Altered expression of GABA_A receptors (α4, γ2 subunit), potassium chloride cotransporter 2 and astrogliosis in tremor rat hippocampus

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ABSTRACT

Impaired GABAergic inhibitory neurotransmission plays an essential role in the pathogenesis of epilepsy. GABA_A receptor (GABA_AR), potassium chloride cotransporter 2 (KCC2) and astrocytes are of particular importance to GABAergic transmission and thus involved in the development of increased seizure susceptibility.

The tremor rat (TRM: tm/tm), a genetic mutant discovered in a Kyoto–Wistar colony, can manifest both absence-like seizures and tonic convulsions without any external stimuli. So far, there are no reports that can elucidate the effects of GABA_AR (α4, γ2 subunit), KCC2 and astrocytes on TRMs. The present study was undertaken to detect the expressions of GABA_AR α4, GABA_AR γ2 and KCC2 in TRMs hippocampus at mRNA and protein levels. In this work, mRNA and protein expressions of GABA_AR α4 were significantly elevated while GABA_AR γ2 and KCC2 were both evidently decreased in TRMs hippocampus by real-time RT-PCR and western blot, respectively. Furthermore, a dramatic elevation of KCC2 protein level was found after cerebroventricular injection with K252a to TRMs than that in the DMSO-treated TRMs. Besides, our present study also demonstrated that GFAP (a major component of astrocyte) immunoreactivity was much more intense in TRMs hippocampal CA1, CA3 and DG regions than that in control group with immunochemistry and confocal microscopic analyses. The protein expression of GFAP was also markedly elevated in TRMs hippocampus, suggesting that astrogliosis appeared in the TRM model. These data demonstrate that altered expressions of GABA_AR (α4, γ2) and KCC2 and astrogliosis observed in TRMs hippocampus may provide us good therapeutic targets for the treatment of genetic epilepsy.

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1 Introduction

Epilepsy, which is characterized by the recurrent seizures, is among the most prevalent neurological disorders, affecting over 50 million people worldwide [25]. Nearly 30% of them are inadequately controlled or controlled with severe side effects. It is well established that epileptic seizures are associated with excessive excitation or insufficient inhibitory neurotransmission [15,24]. γ-Aminobutyric acid (GABA) is a central inhibitory neurotransmitter in the adult brain [8,29]. A disruption of the inhibition mediated by GABA is a principal reason for the occurrence of neuronal hyperexcitability during epileptic seizures [29].

GABA_A receptors (GABA_ARs) mediate most fast inhibitory synaptic transmission in the central nervous system (CNS). The evidence that altered function of GABA_A receptor subtypes is closely related to epileptogenesis has been elucidated in animal models and human epilepsy [5,27]. Pentameric GABA_ARs are ligand-gated ion channels assembled from a diversity of polypeptide subtypes (α1–α6, β1–β3, γ1–γ3, δ, ε, π, θ and p1–p3) [20]. They maintain two forms of inhibition, namely, phasic inhibitory synaptic transmission and tonic perisynaptic inhibition. Both forms of GABA signaling are modulated by distinct GABA_A subunits that typically comprise two α and two β subunits together with the γ2 subunit [9]. Receptors containing α1 and γ2 subunits are preferentially targeted...
to the synapse and involved in phasic inhibition [38], whereas α4 isoform is predominantly located at extrasynaptic site and is critical for tonic inhibition in hippocampus [32].

In addition, GABABR-mediated fast-hyperpolarizing inhibition is dependent upon the low intracellular concentration of chloride. The neuron-specific potassium chloride cotransporter 2 (KCC2) is the major neuronal chloride extruder in the adult CNS [18]. Cumulative evidence has elucidated that KCC2 is responsible for an inwardly directed electrochemical gradient of chloride and hence for the generation of hyperpolarizing GABAB receptor-mediated postsynaptic currents in the adult brain [10,34]. The expressional change of KCC2 was described previously in the pilocarpine-induced epilepsy as well as in cortical epilepsy-associated malformations [1,23]. However, the underlying mechanism that regulates this change in KCC2 expression is not clear.

Astrocytes, one class of glial cells, also play a critical role in the regulation of synaptic transmission throughout the brain [30]. Previous report has demonstrated that hippocampal astrocytes also respond to GABA, finally regulating GABAergic inhibitory synaptic transmission [42]. Reactive astrocytosis is pathologically characterized by gross hyperplasia and hypertrophy of astrocytes in injured or diseased areas of the brain, accompanied by the up-regulation of glial fibrillary acidic protein (GFAP), a predominant component of neurofilaments [31]. The enhanced expression of GFAP has been observed in temporal lobe epilepsy [43], however, the potential mechanism involved in the reactive gliosis linked with epilepsy remains to be elucidated.

The tremor rat (TRM: tm/tm), a genetic mutant discovered in a Kyoto–Wistar colony, can manifest both absence-like seizures and tonic convulsions without any external stimuli [26,36]. Previous study has illustrated that the absence-like seizures in the TRM are characterized by paroxysmal occurrence of 5–7 Hz spike-wave complexes in hippocampal electroencephalograms (EEGs) after 8 weeks (data not shown) [37]. Furthermore, it was reported that the spike and wave complex appeared 0.8–1.9 times per minute and lasted for 1–17 s. However, in the intervening periods, normal EEG recording free of absence-like seizure was noted in the TRM examined [37]. Thus, this TRM is regarded as a very useful model for the research of human absence seizures. So far, the generating effects of GABAergic α4 and GABAB γ2 subunits as well as KCC2 in TRMs have not yet been well elucidated. Besides, although recent studies have demonstrated that there are evident alterations in the expression of KCC2 in the hippocampus of pilocarpine-induced epilepsy and temporal lobe epilepsy patients [23,28], the underlying mechanism that regulates KCC2 expression in epileptogenesis remains unclear. Furthermore, as to the changes of astrocytes in TRMs, there are no relevant publications. Analysis of GABAergic α4 and GABAB γ2 isoforms and KCC2 expression patterns together with the alterations of astrocytes will help to explore the mechanisms of GABAergic inhibitory neurotransmission in the epileptogenesis. In the current study, we studied the expression of GABAergic α4, GABAB γ2 and KCC2 at transcript and protein levels in TRM hippocampus by real-time quantitative RT-PCR and Western blot, respectively. In addition, the role of BDNF/TrkB signaling pathway in regulating KCC2 expression in TRM hippocampus was also investigated. Meanwhile, the protein distribution and expression of GFAP were evaluated using this TRM model.

2. Materials and methods

2.1. Experimental animals

Wild control rats and TRMs at the age of 9–12 weeks were used in this study. The rats were housed under a controlled environment (12:12 light/dark cycle, 50–70% humidity, 24 °C), with free access to food and water. All procedures involving animals were in strict accordance with the guidelines established by the NIH in the USA and approved by Animal Care Committee of China Medical University. Efforts were made to minimize suffering and reduce the number of animals used.

2.2. Real-time quantitative RT-PCR

Total RNA was extracted from the whole hippocampus tissues of TRMs (n = 6) and control rats (n = 6) using Trizol reagent (Invitrogen, CA) according to manufacturer’s instructions. The concentration of RNA was measured by spectrophotometer, with an expected A260/A280 ratio close to 1.8–2.0, denoting an acceptably pure nucleic acid sample. 500 ng of RNA from each sample was reverse-transcribed in a volume of 10 μl in the presence of TaqMan Reverse Transcription Reaction Mix (AMV; Applied Biosystems, CA). Reaction mixture for reverse transcription reactions were carried out at 37 °C for 15 min and 85 °C for 5 s. SYBR Green I-based detection was conducted using ABI PRISM 7300 instrument with thermal cycler conditions of: 95 °C for 30 s, followed by 45 cycles (95 °C for 0 s and 30 s for 31 s). Standard curves plotting the threshold amplification cycle number (Ct) values against input quantity for each gene were constructed using five-fold serial dilutions of RT product. GAPDH was served as an endogenous internal standard control. All experiments were repeated twice and, in each experiment, samples were assayed in duplicate. Data were expressed as a ratio: relative quantity of GABAB α4, GABAB γ2 and KCC2 mRNA/relative quantity of GAPDH mRNA, respectively. Primer sequences for GABABR α4, GABABR γ2, KCC2 and GAPDH were as follows: (GABABR α4) forward: 5′-GCG CAG AAA ATT TTA CCC GTA TC3′ and reverse: 5′-GAG CTG TCG TTA TGT GAG AGA C3′; (GABABR γ2) forward: 5′-CTT CTG GAA GGG TAC GAC AAC-3′ and reverse: 5′-AGC ATC CTG TCG GGA GTC G3′; (KCC2) forward: 5′-AGG TGG AAC TAC AGA TCT CGT GCC TGA TTC TT3′; (GAPDH) forward: 5′-CCA TTG CTC TCA ATG ACA ACT T3′ and reverse: 5′-GCC TTC TCT CTT GTC ACT AGT-3′.

2.3. Western blot analysis

Western blot analysis was performed on samples of the whole hippocampus of TRMs (n = 6) and control rats (n = 6). In brief, samples were washed twice with PBS buffer and homogenized in cold lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% glycercer, 1% Nonident P-40, 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride). The lysates were spun at 13,200 × g for 20 min at 4 °C. The supernatant was collected and quantified for total protein levels using a BCA protein assay kit with bovine serum albumin as the standard (Beyotime Institute of Biotechnology, China).

Equal amounts of protein (60 μg) were subjected to SDS-PAGE using 8% or 10% acrylamide gels and electrophoresed onto nitrocellulose membranes (Millipore, MA). The membranes were blocked with 5% fat-free milk in TBS containing 0.1% Tween-20 for 1 h at room temperature. Blots were incubated with the following primary antibodies: rabbit anti-GABAγ2 (1:300, Santa Cruz, USA), goat anti-GABAα4 (1:200, Santa Cruz, USA), rabbit anti-KCC2 (1:1500, Upstate Biotechnology, NY), rabbit anti-GFAP (1:200, Santa Cruz, USA), mouse anti-β-actin (1:2000, Santa Cruz, USA) and mouse anti-GAPDH (1:2000, Kang Chen, China), respectively, overnight at 4 °C. After the membranes were washed, they were incubated with horseradish peroxidase-horseradish peroxidase-conjugated secondary antibody (1:1000, Santa Cruz, USA) for 2 h at room temperature. Immunolabeled protein bands were detected using an enhanced chemiluminescence kit (Pierce, CA) and exposed on an X-ray film. β-Actin or GAPDH was used as an internal reference for relative quantification. Immunoblots scanned by the densitometer were subjected to the grey value analysis using Quantity One software (BioRad, USA).

2.4. Intracerebroventricular drug application

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and placed in a stereotaxic device. Each rat was prepared with a 0.4 mm external diameter hypodermic needle extending into the lateral ventricle of the brain (coordinated from bregma, AP: 0.8 mm, ML: 1.5 mm, DV: 4.2 mm) [7]. The guide cannula was fixed well with dental cement over the surface of the skull. Animals were cerebroventricular injected with 250 μl (2 μg dissolved in 10 μl PBS with 0.01% DMSO, Sigma, USA) or 10 μl phosphate-buffered saline (PBS) with 0.01% DMSO as a vehicle control once a day for two days. The position of injection cannula tip in the ventricle was verified after experiment by injecting blue ink and checking for distribution.

2.5. Tissue preparation for immunohistochemical and confocal laser scanning microscopic analysis

TRMs (n = 6) and control rats (n = 6) were deeply anesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg) and were transcardially perfused with 0.1 M PBS (pH 7.4), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After perfusion, the brains were dissected, immersed in the same fixative for 12 h and cryoprotected in 30% sucrose solution for 3 days at 4 °C. 8 μm frozen sections containing the hippocampus were cut coronally using Cryostat (CM1900 UV, Leica, Germany).

2.6. Immunohistochemistry

Immunohistochemical analysis was carried out according to the standard ABC method. Briefly, tissue sections were rinsed in PBS and then treated with 10% normal goat serum for 30 min to reduce nonspecific staining. They were incubated in the primary antibody anti-GFAP (1:50, Santa Cruz, USA) overnight at 4°C. Sections were stained using avidin-biotin peroxidase complex method with 3,3'-diaminobenzidine as a chromogen. The stained sections were then dehydrated in graded alcohol, cleared in xylene, oversupplied with neutral balsam and examined under a light microscope equipped with a computerized image analysis system.

Control sections which were incubated with 10% normal goat serum instead of the primary antibodies followed by all subsequent incubations as described above were performed and no positive immunoreaction was observed.

The distribution of GFAP was examined in different hippocampal regions (CA1, CA3 and DG) of control rats and TRMs. The expression levels of GFAP were quantified via the analysis of integrated optical density values using a computerized image analysis system. Briefly, every sixth section spanning the hippocampus was examined for the density of astrocyte and six sections were analyzed in total per animal. All sections were coded and examined blind during the experiment. Results were presented as integrated optical density value of control and TRM groups in hippocampal CA1, CA3 and DG regions.

2.7. Confocal laser scanning microscopy

Frozen sections were preincubated with 5% bovine serum albumin (BSA) for 1 h at room temperature and then incubated with the primary antibody anti-GFAP (1:50, Santa Cruz, USA) overnight at 4°C. After the sections were washed in PBS, they were incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (1:200, Zhongshan Golden Bridge Biotechnology, China) for 2 h at room temperature. Nuclear DNA was counterstained with DAPI (1:500, Jackson Immunoresearch, USA). Sections were coveredlipped with an anti-fading mounting medium and examined using a confocal laser scanning microscope (CL, Nikon, Japan).

2.8. Statistics

All values were presented as mean ± SD. Statistical evaluation was carried out using a program SPSS 13.0. Student’s t-test or one-way ANOVA was used to evaluate the differences between two groups or among more than three groups, respectively. Statistical significance was set at p < 0.05.

3. Results

3.1. aberrant gene expression of gabarα4, gabarγ2 and kcc2 in trms and control rats hippocampus

Since the objective of this investigation was to explore whether gabarα4, gabarγ2 and kcc2 were involved in gabaeergic inhibition of TRMs, we first detected the mRNA expressions of gabarα4, gabarγ2 and kcc2 using real-time RT-PCR. GAPDH was considered as a reference gene that was stably expressed in many tissues including brain. The excellent linear correlation (r = 0.996 for gabarα4, r = 0.995 for gabarγ2 and r = 0.993 for kcc2) was indicated between the Ct value and the logarithm of the DNA copy number. Fig. 1(a) showed that the mRNA expression of gabarα4 was markedly elevated in TRMs hippocampus from 0.74 ± 0.22 to 1.74 ± 0.43 (p < 0.01, n = 6) than that of control group. However, the evident reduction of gabarγ2 mRNA was observed in TRMs hippocampus from 1.64 ± 0.23 to 0.63 ± 0.10 (p < 0.01, n = 6) compared to that in control rats, as shown in Fig. 1(b). In addition, the mRNA expression level of kcc2, which played an important role in regulating postsynaptic gabaeergic responses were also detected and indicated in Fig. 1(c). KCC2 mRNA in TRMs hippocampus was expressed much lower from 1.88 ± 0.36 to 0.75 ± 0.16 (p < 0.01, n = 6) in comparison to the control group.

3.2. Differential protein expression of gabarα4, gabarγ2 and kcc2 in trms and control rats hippocampus

Western blot analysis was further conducted to evaluate whether protein levels of gabarα4, gabarγ2 and kcc2 in TRMs hippocampus were different from those in control rats. Fig. 2(a) showed the result with antibody specific to gabarα4 by western blot. As illustrated in Fig. 2(d), the protein expression of gabarα4 in TRMs hippocampus was abundantly increased from 0.77 ± 0.06 to 1.13 ± 0.04 compared to that in control group. Immunoblotting for gabarγ2 revealed the anticipated band at 54 kDa in both experimental and control groups (Fig. 2(b)). Interestingly, the protein level of gabarγ2 in TRMs hippocampus was apparently decreased from 1.19 ± 0.05 to 0.85 ± 0.01 (p < 0.01, n = 6) in comparison with control rats, as indicated in Fig. 2(e). In the mean time, Fig. 2(c) illustrated that the western blot with kcc2 antibody exhibited a single band of 140 kDa and there was an obvious decline in the kcc2 protein expression in TRMs hippocampus from 1.84 ± 0.09 to 1.38 ± 0.10 (p < 0.01, n = 6) than that in control group, as shown in Fig. 2(f). Taken together, these results by western blot analysis further confirmed the distinct protein expressions of gabarα4, gabarγ2 and kcc2 in TRMs hippocampus compared with control rats, which were in line with our real-time RT-PCR results.

3.3. BDNF/TrkB signaling pathway suppressed kcc2 expression in TRMs hippocampus

In order to further investigate the possible regulating mechanism of kcc2 during the epileptogenesis, K252a, a specific TrkB receptor blocker and DMSO (as the vehicle) were cerebroventricularly injected in the rats. Fig. 3(a) displayed the representative image of immunoblots with antibodies against KCC2 and β-actin after cerebroventricular injection with DMSO or K252a in control and TRMs. The protein expression of KCC2 in the TRMs treated with DMSO was significantly decreased from 1.04 ± 0.05 to 0.88 ± 0.04 (p < 0.01, n = 6) compared with the DMSO-treated control group. Additionally, after injection with K252a to TRMs, a dramatic elevation of KCC2 protein level was found from 0.88 ± 0.04 to 1.53 ± 0.07 (p < 0.01, n = 6) than that in the DMSO-treated TRMs, as indicated in Fig. 3(b).

3.4. Reactive astrocytes activation in TRMs hippocampus

Astrocyte activity is mainly characterized by the expression of GFAP, the primary glial cytoskeletal protein. Enhanced expression of GFAP is closely linked with the morphological changes of astrocytes in epileptogenesis. Thus, in the current study, we utilized the immunoreactivity of GFAP to represent the activity of astrocytes. The distribution of GFAP in TRMs and control rats hippocampus was firstly examined by immunohistochemical analysis. Our results showed that GFAP protein was localized widely in the hippocampus including CA1, CA3 and DG regions of TRMs and control rats (Fig. 4(a)). With the help of the resultant analysis of immunohistochemistry, the integrated optical density values of GFAP in TRMs hippocampus were much higher than those in control group including CA1, CA3 and DG sectors (Fig. 4(b): CA1, CA3 and DG regions, p < 0.01). In order to confirm the results, confocal microscopic method was further employed. Fig. 5 displayed the protein distribution of GFAP in TRMs and control rats hippocampal CA1, CA3 and DG subfields by confocal microscopic analyses. It was noteworthy that GFAP proteins in the hippocampal CA1, CA3 and DG regions of TRMs were distributed much more than those in control group, which were in good agreement with our immunohistochemical results.

To quantify the difference of GFAP expression in the hippocampus between TRMs and control rats at protein level, western blot analysis was further carried out. Fig. 6(a) showed that GFAP protein was detectable as 50-kDa-immunostained polypeptide in experimental and control groups. We found that the protein expression of GFAP in TRMs hippocampus was markedly elevated.
4. Discussion

It has been well recognized that several animal models has been employed to study the pathogenesis of epilepsy. The inbred Wistar Albino Glaxo Rats from Rijswijk (WAG/Rij) and the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) are two validated genetic models of absence epilepsy [21,41]. Spike and wave complexes of 7–10 Hz and 7–11 Hz accompanied by behavioral absence-like seizure have been noticed in WAG/Rij and GAERS stains, respectively [11,41]. However, these animals simultaneously exhibited both twitching of the vibrissae and mouth. Besides, genetic studies have recently shown loci on chromosomes 5 and 9 in WAG/Rij strain and on chromosomes 4, 7 and 8 in GAERS that control the features of SWDs [13,35].

Unlike these rats, our TRM selected in the present study was characterized by (1) paroxysmal discharges of a 5–7 Hz spike and wave complex associated with behavioral absence seizure, (2) no other abnormality of EEG recording, (3) a normal EEG activity in the intervening periods free of absence seizure and (4) seizures linked with tm mutant which was mapped to rat chromosome 10 [22]. These EEG and behavioral characteristics in our TRM seem to resemble the symptoms featured with a 3 Hz spike and wave.

Fig. 4. Protein distribution of GFAP in TRMs and control rats hippocampus by immunohistochemistry. (a) showed the representative image GFAP protein distribution in TRMs and control rats hippocampus by immunohistochemistry and (b) was the statistical analyses of the integrated optical density stained with GFAP antibody in TRMs and control rats hippocampus (mean ± S.D., n = 6). The arrows indicated the positive reactions. Scale bar: 10 μm. **p < 0.01 vs. control group.

Fig. 5. Protein distribution of GFAP in TRMs and control rats hippocampus by confocal microscopic analysis. The arrows indicated the positive reactions. Cell nucleus was counterlabeled with DAPI (in blue). Scale bar: 10 μm.

Fig. 6. Protein expression of GFAP in TRMs and control rats hippocampus by western blot analysis (mean ± S.D., n = 6). (a) showed representative bands of GFAP. GFAP: 50kDa; GAPDH: 36kDa and (b) was the quantitative analysis of the protein levels of GFAP between TRMs and control groups. The data were normalized to the loading control GAPDH. **p < 0.01 vs. control group.
complex and transient absence in petit mal epilepsy in humans. On the basis of these experimental data, TRM has been proposed as a very useful animal model for the research of human absence epilepsy.

Seizures can be induced by an alteration in the inhibitory neuronal networks within the rodent brain wherein impaired GABAergic inhibitory neurotransmission plays an essential role in the pathogenesis of increased seizure susceptibility [40]. Among this, alteration in GABAARs which is responsible for the majority of fast inhibition is closely linked to the onset of epileptogenesis. Marked changes in GABAAR expression have been observed in patients with temporal lobe epilepsy (TLE) [4] and animal models of TLE [5,6]. Most notably, dentate granule cells in chronically epileptic rats displayed an increase in GABAAR α4 subunit gene expression and a decrease in GABAAR α1 [5]. Our present study report this consistent alteration in GABAAR α4 mRNA and an elevation of protein level, possibly due to the transcriptional mediation. GABAAR α2 subunit is a major component of the GABAAR (over 80% of GABAARs contain α2 subunit). The change in GABAAR α2 subunit composition has been also greatly associated with epilepsy. Genetic evidence for the importance of GABAAR α2 in epileptogenesis came from the fact that a K289M mutation, located in the extracellular loop of the α2 subunit, could contribute to the generation of generalized epilepsy with febrile seizures [2]. Our findings suggested the reduced expression of GABAAR α2 isoform at gene and protein levels in TRMs hippocampus. Based on the critical role of GABAAR (α4, γ2) dysfunction in the generation of epilepsy, it is plausible that altered expressions of GABAAR subunits may facilitate, at least in part, the development of elevated seizure susceptibility, as noticed in our study.

Besides, GABAAR-mediated inhibition is also attributable to neuronal Cl− regulation in which KCC2 exerts a leading role. Recently, it was reported that KCC2 was sufficient to induce hyperpolarizing GABA currents [12]. The importance of KCC2 for inhibitory synapses was exemplified in KCC2 knock-out mice that died perinatally [17]. Notably, in the kindling model, the mRNA expression level of KCC2 was found to be down-regulated after seizures [33]. We showed that a similar mRNA switch occurred in TRM hippocampus and KCC2 protein expression was further decreased in this model, indicating that epilepsy appears to take place at the level of transcription of KCC2. The mechanisms that underlie this alteration in KCC2 expression might involve BDNF action since the protein expression of KCC2 was remarkably elevated in TRM after cerebroventricularly injected with K252a, a TrkB receptor blocker. A recent report has shown that this down-regulation of KCC2 depends on the activation of TrkB receptor via BDNF [33]. The continuous effect of KCC2 was also blocked by K252a in hippocampal slices model [33], which was in accordance with our investigation. We speculate that BDNF/TrkB signaling pathway could suppress KCC2 expression, finally regulating GABAergic inhibitory responses. Further investigation needs to be performed on how KCC2 modulates GABA-mediated inhibition in TRM model via BDNF/TrkB signaling mechanism.

Reactive changes of astrocytes in the hippocampus, including the increase of size and number, have been greatly associated with seizure in animal models and epileptic disorders in humans [14,43]. They medulate neuronal excitability not only via the clearance of extracellular potassium and glutamate but also via the release of inhibitory neurotransmitters. Recent findings have suggested that certain characteristics of astrocytes play a pivotal role in maintaining normal neuronal activity and that the disruption of astrocytic function may contribute to the generation of seizures. As the major intermediate filament protein in astrocytes of mammalian CNS, GFAP has been widely used to assess the ‘reactive state’ of astrocytes. Our present study demonstrated that GFAP immunoreactivity was much more intense in TRMs hippocampal CA1, CA3 and DG regions than that in control group with immunochemistry and confocal microscopic analyses. Previously, Khurgel and Ivy [19] reported that an apparent increase was observed in the hippocampal DG region of the kindling model of epilepsy, which was in part consistent with our study. Furthermore, the number of GFAP immunopositive astrocytes was also reported to be significantly higher in the hippocampus of epileptic EL mice in comparison with that of non-epileptic controls, however, no change of GFAP immunoreaction in astrocytes in the EL mice prior to the onset of seizures [3], implying that the alteration in GFAP immunoreactivity could be linked with the generation of epilepsy. Additionally, immunoblot analyses for GFAP protein depicted a conspicuous elevation in TRMs hippocampus compared with control. In agreement with our work, there was a significant increase in GFAP expression level in the hippocampus after seizures [16,39], suggesting that astroglisis could occur in the pathogenesis of epilepsy.

In summary, for the first time, the present study illustrated the altered expressions of GABAAR subunit compositions (α4, γ2) and KCC2 in TRM hippocampus, which might represent a modulation of hippocampal GABAergic inhibition in genetic epilepsy and underlie the observed seizure phenotype of TRM. Furthermore, our results disclosed a novel BDNF/TrkB signaling pathway that profoundly affected neuronal Cl− homeostasis since the up-regulation of KCC2 was observed in TRM hippocampus after applying the specific TrkB receptor blocker, K252a. Besides, marked astrogliosis appeared in our TRM model on the basis of the obtained results by western blot, immunohistochemistry and confocal microscopic analysis, implying that increased GFAP expression was associated with epileptogenesis. According to the current study, altered expressions of GABAAR (α4, γ2) and KCC2 and astroglisis was observed in TRM hippocampus. This might provide us good therapeutic targets for the treatment of genetic epilepsy and further work needs to be investigated.

Conflict of interest

We declare that there are no conflicts of competing interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence the current work.

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