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Analysis of the acute neuroinflammatory response and endogenous markers in the amygdala of animals submitted to status epilepticus by intrahippocampal pilocarpine application

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Abstract

Introduction: Experimental evidence and clinical evidence indicate that the inflammatory process is a crucial mechanism in the pathophysiology of epileptic seizures and temporal lobe epilepsy. The amygdala when involved in an atypical processing is associated with multiple moods such as depression and anxiety disorder and psychiatric disorders such as schizophrenia. **Objective:** This study investigated the acute inflammatory process and modulation of the endogenous proteins' galectins and AnxA1 in the amygdala of animals submitted to an experimental model of temporal lobe epilepsy. **Methods:** The experimental procedures were approved by the Ethics Committee on the Use of Animals at UNIFESP (CEUA nº2958050814). The experiments performed in this study used data and materials that were obtained from the project "Neuroprotective and anti-inflammatory role of the mimetic peptide ac2-26 of the annexin a1 protein in intrahippocampal pilocarpine-induced status epilepticus" conducted by the advisor. The experimental model used male Wistar rats that were divided into 3 experimental groups (NAIVE; SHAM, Status Epilepticus or SE - n = 5 animals/group). Once acclimated, the animals in the SHAM and SE groups underwent stereotaxic surgery for implantation of the intracerebral cannula in the right hippocampus. The SHAM animals received sterile saline in all procedures and the NAIVE group only manipulated. The animals were monitored throughout the period and after 24 hours of experiment all animals were euthanized by overdose of thiopental to remove the brain and performed histological and immunohistochemical analysis. **Results and Conclusion:** Initial results demonstrate that SE and the acute inflammatory process cause damage to the amygdala, and there is also modulation of inflammatory markers in this structure. However, further studies are needed to better understand the mechanism of action in neuroinflammation in status epilepticus.

Keywords: Neuroinflammatory. Endogenous markers. Amygdala. Animals.

Introduction

Experimental evidence and clinical evidence indicate that the inflammatory process is a crucial mechanism in the pathophysiology of epileptic seizures and temporal lobe epilepsy. One of the structures of extreme physiological importance affected in this pathology is an amygdala that, together with other regions, forms a basic system in emotional processing, memory formation and affective behavior. Thus, the amygdala when involved in an atypical processing is associated with multiple moods such as depression and anxiety disorder and psychiatric disorders such as schizophrenia [1].

Given this situation, there is an increase in interest in identifying markers and new treatments. In this regard, proteins from the class of galectins (1, 3 and 9) and annexin A1 (ANXA1) evaluated as anti-inflammatory or as participants in this process are interesting targets in the understanding of neuroinflammation and epilepsy. Annexin A1 (AnxA1) is a protein involved in the modulation of molecular and cellular steps in the inflammatory response and its expression has already been demonstrated in ependymal cells, microglia, astrocytes, neurons and vessels [2,3].

In experimental epilepsy in rats, the administration of the peptide Ac2-26 of AnxA1 during the induction of status epilepticus (SE) showed a significant neuroprotective effect in the CA1 region of the hippocampus in rats compared to untreated animals [4]. In that same study, pharmacological treatment with Ac2-26 produced a reduction in the astrocytic reaction, release of cytokines IL-1β, IL-6 and GRO / KC chemokine, in addition to ERK activation. Under normal physiological conditions, galectins also play a role in maintaining CNS homeostasis, participating in neuronal myelination, neuronal stem cell proliferation and apical vesicle transport. The main CNS-expressed galectins are a galectin-1 that is highly expressed in neuronal cells and neural stem cells [5], galectin-3 that is expressed by microglia, and its expression is induced in the ischemic brain, and galectin-9 which can be detected in astrocytes [6].

On the other hand, in CNS injury caused by autoinflammatory diseases or trauma, some galectins are induced in microglial cells and neurons and may contribute to neuronal regeneration or Wallerian degeneration [7]. Together, these data reveal that ANXA1 and galectins 1, 3, and 9 play a significant role in CNS diseases that, although having a varied and often undefined etiology, share a common neuroinflammatory component.

Therefore, this study investigated the acute inflammatory process and modulation of the endogenous proteins' galectins and AnxA1 in the amygdala of animals submitted to an experimental model of temporal lobe epilepsy.

Methods

The experiments performed in this study used data and materials that were obtained from the project "Neuroprotective and anti-inflammatory role of the mimetic peptide ac2-26 of the annexin a1 protein in intrahippocampal pilocarpine-induced status epilepticus" conducted by the advisor. The experimental model used male Wistar rats that were divided into 3 experimental groups (NAIVE; SHAM, Status Epilepticus or SE - n = 5 animals/group). Ten days before the experiment was conducted, the animals were taken to the site so that they could be adapted to the environment, thus avoiding any external interference. Once acclimated, the animals in the SHAM and SE groups underwent stereotaxic surgery for implantation of the intracerebral cannula in the right hippocampus according to the following coordinates of Paxinos, Watson (2006) [8]: AP-5.9mm, ML-4.3mm and DV3.5mm. After seven days of recovery, status was induced by intrahippocampal pilocarpine administration in the SE group and, after 4 hours, Diazepam was administered by intraperitoneal injection to complete this process. The SHAM animals received sterile saline in all procedures and the NAIVE group only manipulated. The animals were monitored throughout the period and after 24 hours of experiment all animals were euthanized by overdose of thiopental to remove the brain and performed histological and immunohistochemical analysis.

Ethics Approval

The experimental procedures were approved by the Ethics Committee on the Use of Animals at UNIFESP (CEUA nº2958050814).

Results and Discussion

By direct analysis over a 24-hour period of the animals submitted to the experiments, it was observed that all animals with SE had generalized seizures (Racine 3 to 5) characterized by paw clonias, body elevation followed by fall and loss of body control. SE caused bilateral neuronal degeneration in the tonsil areas, evidenced by the analysis of positive Fluoro Jade-C cells, demonstrating the effectiveness of damage caused by epileptic seizures. The bilateral lesion present in all post-SE animals corroborates data from other studies, in which neurodegeneration and glial alterations did not only occur ipsilaterally to pilocarpine injection, but in a generalized way, since the neuronal circuitry interconnects various regions of the brain [1,9].

Immunohistochemical analyzes showed the presence of microglia (Iba-1+) and astrocytes (GFAP+) activated in the tonsil of the SE group. Gliosis is a common feature of the brains of patients and animal models of seizures and epilepsy and, if this condition is not resolved in the post-acute or pre-chronic period, it has an inhibitory effect on nervous tissue regeneration after injury [10-12]. Furthermore, it was detected the expression of annexin A1 and its receptor (Fpr2) in the amygdala of all experimental groups and its modulation during SE. Considering these findings, the ANXA1-Fpr2 system should act in the SE model as a tool to control the inflammatory process.

Conclusion

Initial results demonstrate that SE and the acute inflammatory process cause damage to the amygdala, and there is also modulation of inflammatory markers in this structure. However, further studies are needed to better understand the mechanism of action in neuroinflammation in status epilepticus.

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Ethics approval
The experimental procedures were approved by the Ethics Committee on the Use of Animals at UNIFESP (CEUA nº2958050814).

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Data sharing statement
No additional data are available.

Conflict of interest
The authors declare no conflict of interest.

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