The rearrangement of synaptic actin networks after pilocarpine-induced status epilepticus and pentylenetetrazol-induced kindling

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Post status epilepticus (SE) and chemical kindling are two widely used animal models for epileptic studies. The pilocarpine-treated C57BL/6 mice exhibited stereotypical alterations of F-actin networks, including a severe reduction of F-actin in area CA1-CA3 and in the hilum. By contrast, F-actin networks seemed less affected by pentylenetetrazol kindling, and almost no remarkable alterations were noted in area CA1 or in the hilum. The overall labeling of F-actin in the hippocampus was generally consistent with the pathological observations on dendritic spines reported in both the epileptic models. Because the reorganized F-actin network can lead to long-term stabilization of synaptic changes and to consolidation of the enhanced neuronal activity, the alterations of F-actin networks may be related to the aberrant hyperexcitability in the epileptic animals.

Keywords: Chemical kindling; status epilepticus; filamentous actin; synapse; hippocampus


Introduction

As a usual chronic neurological disease, epilepsy is known to show recurrent spontaneous seizures, which is generally believed to result from a shifted balance between the excitation and inhibition in the central nervous system [1]. Post status epilepticus (SE) and chemical kindling are two widely used animal models for epileptic studies [1, 2, 3, 4]. The post SE model is induced by injecting a single convulsive dose of pilocarpine or kainate in the animals, which develop to a life-threatening neurological state with persistent and continuous seizure activity [3, 4]. The kindling model is induced by repeated administrations of a low dosage of convulsive drugs, such as pentylenetetrazol (PTZ), which finally leads to generalized tonic-clonic seizures in the animals [1,2].

Morphological analysis demonstrated severe neuronal damage after pilocarpine treatment, including extensive cell death, glial proliferation and mossy fiber (MF) sprouting in the hippocampus [5, 6, 4, 7, 8]. By contrast, chemical kindling tends to induce less severe alterations in the central nervous system [9,10]. Although the extent to which the brains were damaged is considerably different between the post SE and the kindled animals,
animals of both models have been demonstrated to show significantly enhanced neuronal excitability. For example, the post SE animals have been observed to develop to spontaneous recurrent seizures after several weeks following the injection [11, 12], while the kindled state induced by PTZ has been confirmed to remain for at least 10 months after drug discontinuation [13]. It is obvious that only severe neuronal damage cannot explain the resultant epileptic activity, and subcellular structural modifications facilitating aberrant firing must have occurred in the existing neuronal circuits [17].

F-actin and synaptic plasticity

As one of the major cytoskeletal component in the synapse, actin is known to play both structural and functional roles in the central nervous system. A few studies have reported an acute depolymerization of filamentous actin (F-actin) in the CA1 region of hippocampus immediately after the SE [14, 15], suggesting that seizure-dependent changes in F-actin networks may represent a subcellular mechanism that is relevant to both structural and non-structural abnormalities in the epilepsy.

Actin is perhaps the most abundant protein in nerve cells, and plays various roles in every biological event by way of a dynamic equilibrium between monomeric and filamentous forms (F-actin). In the presynaptic region, actin functions to control the availability of the reserve synaptic vesicle pool, regulate synaptic vesicle exocytosis, and is also necessary for clathrin-mediated endocytosis [16, 17, 18, 19]. In the postsynaptic region, F-actin forms complex networks to maintain the morphology of dendritic spines and control the plastic changes of spines [20, 21]. Given so many important roles of F-actin in the synapse, even a modest change in F-actin may result in profound effect on synaptic activities. So far, the long-term changes of F-actin following SE or kindling have not still been reported.

The structural components of hippocampal trisynaptic pathway are known to display different sensitivities to seizure-induced cell death [5, 6, 4, 7, 8], suggesting that F-actin network may be also heterogeneously alternated in the hippocampus.

The rearrangement of F-actin during epilepsy

Recently we have examined the changes of F-actin in the entire hippocampus in two different epileptic models by use of C57BL/6 mice [22, 23]. A previous study has shown that C57BL/6 mice are susceptible to pilocarpine-induced seizures and that almost all the mice that survived SE developed to spontaneous tonic–clonic seizures [24]. As many other mouse strains did, the post SE C57BL/6 mice exhibited widespread neuronal damage and prominent ectopic MF sprouting in the hippocampus [24]. In our experiments, similar results have been obtained [22].

By contrast, C57BL/6 mice are relatively resistant to the excitotoxic neuronal death induced by PTZ or kainate [25, 26, 27]. They exhibited a significant delayed onset of generalized clonic convulsions in the kindling experiment, compared with other mouse strains [28, 29]. In addition, only limited cell loss was observed in area CA1-CA3 and in the hilum in this strain of mice after kindling. On the other hand, the PTZ-kindled mice did exhibit aberrant hyperexcitability.

Examinations of F-actin in the hippocampus showed that the pilocarpine-treated mice exhibited stereotypical alterations in F-actin networks, including a severe reduction of F-actin in area CA1-CA3 [22]. Since F-actin is localized mainly in dendritic spines, the overall labeling of F-actin is thought to reflect the extent to which dendritic spines have been damaged. In this regard, the alterations of F-actin in the pilocarpine-treated mice were well consistent with the pathological observations on dendritic spines in the hippocampus. A variety of studies have reported that pilocarpine treatment resulted in severe neuronal death in area CA1-CA3 and in the hilum [4, 5, 6, 7, 8, 15]. Therefore, the loss of dendritic spines due to neuronal death may lead to the overall reduction of F-actin in the hippocampus.

In addition, several studies have documented a significant enlargement of postsynaptic densities in the existing dendrites in the stratum lucidum of area CA3 following pilocarpine treatment [30, 31]. Consistent with this, we also noted a dramatic increase of F-actin in puncta area in the same region [22]. It is very likely that the enlargement of F-actin puncta was an accommodated reaction of the postsynaptic structures to the relatively more presynaptic terminals.

In contrast with the dramatic alterations of F-actin in the pilocarpine-treated mice, F-actin networks seemed less affected by PTZ injections [23]. Almost no remarkable alterations of F-actin were noted in area CA1-CA3. We did find that the fluorescence became a little weakened in the CA3 regions after PTZ treatment, but quantitative analysis has revealed an unexpected increase of F-actin in labeling density (positive puncta area) in the CA3 stratum lucidum.

Given that F-actin is enriched in dendritic spines, the overall labeling of F-actin should have been decreased if the dendritic spines were severely damaged. Because only limited cell loss was observed in area CA3 in the experimental mice, one of possible explanations is that
the slight increase of F-actin was due to the overcompensation of the existing dendritic spines.

**In summary**

Since the pilocarpine-induced post SE induced more stereotypical alterations of F-actin networks in the hippocampus relative to the PTZ-induced kindling, these studies provided a piece of evidence that the seizure-induced modulations in F-actin networks are related to the severity and kind of the convulsive stimulus. Because the reorganized F-actin network can lead to long-term stabilization of synaptic changes and to consolidation of the enhanced neuronal activity \[32, 33\], the alterations of F-actin networks found here may be related to the aberrant hyperexcitability in the epileptic models.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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