Neurobiology of Disease 44 (2011) 259–269

Contents lists available at ScienceDirect

Neurobiology of Disease

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

IL-1β is induced in reactive astrocytes in the somatosensory cortex of rats with genetic absence epilepsy at the onset of spike-and-wave discharges, and contributes to their occurrence

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ARTICLE INFO

Article history:
Received 2 December 2010
Revised 21 April 2011
Accepted 20 May 2011
Available online 30 May 2011

Keywords:
Inflammation
Absence seizures
Interleukins
SWD

ABSTRACT

Interleukin (IL)-1β plays a crucial role in the mechanisms of limbic seizures in rodent models of temporal lobe epilepsy. We addressed whether activation of the IL-1β signaling occurs in rats with genetic absence epilepsy (GAERS) during the development of spike-and-wave discharges (SWDs). Moreover, we studied whether inhibition of IL-1β biosynthesis in GAERS could affect SWD activity.

IL-1β expression and glia activation were studied by immunocytochemistry in the forebrain of GAERS at postnatal days (PN)14, PN20, and PN90 and in age-matched non-epileptic control Wistar rats. In PN14 GAERS, when no SWDs have developed yet, IL-1β immunostaining was undetectable, and astrocytes and microglia showed a resting phenotype similar to control Wistar rats. In 3 out of 9 PN20 GAERS, IL-1β was observed in activated astrocytes of the somatosensory cortex; the cytokine expression was associated with the occurrence of immature-type of SWDs. In all adult PN90 GAERS, when mature SWDs are established, IL-1β was observed in reactive astrocytes of the somatosensory cortex but not in adjacent cortical areas or in extra-cortical regions.

An age-dependent c-fos activation was found in the somatosensory cortex of GAERS with maximal levels reached in PN90 rats; c-fos was also induced in some thalamic nuclei in PN20 and PN90 GAERS.

Inhibition of IL-1β biosynthesis in PN90 GAERS by 4-day systemic administration of a specific ICE/Caspase-1 blocker, significantly reduced both SWD number and duration.

These results show that IL-1β is induced in reactive astrocytes of the somatosensory cortex of GAERS at the onset of SWDs. IL-1β has pro-ictogenic properties in this model, and thus it may play a contributing role in the mechanisms underlying the occurrence of absence seizures.

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Introduction

Typical absence epilepsy, a prototype of generalized idiopathic epilepsies, is characterized by several daily episodes of absence seizures which consist of a brief interruption of behavioral activity and a simultaneous electroencephalographic (EEG) expression of bilateral, synchronous, and symmetrical spike-and-wave discharges (SWDs). Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a well defined animal model of typical absence epilepsy in humans, display non-convulsive seizures and SWDs within the cortico-thalamo-cortical circuit involving the somatosensory cortex, the ventrobasal thalamus and the reticular thalamic nucleus (see for review Danober et al., 1998). SWDs in GAERS appear to have an initiation site within the somatosensory cortex, namely in the "the upper lip and nose area of the somatosensory cortex" (*S1Ulp*) (Meeren et al., 2002; Paxinos and Watson, 2005; Polack et al., 2009). In GAERS, immature-type of SWDs are observed around postnatal day (PN)30 (Carcak et al., 2008; Vergnes et al., 1986) and subsequently the number, duration and frequency of SWDs increase with age, reaching a mature pattern in 3–4 month-old rats (Carcak et al., 2008; Marescaux et al., 1992; Vergnes et al., 1986).

Glial cells have been suggested to play a role in the mechanisms underlying SWDs in GAERS: in particular, it has been shown that the glutamine supply to GABAergic neurons in the cortex and subcortical areas of PN30 GAERS is lower than in control rats (Melo et al., 2006); in adult GAERS, the production of glutamate from astrocytic

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2011.05.015

glutamine was enhanced indicating increased astrocytic metabolism (Melo et al., 2007). Moreover, Dutuit et al. (2000) have described astrocytic activation, as shown by increased levels of glial fibrillary acidic protein (GFAP) in cortex and thalamus of PN30 GAERS before the occurrence of absence seizures, as well as in adult epileptic GAERS as compared to non-epileptic rats.

Activated astrocytes and microglia are major sources of inflammatory molecules in the brain during epileptic activity induced in experimental models of limbic seizures and temporal lobe epilepsy (TLE) (reviewed in Vezzani et al., 2011). In particular, the prototypical pro-inflammatory pathway activated by interleukin-1(IL)β via its functional receptor IL-1R1, is upregulated both in experimental models as well as in human epileptogenic tissue in TLE and in epilepsies associated with malformations of cortical development (Ravizza et al., 2006a, 2008a). Moreover, the activation of IL-1β signaling in adult rodent forebrain exacerbates limbic seizures (Balosso et al., 2008; Vezzani et al., 2000, 2008) and contributes to precipitation of febrile-like convulsions in immature rodents (Dubé et al., 2005; Heida and Pittman, 2005). Whether IL-1β system is activated and has a proconvulsant role in absence epilepsy is still unknown.

In the present study, we show that IL-1β is induced in activated astrocytes specifically in the somatosensory cortex of adult GAERS but not in non-epileptic control Wistar rats. Importantly, the changes in IL-1β anticipate the age-related onset of mature SWDs, thus suggesting a possible contributing role of this cytokine in SWD generation. We further demonstrate that pharmacological treatment with VX-765, which selectively blocks IL-1β biosynthesis (Randle et al., 2001; Ravizza et al., 2006b; Stack et al., 2005), decreases the frequency of SWDs in adult GAERS, highlighting a contributing role of this cytokine to SWD activity.

Materials and methods

Experimental animals

Postnatal day (PN) 14, 20 and adult (PN90) male non-epileptic Wistar rats and GAERS were used (n = 5–9 for each experimental group). PN0 was defined as the day of birth. Wistar rats were obtained from Marmara University Experimental Animal Unit, and GAERS were provided from the breeding colony of Marmara University School of Medicine Department of Pharmacology and Clinical Pharmacology. The rats were housed with their dams at a constant temperature (21 ± 3 °C) and relative humidity (60%) with a fixed 12 h light–dark cycle and free access to food and water. The pups were housed with their dams until weaning at PN21. Older animals were housed in groups of two per cage. For each experimental group, male pups were taken from three separate litters. Procedures involving animals and their care were approved by the Marmara University Ethical Committee for Experimental Animals (41.2009.MAR).

Immunocytochemistry

The changes in the expression of glia markers and alterations in cell morphology indicative of glia activation, as well as the expression of IL-1β, were investigated at three different stages of SWD development in GAERS, namely at PN14, when no SWDs have yet developed; at PN20, when "mature" SWDs have not developed yet, and at PN90, when "mature" SWDs are established (Carcak et al., 2008; Vergnes et al., 1986). We did not perform routine EEG analysis to detect the occurrence of SWDs in GAERS used for histological evaluations to avoid compromising the quality of the histological preparations in brain tissue surrounding implanted electrodes. However, 4 out of 9 PN20 GAERS were EEG recorded to evaluate whether "immature-type" of SWDs occurred (Fig. 6).

Transcardial perfusion

Rats were deeply anesthetized with ketamine (100 mg/kg intraperitoneally, i.p.) and xylazine (10 mg/kg i.p.), and perfused through the ascending aorta with 50 mM cold phosphate-buffered saline (PBS, pH 7.4) followed by chilled 4% PAF in PBS. The brains were post-fixed at 4 °C for 90 min, and then transferred to 20% (PN90 rat) or 30% (PN14 and PN20 rats) sucrose in PBS at 4 °C for 24 h. Then, the brains were immersed in −40 °C (PN14 and PN20 rats) or −50 °C (PN90 rats) isopentane for 3 min and stored at −80 °C until assayed.

Serial coronal sections (40 μm) were cut from 0 to −6.6 mm from bregma according to Paxinos and Watson(2005), and collected in 100 mM PBS. We selected 7 different brain levels for the subsequent immunocytochemical analysis: bregma 0, −1.4, −2.5, −3.6, −4.8, −5.7 and −6.6. We prepared 7 series of 8 sections each: the .first section was stained for IL-1β, the 2nd for GFAP, the 3rd for OX-42, the 4th for c-fos, while the other sections were used for a double-immunostaining analysis (see below).

IL-1β

IL-1β immunostaining was carried out as previously described (Ravizza et al., 2008a). Briefly, slices were incubated at 4 °C for 10 min in 70% methanol and 2% H_2O_2 in Tris–HCl-buffered saline (TBS), followed by 30 min incubation in 10% fetal bovine serum (FBS) in 1% Triton X-100 in TBS. The, slices were incubated overnight at 4 °C in the same medium with the primary antibody against IL-1β (1:200, Santa Cruz Bio., CA, USA). Immunoreactivity was tested by the avidin–biotin–peroxidase technique (Vector Labs, Burlingame, CA, USA) using 3′,3′-diaminobenzidine (DAB; Sigma-Aldrich, Munich, Germany) as chromogen, and the signal was amplified by nickel ammonium.

Glial markers

Slices were incubated at 4 °C for 30 min in 0.4% Triton X-100 in PBS followed by a 15 min incubation in 3% FBS in 0.1% Triton X-100 in PBS. They were subsequently incubated overnight at 4 °C in 3% FBS in 0.1% Triton X-100 in PBS, with the following primary antibodies: mouse anti-CD11b (complement receptor type 3, OX-42, 1:100, Serotec Ltd, Oxford, UK), a marker of microglia/macrophages, or with mouse anti-glial fibrillary acidic protein (GFAP, 1:2500, Chemicon Int. Inc., Temecula, USA), a selective marker of astrocytes. Immunoreactivity was tested by avidin–biotin–peroxidase technique (Vectastain ABC kit, Vector Labs, USA), using 3′,3′-diaminobenzidine (DAB; Sigma-Aldrich) as chromogen.

c-fos

This analysis was done since c-fos is a well established surrogate marker of neuronal network activation (Morgan et al., 1987). Slices were incubated at room temperature for 30 min in 0.3% H_2O_2 in PBS followed by a 2 h incubation in 3% FBS in 0.25% Triton X-100 in PBS. They were subsequently incubated for 48 h at 4 °C with the primary antibody against c-fos (1:10,000, Oncogene, Cambridge, MA, USA) in 3% FBS in 0.25% Triton X-100 in PBS. Immunoreactivity was tested using signal amplification as described above.

No immunostaining was observed when the slices were incubated with the primary antibodies pre-absorbed with the corresponding peptides (Marcon et al., 2009; Ravizza et al., 2008a), or without the primary antibodies.

Double-immunostaining

Co-localization studies were carried out to identify the cell types expressing IL-1β. Two slices were used from each brain for each cell type marker. After incubation with the primary antibody against IL-1β, slices were incubated in biotinylated secondary anti-goat antibody (1:200, Vector Labs), then in streptavidin-HRP and the signal was revealed with tyramide conjugated to Fluorescein using a TSA amplification kit (NEN Life Science Products, Boston, MA, USA).

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