Increased expression of calponin-3 in epileptic patients and experimental rats

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Abstract

Calponin-3 is an actin-interacting protein and is expressed in the brain. Our previous microarray scan has found an up-regulation of calponin-3 gene CNN3 in the temporal lobe of patients with drug-resistant epilepsy. Here we investigated in epileptic patients the changes of brain and cerebrospinal fluid (CSF) calponin-3 expressions, and assessed calponin-3 expression pattern in a rat model of pilocarpine-induced epilepsy. We showed that in the temporal neocortices of 30 patients with drug-resistant epilepsy, both mRNA and protein level of calponin-3 were significantly increased. In addition, the augmentation of CSF calponin-3 from 126 epileptic patients was closely correlated with disease duration. Moreover, in the cortices of temporal lobes of pilocarpine-treated rats, calponin-3 increased along with the time and maintained at significant high levels for up to 2 months, while the up-regulation of hippocampal calponin-3 only occurred at 24 h and 1 week. The elevated calponin-3 suggests that deregulation of actin filament dynamics in axonal and dendritic outgrowth and synaptic rearrangement may contribute to pathophysiology of epilepsy.

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Introduction

Epilepsy is a common and chronic brain disorder, affecting approximately 50 million people worldwide (Hauser et al., 1993). Patients with epilepsy display an enduring predisposition to generate unprovoked seizures (Schuele and Luders, 2008). Animal and human tissue studies have suggested that development of epilepsy involves a cascade of molecular, cellular and structural alterations (Rakhade and Jensen, 2009). Recurrent neuronal firings are accompanied with granule cell axon (mossy fiber) sprouting, morphological and structural property changes of dendritic spines and network reorganization (Rakhade and Jensen, 2009; Wong, 2005).

Lines of evidence have shown that disruption of cytoskeleton dynamics contributes to the pathophysiology of epilepsy (Gardiner and Marc, 2010). For example, mutations of fimatin A gene that encodes an actin-binding phosphoprotein are associated with periventricular heterotopias, and epilepsy is one of the major clinical features (Parrini et al., 2006). Deficiency of actin-serving protein gelsolin exacerbates seizure-induced damage to hippocampal neurons (Furukawa et al., 1997). Conversely, seizure induces activation of actin-interacting protein coflin and loss of neuronal F-actin in an animal model of epilepsy (Zeng et al., 2007). In patients with intractable epilepsy, neuronal Wiskott-Aldrich syndrome protein (N-WASP) that promotes actin polymerization is up-regulated (Xiao et al., 2008). It is general consensus that actin filaments are actively involved in axonal and dendritic outgrowth and play a critical role in cellular process such as endocytosis and exocytosis (Kuromi and Kidokoro, 2005; Witte and Bradke, 2008). However, the functional role of actin and its binding partners in the pathophysiology of epilepsy has not been well studied.

Calponin-3, also called acid calponin, is encoded by calponin-3 gene (CNN3) that was first identified in 1995 (Maguchi et al., 1995). As one of the three isoforms of calponin family that functions as calmodulin and actin binding partners, calponin-3 is expressed in the central nervous system (Plantier et al., 1999; Rozenblum and Gimona, 2008). Study has demonstrated that the interaction of calponin-3 with actin filament reduces the sliding of actin on myosin, leading to depressed retraction of spine heads (Rami et al., 2006). In neurons calponin-3 colocalizes with excitatory synaptic markers PSD95 and glutamatergic receptors GluR1 and NR1 (Rami et al., 2006). Over-expression of calponin-3 in the cultured hippocampal neurons results in elongation of dendritic spines and enhanced excitatory postsynaptic current (Rami et al., 2006). Our previous study has found that the CNN3 gene is up-regulated in the anterior temporal neocortices of patients with drug-resistant epilepsy (Xi et al., 2009). Therefore, we hypothesized that calponin-3 may be deregulated in patients with epilepsy. In this study, we first measured the expression...
level of calponin-3 in the temporal cortices and CSF of patients with epilepsy; to rule out the possibility that antiepileptic drugs (AEDs) may have impact on calponin-3 expression, we investigated the dynamic changes of calponin-3 in the brain of a rat model.

Methods

Human brain tissue collection

All temporal neocortical samples were drawn randomly from our human brain bank. This bank consisted of 200 epileptic tissues and 60 control tissues, which were collected from the Departments of Neurosurgery of Beijing Tiantan Hospital, Xuanwu Hospital of the Capital University of Medical Sciences, Xinqiao Hospital of the Third Military Medical University and The First Affiliated Hospital of Chongqing Medical University from year 2004 to 2007.

Thirty brain samples that were used for mRNA and protein expression profiles were obtained from the patients with drug-resistant epilepsy (20 males and 10 females with a mean age of 21.73 ± 6.59 years; range: 16–52 years). The mean age at seizure onset was 9.05 ± 6.59 years (range: 0–25 years) and the mean epilepsy duration was 12.55 ± 7.06 years (range: 3–30 years). The procedures for the location of epileptogenic foci and the diagnosis of drug-resistant epilepsy have been described previously in our recent study (Xi et al., 2009). Samples were taken following anterior temporal lobectomy for patients who were seizure-free after 2 years of surgery. Neuronal loss and gliosis were the common histopathological findings in all samples. Patients with hepatic or renal insufficiency, active psychosis, severely mental retardation, cardiovascular disease, hematological disease and brain tumors including glioma were excluded from this study.

Ten control brain samples with normal histological manifestation were obtained from age- and gender-matched individuals (6 males and 4 females with a mean age of 27.30 ± 11.07 years; range: 16–52 years). The control patients underwent therapeutic surgical resection after increased intracranial pressure due to head trauma. These patients never manifested seizures and were not exposed to AEDs.

Samples were immediately treated and divided into two portions. One portion was placed into buffered diethylpyrocatechol (1:1000) for 24 h and frozen in liquid nitrogen for reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting analysis. Another portion was fixed in 10% buffered formalin for 48 h and embedded in paraffin for immunohistochemical staining. The different experiments were conducted blindly.

Measurement of brain calponin-3 mRNA level

RT-PCR method for determining the expression of the CNN3 gene was previously described (Xi et al., 2009). A fragment of 498 bp of the CNN3 gene was amplified using primer pair 5′-CTCTGTTGG-CTTCGGACGGTC-3′/5′-CTTCGCCCTCGTGATGAAATG-3′. The following PCR conditions were used: denaturing at 94 °C for 5 min; 35 cycles of 30 s at 94 °C, 30 s at 55 °C, 45 s at 72 °C, and ended with a final 5 min extension at 72 °C. All reactions were repeated in triplicate. Human β-actin was chosen as an internal control. Optical density (OD) ratio of CNN3 over β-actin was calculated.

Determination of calponin-3 expression pattern in the brain

Immunohistochemical staining of calponin-3 was performed as previously described (Pan et al., 2010). The polyclonal rabbit calponin-3 antibody (diluted 1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as the primary antibody. After visual fields in every section were selected randomly, the ratio of the cumulative OD value and the cumulative area was calculated to semi-quantify calponin-3 level.

Double-immunofluorescence labeling of calponin-3 and glial fibrillary acidic protein (GFAP, an astrocyte marker) or microtubule-associated protein-2 (MAP2, a neuron marker) was carried out to determine calponin-3 immunoreactivity in different cell types, as previously described (Pan et al., 2010). The primary antibodies included a polyclonal rabbit calponin-3 antibody (diluted 1:50) and a mouse monoclonal MAP2 antibody (1:50, Wuhan Boster Biological Technology, Wuhan, China) or a mouse monoclonal GFAP antibody (1:50, Boster Bioengineering, Wuhan, China).

Measurement of brain calponin-3 protein level

Western blotting was performed as previously described (Xi et al., 2009). Some modification included that the electrotransformation of protein was at 250 mA for 45 min and the primary antibody was a polyclonal rabbit calponin-3 antibody (diluted 1:200).

Collection of CSF sample

164 patients (including 126 patients with epilepsy and 38 controls) were examined between May 2008 and March 2010 at the Epilepsy Clinic or wards of the Department of Neurology, The First Affiliated Hospital of Chongqing Medical University. Epilepsy was diagnosed and classified according to the criteria proposed by the International League Against Epilepsy (Blume et al., 2001). All patients recruited in this study manifested at least 2 episodes of epileptic attacks. Standard evaluation included medical history, physical and neurological examination, blood and CSF screening tests, electroencephalography (EEG) and brain magnetic resonance imaging (MRI) study for diagnosis or differential diagnosis. Only patients who displayed normal blood and CSF profiles were included in this study. The following situations were excluded from study: 1) patients recently presented with the neurological diseases that might increase the permeability of blood–brain barrier, such as encephalitis or meningitis, stroke and multiple sclerosis; 2) patients with active psychosis, severely mental retardation or psychotropic treatment; 3) patients with cardiovascular disease or tumor including glioma which might cause high calponin levels in the blood; 4) patients with hepatic or renal insufficiency that might influence protein synthesis or hemoglutination. Age- and gender-matched controls were without seizures, family history of epilepsy or exposure to any medications.

CSF samples were obtained by lumbar puncture. The pink CSF samples were not included in this study. The samples were centrifuged at 2000 rpm for 10 min at 4 °C. Aliquot of supernatant was stored at −80 °C.

Measurement of CSF calponin-3 level

Calponin-3 levels in CSF samples were determined by a double-antibody sandwich ELISA (enzyme-linked immunosorbent assay) according to the manufacturer’s recommendations (Baili Biotech., Shanghai, China). All standard materials and CSF samples were detected in duplicate. The OD of each well was measured at 450 nm with a Multiskan® Spectrum Microplate Spectrophotometer (Thermo Fisher Scientific). The conductor was blinded to the information of diagnosis.

Rat model of epilepsy

Healthy 6–8-week-old male Sprague Dawley rats were randomly divided into the epileptic group or the control group. 72 rats were randomly divided into nine subgroups (control, 6 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 1 month, 2 months after pilocarpine-induced
seizures, respectively, n=8 per subgroup). Epilepsy was induced by lithium chloride and pilocarpine injection (Hanaya et al., 2007). Control rats were injected with saline. Most rats began to display seizures about 15–30 min after pilocarpine injection. The evoked seizures were scored according to Racine (Racine, 1972): Stage 0 = arrest, wet dog shakes, normal behavior; Stage 1 = facial twitches (nose, lips, eyes); Stage 2 = chewing, head nodding; Stage 3 = forelimb clonus; Stage 4 = rearing, falling on forelimbs; Stage 5 = imbalance and falling on side or back. Only those rats that reached Racine's stages 4–5 were included in later experiments. Diazepam (10 mg/kg, i.p., Sigma, USA) was injected to control status epilepticus. After this all rats were video-monitored until the end of experiment. Rats underwent intermittently seizures of Racine's stages 3–5 in 2 to 6 days (the acute stage), followed by a seizure-free period of 7 to

Fig. 1. Changes of calponin-3 gene and protein in the temporal neocortices of patients with epilepsy. (A) Representative RT-PCR results of calponin-3 gene (CNN3) mRNA in patients with epilepsy and in the control. (B) Statistical analysis of CNN3 over β-actin mRNA ratios between the two groups. (C) Immunohistochemical staining showed more strongly stained cells in the epileptic brain compared with those in control. (D) Semi-quantified analysis showed significantly increased mean optical density value of calponin-3 in the epileptic group compared to control group. (E) Double-immunofluorescence labeling showed that calponin-3 (green) was co-expressed with GFAP and MAP2. In control, faint calponin-3-immunoreactivity was found and co-expressed with GFAP in the astrocytes (yellow). In epilepsy, calponin-3-immunoreactivity (green) was strong and co-expressed with MAP2 (yellow). (F) Representative Western blotting results of calponin-3 in patients with epilepsy and control. (G) Semi-quantified analysis of Western blotting showed a significant difference of calponin-3 between the two groups. Ctl: control; EP: epilepsy; GFAP: glial fibrillar acidic protein; MAP2: microtubule-associated protein-2; OD: optical density. *p<0.05; **p<0.01; ***p<0.001.
21 days (the silent stage), and then again showed recurrent seizures of Racine’s stages 2–5 after 8 to 21 days (the chronic stage). Our results were in agreement with published reports (Okamoto et al., 2010; Turski et al., 1989).

Rats were anesthetized by chloral hydrate and sacrificed. Both sides of temporal cortices and hippocampi were quickly removed and stored at −80 °C for Western blotting analysis. The protocols and the polyclonal rabbit calponin-3 antibody (diluted 1:50) used in the experiment were the same as those in the study of human.

**Ethics**

All brain tissues and CSF samples from humans and animals were obtained and used with the approval of the ethics committee of Chongqing Medical University. All patients or their relatives presented written informed consent after adequate explanation of the aims, methods, and potential hazardous consequences of the study. All of the experiments were performed in compliance with the Declaration of Helsinki.

**Statistical analysis**

SPSS version 16.0 (SPSS Inc, Chicago, IL) was used for statistical analyses. All data values were first tested for the approximation of population distribution to normality. The asymmetrically distributed ELISA results were presented as median values and interquartile ranges (Comabella et al., 2010; Tapiola et al., 2009). All other data results were symmetrically distributed and thus were expressed as mean ± standard deviation (SD). Student’s t-test was used to assess the differences of calponin-3 in human or animal brain tissues. Mann–Whitney U test and Kruskal–Wallis U test were performed to analyze CSF calponin-3 levels. Correlations between calponin-3 CSF levels and clinical parameters in epileptic patients were assessed by Spearman’s rank correlation coefficient (Comabella et al., 2010; Tapiola et al., 2009).

**Results**

**Calponin-3 mRNA and protein expression in the temporal cortices of patients**

RT-PCR results showed that CNN3 mRNA levels in the temporal cortices were significantly higher in patients with epilepsy than in non-epileptic controls ($p < 0.001$) (Figs. 1A and B). Immunohistochemistry images showed a weak immunostaining in the control cortices. However, the epileptic tissues showed an abundant and strong positive immunostaining for calponin-3 (Fig. 1C). Quantification analysis of calponin-3 protein expressions showed that there was a significant difference in the mean OD values ($p = 0.024$) (Fig. 1D) between the two groups. To determine cell-type specific expression of calponin-3, we assessed calponin-3-immunoreactivity by double-
Calponin-3 was expressed in astrocytes under normal conditions. Calponin-3 was normally presented in MAP2 positive neurons ([Ferhat et al., 2003], data not shown). In the epileptic tissues, calponin-3 was strongly stained and colocalized with MAP2 (Fig. 1E), suggesting a marked increase of calponin-3 expression in the neurons in the epileptic cortices. To quantitatively analyze the differences of calponin-3 between the epilepsy and control, we performed Western blotting study. Calponin-3 protein presented as a single band at approximately 37 kDa. The protein expression was significantly higher in patients with epilepsy than in control ($p = 0.006$) (Fig. 1F). These results demonstrated that calponin-3 was significantly increased in the brain tissues of patients with epilepsy.

### Group characteristics in CSF study

The CSF study enrolled 126 patients with epilepsy and 38 controls. There was no statistically difference in the age and gender between the two groups (Table 1).

In the epileptic group, the onset age ranged from 1 to 73 years (average $25.59 \pm 16.30$ years), epilepsy duration ranged from 1 day to 35 years (average $7.73 \pm 9.31$ years), and the sampling time to last seizures ranged from 6 h to 120 days. Among 126 patients with epilepsy, 18 patients (14.29%) presented with complex partial seizures (CPS), 52 (41.27%) with secondarily generalized tonic clonic seizures (SGTCS), 48 (38.10%) with generalized tonic clonic seizures (GTCS), and 8 (6.35%) presented with other seizure types.

### Table 3

CSF calponin-3 in subgroups of patients with epilepsy. Note: There were no statistical differences in median CSF calponin-3 concentrations among different epileptic subgroups classified according to gender, seizure type, MRI finding and AED treatment with same duration. Abbreviation: AEDs, antiepileptic drugs; CPS, complex partial seizures; CSF, cerebrospinal fluid; GTCS, generalized tonic clonic seizures; IQR, interquartile range; MRI, magnetic resonance imaging; N, number of cases; SGTCS, secondarily generalized tonic clonic seizures SPS, simple partial seizures.

<table>
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<th>Subgroups</th>
<th>N</th>
<th>Median CSF calponin-3 (IQR), ng/L</th>
<th>P Value</th>
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<tr>
<td>Male</td>
<td>82</td>
<td>1136.30 (939.41)</td>
<td>0.276</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>1251.50 (650.22)</td>
<td></td>
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<tr>
<td>Seizure type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPS</td>
<td>18</td>
<td>1073.10 (474.77)</td>
<td>0.665</td>
</tr>
<tr>
<td>SGTCS</td>
<td>52</td>
<td>1201.30 (986.36)</td>
<td></td>
</tr>
<tr>
<td>GTCS</td>
<td>48</td>
<td>1197.00 (629.24)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
<td>633.60</td>
<td></td>
</tr>
<tr>
<td>Brain MRI scan</td>
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<td>0.799</td>
</tr>
<tr>
<td>Positive findings</td>
<td>44</td>
<td>1117.70 (699.14)</td>
<td></td>
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<tr>
<td>Negative finding</td>
<td>82</td>
<td>1197.00 (629.24)</td>
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<tr>
<td>Duration ≤2 years</td>
<td>68</td>
<td>566.77 (633.60)</td>
<td>0.761</td>
</tr>
<tr>
<td>No AED</td>
<td>45</td>
<td>783.18 (287.33)</td>
<td></td>
</tr>
<tr>
<td>With AEDs</td>
<td>23</td>
<td>1141.00 (401.92)</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 3. CSF calponin-3 profiling in epileptic patients. (A–C) showed there was no significant difference among different epileptic subgroups of seizure types (A), MRI finding (B) and AED treatment with duration ≤2 years (C). (D–F) showed the correlation between CSF calponin-3 and disease duration (D), onset age (E) and the interval between last seizure and CSF collection (F). AED: antiepileptic drug; CPS, complex partial seizures; CSF: cerebrospinal fluid; GTCS, generalized tonic clonic seizures; SGTCS, secondarily generalized tonic clonic seizures.
seizures (SGTCS), 48 (38.10%) with generalized tonic clonic seizures (GTCS), and 8 (6.34%) with other seizure types (including simple partial seizures (SPS), absence, and myoclonic seizures). In addition, MRI results showed that there were 82 patients with negative finding and 44 patients with positive findings, including hippocampal sclerosis (n=10), brain atrophy (n=14), cerebral ischemia (n=10), encephalomalacia (n=6), demyelinated foci (n=2) and gliosis (n=2). Before lumbar puncture, 54 patients were without having any AEDs while 72 patients were treated with AEDs. In total 68 patients whose disease durations were equal or less than 2 years, 45 patients were with no AED and 23 patients with AEDs treatment.

**CSF calponin-3 levels**

The median CSF calponin-3 concentration in patients with epilepsy showed three-folds more than that in control, and Mann–Whitney U test displayed a significant difference (Table 1, Fig. 2A). Although patients with epilepsy showed a significantly higher calponin-3 in both male and female subgroups compared with control (Fig. 2B), there was no significant difference of calponon-3 between genders in both control and epileptic groups (Table 2). To test if calponin-3 level is related to age, we examined correlations between CSF calponin-3 levels and age. As shown in Figs. 2C and D, no correlation was found in either control (r = 0.133, p = 0.426) (Fig. 2C) or epileptic group (r = –0.103, p = 0.250) (Fig. 2D). These results suggest that the increased CSF calponin-3 is not a gender or age-related phenomenon.

To clarify if AED and brain pathology such as hippocampal sclerosis and brain atrophy may have an effect on calponin-3 function, we further investigated CSF calponin-3 in different epileptic subgroups. First, we compared the median CSF calponin-3 concentrations of different epileptic subgroup classified according to seizure type, brain lesions viewed by MRI and AED treatment with same duration. We found that there was no difference among these subgroups (Table 3, Figs. 3A–C), suggesting that CSF calponin-3 is not influenced by original diseases and AEDs. Secondly, we examined correlations between the CSF calponin-3 levels of patients with epilepsy and remaining clinical variables. Spearman’s correlation test showed that the elevated CSF calponin-3 concentrations in the patients with epilepsy were directly correlated with disease duration (r = 0.396, p < 0.001) (Fig. 3D), whereas no correlation between CSF calponin-3 levels with onset age (r = –0.172, p = 0.057) (Fig. 3E) and the interval between last seizure and CSF collection (r = –0.084, p = 0.603) (Fig. 3F) was found.

**Dynamic changes of calponin-3 in the brain of epileptic rats**

To rule out the possibility that AEDs may have an impact on calponin-3 expression and to validate the finding in human that CSF calponin-3 was closely related to disease duration, we performed an animal study using a rat model of pilocarpine-induced epilepsy. Western blotting analysis demonstrated that the calponin-3 expressions in the temporal cortices of the epileptic rats began to increase at 6 h, reached a peak at 48 h, decreased gradually until the silent stage (1 and 2 weeks) and then increased again during the chronic stage (1 and 2 months). Student’s t-test showed that the calponin-3 levels were significantly higher in the epileptic group than in the control group 6 h after pilocarpine-induced seizures (Fig. 4A). In the hippocampus, calponin-3 levels were significantly higher in epileptic rats compared with controls at 24 h and 1 week (especially at 1 week), but returned to control level in 1 and 2 months after pilocarpine-induced seizures (Fig. 4B). These results demonstrated that while calponin-3 in the cortex of temporal lobe was increased along with the time after pilocarpine-induced seizures, the increase of calponin-3 occurred only at early stage in hippocampus.

**Discussion**

In the present study, we have found that calponin-3 was significantly higher in the brain and CSF of patients with epilepsy. The elevation of CSF calponin-3 was highly correlated with disease duration. In a rat model of epilepsy, the cortical up-regulation of calponin-3 occurred at 24 h and thereafter, while the expression of hippocampal calponin-3 only increased at 24 h and 1 week after seizures, respectively.

![Fig. 4. Dynamic changes of calponin-3 in the temporal lobe cortices and hippocampi after pilocarpine-induced epilepsy in rats. (A) Western blotting study showing representative blots (left) and statistical analysis of calponin-3 (right) in the rat temporal cortex before (Ctl) and after different time points following pilocarpine-induced seizures. Significant differences were shown at all time points except at 6-hour point (right). (B) Western blotting study showing representative blots (left) and statistical analysis of calponin-3 (right) in the rat hippocampus before (Ctl) and after different time points following pilocarpine-induced seizures. Significant differences were shown at 24 h and 1 week after seizures. Ctl: control; d, day; EP: epilepsy; h, hour; w, week. *p<0.05; **p<0.01; ***p<0.001.](image-url)
Augmentation of CNN3 mRNA and calponin-3 protein in the epileptic brain was consistent with our previous cDNA microarray results that CNN3 gene was up-regulated in patients with drug-resistant epilepsy (Xi et al., 2009). Seizure attacks are associated with hypersynchronous excitatory synaptic transmission (Gardiner and Marc, 2010; Hotulainen and Hoogenraad, 2010). This might lead to changes in the structure and function of dendritic spine, including those of local protein synthesis (Bourne and Harris, 2008; Wang and Zhou, 2010). It is known that calponin-3 is localized in the dendritic spines and dendritic injury are correlated with a epilepsy. Report has shown that in kainate-induced seizures, loss of currently not known whether co-
leptic brain was consistent with our previous cDNA microarray in the silent and chronic stages in this study, further study is needed 
atory activities as mentioned above (Rami et al., 2006). Thus it is calponin-3 in the hippocampus is associated with enhanced excit-
resistant epilepsy (Xi et al., 2009). Seizure attacks are associated 
AEDs before temporal lobe resections. Owing to ethical consider-
not in need of surgery for symptom control. For 70–80% of patients with epilepsy, AEDs can control their seizure activity, and both reg-
seizure attacks instead of AEDs. (Majores et al., 2007). Thus we used this model to further validate calponin-3 changes found in patients with epilepsy. The up-
ulations of calponin-3 in the epileptic cortices after pilocarpine-
seizure attacks of recurrent seizure attacks instead of AEDs.

Lithium/pilocarpine rat model shows similar patterns of seizures and molecular/neuropathological alterations to human epilepsy (Majores et al., 2007). Thus we used this model to further validate calponin-3 changes found in patients with epilepsy. The up-regulation of calponin-3 in the epileptic cortices after pilocarpine-induced seizure provides additional information that calponin-3 is involved in epilepsy. However, it is difficult to conclude from current study whether calponin-3 is simply a cause or a result of epilepsy. Evidences supporting the former include that over-expression of calponin-3 results in hyperactivity of hippocampal neurons (Rami et al., 2006), and that gene mutations in fimalin A (an actin-binding protein) lead to periventricular heterotopias and seizures (Parrini et al., 2006). On the other hand, evidence supporting the latter comes from a study demonstrating that seizures lead to activation of cofilin (actin-interacting protein) (Zeng et al., 2007). In our study, calponin-3 was significantly increased for up to 2 months, in which the silent stage is considered to be critical in epileptogenesis (Hanaya et al., 2007; Majores et al., 2007) involving cellular and structural changes (Rakhade and Jensen, 2009). Studies have shown that while alterations of some genes present only in acute or chronic stage (Majores et al., 2007), some common genes, including cell mobility genes, remain significantly changed through 15 days after pilocarpine-induced epilepsy (Okamoto et al., 2010). These results suggest that at least some of the molecular changes resulting from epilepsy may also be actively involved in epileptogenesis (causing epilepsy) (Okamoto et al., 2010). Although calponin-3 was elevated in the silent and chronic stages in this study, further study is needed to clearly define its role in epilepsy.

Notably, not all actin-related proteins are elevated after epilepsy. Report has shown that in kainate-induced seizures, loss of dendritic spines and dendritic injury are correlated with a decrease of cofilin, an actin-binding protein (Zeng et al., 2007). It is currently not known whether cofilin is also decreased in patients with epilepsy.

The pattern of calponin-3 change in the rat hippocampus after seizure was not closely as that seen in temporal cortex. While calponin-3 in cortex was consistently higher through almost all experimental time points, its level in hippocampus was increased at 24 h and 1 week after seizure and returned to control level at 1- and 2-month points. Similar finding in hippocampus was reported by Ferhat et al. (2003), in which calponin-3 levels were increased in the hippocampal dendritic spines 1 and 2 weeks after pilocarpine-induced seizures and returned to baseline thereafter. Whether different brain regions contribute differently to the pathophysiology of epilepsy requires future investigation.

Acknowledgments

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References


