainate model of status epilepticus in adult Wistar rats. After a 3-month recovery period, all kainate-treated rats that had experienced status epilepticus showed spontaneous seizures and reduced sucrose preference (anhedonia), a core symptom of depression. No changes in the forced swim test were detected. The total numbers of 5-HT immunoreactive cells were estimated in all DR subdivisions of control and epileptic rats. Interestingly, epilepsy-related loss of 5-HT neurons (approximately 35%) was observed only in the interfascicular part of the DR complex, which is known to innervate brain regions involved in depression. These findings support the notion that mental health impairments observed in epilepsy may be related to loss of a specific population of the DR 5-HT neurons projecting to limbic brain areas.

1. Introduction

Clinical evidence indicates that epilepsy can be accompanied by comorbid psychiatric disorders, such as anxiety, depression, and mood impairments [1–3]. In particular, it has been reported that the prevalence rates for major depression disorder (MDD) in patients with temporal lobe epilepsy (TLE) may reach 30–35% and prevalence rates of mood disorders range between 24 and 72% [for review, see Ref. 4]. Furthermore, a growing number of experimental studies have confirmed the presence of anxiety and depression-like behaviors in epileptic animals [5,6]. It has been hypothesized that seizure disorders and affective symptomatology can be interrelated via common neurobiological mechanisms, which include dysregulation of the hypothalamo–pituitary–adrenal (HPA) axis, changes in monoamine neurotransmission, neuroinflammation or neurodegenerative processes in specific brain regions [7,8]. However, the precise mechanisms underlying the comorbidity of epilepsy with mental health impairments remain to be clarified.

It has long been known that dysfunction of ascending serotonergic pathways is crucially implicated in psychiatric disorders, such as panic, depression, and suicide. Several lines of evidence

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support this viewpoint, including that (1) low levels of serotonin (5-hydroxytriptophan; 5-HT) metabolites were found in depressed suicides [9], (2) depletion of 5-HT in volunteers triggers relapse of depressive episodes [10], (3) affective disorders appear to be linked to changes in the activity of serotonin transporter (SERT) [11], and (4) treatment with 5-HT reuptake inhibitors mitigates depressive symptoms, at least in a subpopulation of patients [12]. In addition, in depressed patients a number of structural defects were observed in the dorsal raphe nucleus (DRN), the brainstem region which provides the majority of cortical serotonergic fibers. They include an overall neuron number deficit [13,14], reduced cross-sectional area of DRN in MDD, but increased in depressed suicides [15], loss of SERT-expressing cells [16], increased [17] or unaltered [18] number of neurons immunoreactive to TPH (tryptophan hydroxylase, enzyme required for 5-HT synthesis), and changes in binding properties of the presynaptic serotonin autoreceptor 5-HT1A [19,20]. Finally, that depressive disorders are characterized by respective changes in the postsynaptic 5-HT receptors located in target corticolimbic brain areas implicated in affective functions has been also reported [21,22].

It is perhaps not surprising that many of the above-mentioned serotonergic abnormalities, particularly changes in 5-HT receptor binding, have also been found in epilepsy patients with comorbid depression and mood disorders [1,23,24]. With respect to animal studies, Mazarati et al. reported that the induction of epilepsy in rats, in addition to producing depression-like behaviors, results in compromised neurotransmission in the DRN—hippocampal serotonergic pathway [6]. However, whether or not epilepsy-related depression is likewise attributable to structural alterations in the DRN is still an open question. In this study, we hypothesized that chronic epilepsy in rats can be associated with loss of serotonin-producing neurons in the DRN, which would help to explain the compromised raphe-hippocampal transmission as well as respective behavioral impairments. To address this issue, we estimated the total number of 5-HT-immunoreactive neurons in all subdivisions of the DRN of control and chronically epileptic rats. Epilepsy was induced using the kainate model of status epilepticus. Behavioral changes were assessed using two common tests for depression in rodents, the forced swim test and the sucrose preference test.

2. Material and methods

2.1. Ethical statement

The handling and care of the animals were conducted according to the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The experimental protocol has been approved by the Ethics Committee of the Faculty of Medicine of Porto and the General Veterinary Direction (03.04.2012) for the FCT application grant PTDC/SAU-NSC/115506/2009. All efforts were made to minimize the number of animals used and their suffering.

2.2. Animals and treatments

Male Wistar rats, maintained individually under standard laboratory conditions, were used in this study. At 10 weeks of age, they were randomly divided into two groups: KA group ($n = 10$) and control group ($n = 8$). In the first group, the rats were injected with 9.5 mg/kg of KA (i.p., Sigma) to induce convulsive SE, which was defined as the appearance of behavioral symptoms corresponding to stage 3, 4 or 5 seizures on the Racine scale [25], i.e., bilateral forelimb clonus, rearing, and rearing with falling. Rats in this group initially demonstrated numerous wet-dog-shake seizures, which culminated in SE lasting 3–6 h. The animals were periodically injected with saline (s.c.) during the first 48 h of the recovery period. On the following days, the rat diet was supplemented with apples that were sliced and left at the bottom of the cage. The rats that refused to eat or drink were hand-fed using a plastic syringe. Rats in the control group were injected with saline alone.

2.3. Surgery, behavioral monitoring and electroencephalographic (EEG) recording

Following the treatments, all animals were given a 3-month recovery period. In the beginning of the third month, the rats received stereotaxic surgery conducted under isoflurane anesthesia. Rats were placed in a Kopf stereotaxic apparatus and the scalp was incised along the midline and retracted to the side. Three epidural stainless steel electrodes (E363/20 Plastics One Inc., Roanoke, VA, USA) were implanted above the right prefrontal cortex (3 mm anterior to bregma, 2 mm lateral to midline), left parietal cortex overlaying the hippocampus (4.3 mm posterior to bregma, 2.0 mm lateral to midline) and right occipital cortex (1.0 mm anterior to lambda, 3.5 mm lateral to midline). Two additional screw electrodes were placed over the cerebellum to serve as a reference electrode and as a ground. All of the electrodes were connected to a plastic pedestal (Plastics One, Inc.) that was cemented to the skull using dental acrylic.

Starting from the sixth week following the treatments, the rats were daily (except weekends) observed for spontaneous behavioral seizures during 2 h intervals between 09:00 h and 11:00 h by a person blind to treatment groups.

Video-EEG recording was performed in the last two weeks of the recovery period in order to confirm the presence of electrographic seizures in KA-treated rats. Recordings were simultaneously performed in pairs of rats randomly selected from each group and were 24 h in duration. For this purpose, rats were placed in Plexiglas cages where they could move freely (one rat per cage). EEG activity was continuously registered from the epidural electrodes using the Truscan-32 acquisition system (Deymed Diagnostic, Hronov, Czech Republic) connected to computer via a universal serial bus port amplifier (Deymed Diagnostic). Recordings were sampled at 256 Hz, high- and low-pass-filtered at 1 Hz and 100 Hz, respectively, and stored on the computer disk for offline seizure review using the TruScan Explorer software (Deymed Diagnostic). The behavior of the animals was simultaneously recorded using a digital video camera Sony DCR-SR58E (Sony Corporation, Japan), which was positioned above the cages. The video-EEG recordings were analyzed by a study-blinded clinical neurophysiologist. Electrographic seizures were defined by the presence of sustained spike and poly-spike activity longer than 3 s. Behavioral seizures were defined according to the Racine scale.

2.4. Behavioral testing

Three months after induction of SE, the animals were handled 3 min per day during 5 days and subjected to the forced swim test (FST) and sucrose preference test (SPT). The rats were counterbalanced so that, in each group, half received the FST first and the other half received the SPT first. There was a 7-day interval between the two tests.

The procedure for the FST was essentially as described by Porsolt et al. [26]. The apparatus used in this task consisted of a transparent glass cylinder, 20 cm in internal diameter and 50 cm in height. It was filled with tap water (25 °C) to a depth of 30 cm. On the first day, the rats were forced to swim in the apparatus for 15 min. During this session, animals progressively reduce their attempts to escape from the water and spend more time without movement.