Epilepsy Research (2014) **108**, 379—389

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

Pilocarpine-induced epilepsy is associated with actin cytoskeleton reorganization in the mossy fiber-CA3 synapses

Yan-Feng Zhang[a,c], Tian-Qing Xiong[a], Bai-Hong Tan[b], Yan Song[d], Shu-Lei Li[a], Li-Bin Yang[c], Yan-Chao Li[a,*]

[a] *Department of Histology and Embryology, College of Basic Medical Sciences, Norman Bethune Health Science Center of Jilin University, Changchun, Jilin Province 130021, PR China*
[b] *Laboratory Teaching Center of Basic Medicine, Norman Bethune College of Medicine, Jilin University, Changchun, Jilin Province 130021, PR China*
[c] *Pediatric Neurology, First Hospital of Jilin University, Changchun, Jilin Province 130021, PR China*
[d] *Nursing College, Beihua University, Jilin, Jilin Province 132013, PR China*

Received 13 July 2013; received in revised form 21 January 2014; accepted 24 January 2014
Available online 3 February 2014

KEYWORDS
Filamentous actin;
Mossy fiber;
Status epilepticus;
Pilocarpine

Summary Dramatic structural changes have been demonstrated in the mossy fiber-CA3 synapses in the post status epilepticus (SE) animals, suggesting a potential reorganization of filamentous actin (F-actin) network occurring in the hippocampus. However, until now the long-term effects of SE on the synaptic F-actin have still not been reported. In this study, phalloidin labeling combined with confocal microscopy and protein analyses were adopted to investigate the effects of pilocarpine treatment on the F-actin in the C57BL/6 mice. As compared to the controls, there was ~ 43% reduction in F-actin density in the post SE mice. Quantitative analysis showed that the labeling density and the puncta number were significantly decreased after pilocarpine treatment ($p < 0.01$, $n = 5$ mice per group, Student's t-test). The puncta of F-actin in the post SE group tended to be highly clustered, while those in the controls were generally distributed evenly. The mean puncta size of F-actin puncta was $0.73 \pm 0.19\,\mu m^2$ ($n = 1102$ puncta from 5 SE mice) in the experimental group, significantly larger than that in the controls ($0.51 \pm 0.10\,\mu m^2$, $n = 1983$ puncta from 5 aged-matched control mice, $p < 0.01$, Student's t-test). These observations were well consistent with the alterations of postsynaptic densities in the same region, revealed by immunostaining of PSD95, suggesting the reorganization of F-actin occurred mainly postsynaptically. Our results are indicative of important cytoskeletal changes in the mossy fiber-CA3 synapses after pilocarpine treatment, which may contribute to the excessive excitatory output in the hippocampal trisynaptic circuit.
© 2014 Elsevier B.V. All rights reserved.

* Corresponding author. Tel.: +86 431 85619477.
E-mail address: liyanchao@jlu.edu.cn (Y.-C. Li).

0920-1211/$ — see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.eplepsyres.2014.01.016

Introduction

Status epilepticus (SE) is a life-threatening neurological emergency characterized by persistent and continuous seizure activity (Leite et al., 2002). One potential result of SE is the development of temporal lobe epilepsy (TLE), which is characteristic of spontaneous seizures and persistent deficits in hippocampus-dependant learning (Lowenstein and Alldredge, 1998; Cornaggia et al., 2006). The SE animal models induced by pilocarpine and kainate are the most commonly used chemical models for the TLE. Animals are administered with a single convulsive dose of pilocarpine or kainate, and typically display spontaneous recurrent seizures after a latent period of several weeks (Leite et al., 1990; Cavalheiro et al., 1996).

In the post SE animals, persistent structural modifications have been found in both pre- and postsynaptically in the MF-CA3 synapses, including a significant spine loss, and a corresponding increase in bouton area (McAuliffe et al., 2011; Upreti et al., 2012). The MF boutons are among the largest and most potent presynaptic terminals in the brain. Their postsynaptic structures consist of complex thorny excrescences, and are much larger than most of other dendritic spines (Blackstad and Kjarheim, 1961; Chicurel and Harris, 1992; Henze et al., 2002). By such unique organization, the MF-CA3 synapses play an important role in the hippocampal trisynaptic circuit, and through them the abnormal excitability within dentate gyrus exerts their principal effect on the downstream CA3 pyramidal cells (McAuliffe et al., 2011). Because the maintenance and plasticity of synaptic structures depend mainly on the dynamics of synaptic actin, the reconstruction of MF-CA3 synapses suggests a potential reorganization of actin cytoskeleton in the post SE animals.

Actin is a major cytoskeletal component in the synapses, where the balance between filamentous actin (F-actin) and monomeric actin (G-actin) is modulated exquisitely by a variety of dynamic events occurring during neuronal communications (Dillon and Goda, 2005). Depolymerizing F-actin by latrunculin A in rat hippocampus has been reported to induce acute epileptic seizures and long-term changes in neuronal excitability, leading to the onset of sporadic spontaneous seizures (Sierra-Paredes et al., 2006). An acute reduction of F-actin has also been demonstrated in mouse hippocampus immediately after the SE induced by kainite (Zeng et al., 2007) and by 4-aminopyridine (Ouyang et al., 2007). These data suggested that not only the seizure activity itself can modulate the dynamics of synaptic actin, but the disturbance of actin network also contributes to the permanent epileptic state.

Although the early alterations of F-actin have been noted in the SE animals, the long-term effects of SE on F-actin have still not been reported. Given the dramatically structural changes at the synaptic level, it is intriguing to know how F-actin is reorganized in the MF-CA3 synapses after the chronic seizure activity was established.

In this study, phalloidin, a highly specific probe for F-actin, together with protein analyses, was adopted to investigate the long-term changes of synaptic actin network in the C57BL/6 mice. Since the alterations of F-actin might occur both pre- and postsynaptically, the cellular localization of F-actin was further examined by combining phalloidin labeling with immunochemical detections of pre- and postsynaptic markers.

Materials and methods

The post SE model of pilocarpine

Experiments were carried out on 10–12 week-old male C57BL/6 mice (20–22 g), which were purchased from the Experimental Animal Center of Jilin University. A total of 150 mice were used, including 120 for pilocarpine injection, 30 for the controls. All the experiments related to this study were approved by the Animal Research Committee of Jilin University. The animals were handled under the control of the Animal Experiment guidelines of Jilin University and in accordance with NIH Guide for the Care and Use of Laboratory Animals.

The animals were kept on a 12-h light-dark cycle and allowed free access to food and water, and the experiments were performed between 8:00 and 12:00 in a silent room at the temperature of 22–26 °C. The mice were injected intraperitoneally with 1 mg/kg methylscopolamine (Sigma–Aldrich, MO, USA) in sterile saline, followed with a single dose of 365 mg/kg of pilocarpine (Sigma–Aldrich, MO, USA) in saline 30 min later. Control mice were administered with a comparable volume of saline after the initial methylscopolamine treatment. After pilocarpine injection, the animals were observed for the development of continuous seizure activity, which was evaluated according to a modified version of the Racine scale (Shibley and Smith, 2002).

All mice that developed SE received 10% chloral hydrate (300 mg/kg, i.p.) 2 h after onset. Mice in the control group received the same dose of chloral hydrate at the same time. The mice that experienced lethal convulsions or did not develop SE were excluded from the study. The animals were supplied with water moistened chow for 4 days after SE induction to help replenish fluids, and were monitored 2 h/day and 5 days/week up to 2 months after pilocarpine injection.

Sample preparations

Two months after pilocarpine treatment, 18 animals, including 9 control and 9 post SE mice, were transcardially perfused with 0.4% sodium sulphide in 0.1 M phosphate buffer (PB, pH 7.2) under deep anesthetization with 10% chloral hydrate. After a brief wash with 20 ml saline, the animals were fixed with 4% paraformaldehyde in 0.1 M PB.

The brains were dissected out, postfixed in the same fixative for 2–6 h at 4 °C, and then placed in a series of cold sucrose solutions of increasing concentration. The samples were embedded in OCT compound (Tissue-Tek, Sakura Finetek), frozen on dry ice, and then cut coronally into 40-μm-thick serial sections using a Leica cryostat.

As a second assay, 10 mice (5 control and 5 post SE mice) were randomly selected, and the brains were dissected out under deep anesthetization. The left and right hippocampi were further separated for the analysis of F/G ratio and for the analysis of synapsin I and PSD95, respectively.

Download English Version:

https://daneshyari.com/en/article/3052100

Download Persian Version:

https://daneshyari.com/article/3052100

Daneshyari.com