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Up-regulation of GABA transporters and $GABA_A$ receptor α1 subunit in tremor rat hippocampus

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ABSTRACT

The loss of GABAergic neurotransmission has been closely linked with epileptogenesis. The modulation of the synaptic activity occurs both via the removal of GABA from the synaptic cleft and by GABA transporters (GATs) and by modulation of GABA receptors. The tremor rat (TRM; tm/tm) is the parent strain of the spontaneously epileptic rat (SER; zi/zi, tm/tm), which exhibits absence-like seizure after 8 weeks of age. However, there are no reports that can elucidate the effects of GATs and $GABA_A$ receptors (GABARs) on TRMs. The present study was conducted to detect GATs and GABAR α1 subunit in TRMs hippocampus at mRNA and protein levels. In this study, total synaptosomal GABA content was significantly decreased in TRMs hippocampus compared with control Wistar rats by high performance liquid chromatography (HPLC); mRNA and protein expressions of GAT-1, GAT-3 and GABAR α1 subunit were all significantly increased in TRMs hippocampus by real time PCR and Western blot, respectively; GAT-1 and GABAR α1 subunit proteins were localized widely in TRMs and control rats hippocampus including CA1, CA3 and dentate gyrus (DG) regions whereas only a wide distribution of GAT-3 was observed in CA1 region by immunohistochemistry. These data demonstrate that excessive expressions of GAT-1 as well as GAT-3 and GABAR α1 subunit in TRMs hippocampus may provide the potential therapeutic targets for genetic epilepsy.

γ-Aminobutyric acid (GABA) is the predominant inhibitory neurotransmitter in the mammalian central nervous system (CNS). The modulation of the synaptic activity occurs both via the removal of GABA from the synaptic cleft and by GABA transporters (GATs) and by modulation of GABA receptors.

GATs located on the plasma membrane of neurons and astrocytes contribute to determining GABA level in the synaptic cleft [16,13]. Up to date, four subtypes of GATs, designated GAT-1, GAT-2, GAT-3 and betaine/GABA transporter (BGT)-1 have been cloned in rat brain [11]. GAT-1 and GAT-3 are the most copiously expressed GATs in the brain and the two types of membrane GATs are most likely to be responsible for regulating the ambient level of GABA [6], finally contributing to epileptogenesis. The fact that changes of GATs activity are closely related both to pathology and therapy of epileptic seizure has been approved in clinical practice [1].

$GABA_A$ receptors (GABARs) mediate the majority of fast inhibitory synaptic transmission in the CNS. Mammalian GABARs are composed of pentameric, ligand-gated ion channels assembled from various (α1–α6, β1–β3, γ1–γ3, δ, ε, π, θ) polypeptide subunits [12]. The α family is the largest with six different subtypes and contributes significantly to the functional characterization of the GABARs. GABAR α1 is the most widely expressed of all the α subtypes and shows a high level of expression in most regions of the brain [19]. The importance of the α1 subunit for inhibitory synaptic transmission is exemplified in α1 knock-in mice that are more susceptible to having anticonvulsant properties of benzodiazepines [23].

Synaptosome is an isolated nerve terminal of a neuron which is adequate for exploring neural function in vitro. Alterations of synaptosomal neurotransmitter levels have been implicated in the cause of neurological diseases, such as epilepsy.

The tremor rat (TRM) (tm/tm) is the parent strain of the spontaneously epileptic rat (SER: zi/zi, tm/tm), which exhibits both absence-like and convulsive seizures without any external stimuli [31]. Previous work has demonstrated that the electroencephalograms (EEGs) recording can show 5–7 Hz spike-wave-like complexes synchronously in hippocampus accompanied by absence-like seizures after 8 weeks (data not shown) [26]. So far, the effects of GATs and GABAR α1 in TRMs have not yet been well elucidated. We speculate that the etiopathogenesis and hypoinhi-

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bition of TRM in genetic epilepsy might be involved in changes of GATs and GABAR α1. Therefore, we measured the synaptosomal GABA concentration in TRM hippocampus by high performance liquid chromatography (HPLC). Furthermore, we investigated the expressions of GATs and GABAR α1 at the mRNA and protein levels of TRM through real time PCR, Western blot and immunohistochemical analysis.

Normal Wistar rats and TRMs at the age of 9–12 weeks were housed in individual cages under a controlled environment (12:12 h light/dark cycle, 50%–70% humidity, 24 °C). Food and water were available *ad libitum*.

The GAT-1, GABAR α1 and β-actin antibodies were purchased from Santa Cruz. The GAT-3 antibody was obtained from Abcam technology. Other reagents were from Sigma–Aldrich.

Purified synaptosomes were isolated according to Dunkley et al [7]. Briefly, the hippocampus was homogenized in 0.32 M sucrose buffer. After the two centrifugations at $1000 \times g$ for 2 min and $15{,}000 \times g$ for 12 min, resultant pellet was resuspended in 0.32 M sucrose and layered on top of Percoll discontinuous gradients (23%, 10% and 3%) in sucrose buffer. Synaptosomal fraction was obtained from the 23%/10% interface and synaptosomal protein was determined by Bradford method [3]. The Synaptosomal (1 mg/ml) sample was separated by HPLC method. The result was measured through the peak areas.

Total RNA was extracted from hippocampus using Trizol (Invitrogen Technology). PCR primers for GAT-1, GAT-3, GABAR α1 and β-actin were as follows: (GAT-1) forward: 5′-TGC AAA CAC GTA CGC ACA TAG AA-3′ and reverse: 5′-AGA TGC CTC AGC CAC ACG AC-3′; (GAT-3) forward: 5′-CGG TCA CTG GAA CAA CAA GGT G-3′ and reverse: 5′-AAC ACC ACG TAA GGA ATC AGG AAT G-3′; (GABAR α1) forward: 5′-CCT GGA CCC TCA TTC TGA GCA-3′ and reverse: 5′-ATC CTC GTG AAG ACA GTG GTG TTG-3′ and (β-actin) forward: 5′-AGG CCC CTC TGA ACC CTA AG-3′ and reverse: 5′-CCA GAG GCA TAC AGG GAC AAC-3′. They produced the PCR products of 144 bp, 135 bp, 94 bp and 118 bp, respectively. Total RNA was reverse-transcribed at 37 °C for 15 min and 85 °C for 5 s. SYBR Green I-based detection was carried out on a real-time PCR instrument with thermal cycler conditions of: 95 °C for 30 s, followed by 40 cycles (95 °C for 5 s and 62 °C for 34 s). Data were expressed as a ratio: relative quantity of GAT-1, GAT-3 and GABAR α1 mRNA/relative quantity of β-actin mRNA, respectively.

Samples were homogenized in RIPA lysis buffer. An equal amount of 60 μg proteins were loaded to each lane, separated electrophoretically by SDS-PAGE and electroblotted onto PVDF membranes. After blockage of 1 h in 5% nonfat dry milk, the membranes were probed respectively with the anti-GAT-1 (1:600 dilution), GAT-3 (1:600 dilution), GABAR α1 (1:500 dilution) or β-actin (1:1000 dilution) antisera conjugated goat anti-rabbit IgG and were detected with enhanced chemiluminesecence (ECL). β-actin was used as an internal reference for relative quantification.

Rats were anesthetized and perfused with 4% paraformaldehyde. The brains were further immersed into 30% sucrose. After blockage with 10% goat serum, free-floating sections were incubated respectively with rabbit anti-GAT-1 (1:150 dilution), anti-GABAR α1 (1:100 dilution), GAT-3 antisera (1:100 dilution) and goat anti-rabbit IgG (1:200 dilution). The reactions were visualized with 3,3-diaminobenzidine tetrahydrochloride (DAB) method.

Statistical analyses were performed using Student's *t*-test, values were expressed as mean ± SD, $p < 0.05$ was considered significant.

It has been recognized that the basal levels of neurotransmitters in the synaptosome were more closely linked with epileptogenesis than those in the whole brain tissue [5,25]. To explore the alteration of GABA in TRM, the GABA concentration was detected in TRMs hippocampal synaptosomes by HPLC. Obviously, the content of synaptosomal GABA in TRMs was definitely decreased compared with that in Wistar rats (59.55 ± 6.38 μg/mg protein in TRMs vs. 102.36 ± 21.90 μg/mg protein in Wistar rats, $p < 0.01$).

In order to evaluate the roles of GAT-1, GAT-3 and GABAR α1 in GABAergic inhibition of TRMs, we subsequently made an analysis of the mRNA expressions of GAT-1, GAT-3 and GABAR α1 in TRMs hippocampus using real time PCR. The data showed that the mRNA expression of GAT-1 in hippocampus was markedly increased than that of control groups (1.58 ± 0.42 in TRMs vs. 0.45 ± 0.33 in control rats; $p < 0.01$; Fig. 1a). In addition, GAT-3 mRNA in the hippocampus of TRMs was expressed much higher compared with that in control rats (1.40 ± 0.13 in TRMs vs. 0.77 ± 0.29 in control rats; $p < 0.05$; Fig. 1b). Moreover, the mRNA level of GABAR α1 in TRMs hippocampus was significantly higher than that in control groups (2.09 ± 0.57 in TRMs vs. 0.90 ± 0.41 in control rats, $p < 0.01$; Fig. 1c).

To investigate whether GAT-1, GAT-3 and GABAR α1 protein expressions in TRMs hippocampus were different from those in control rats, Western blot analysis was further performed. Fig. 1d showed the result with antibody specific to GAT-1 by Western blot. The protein expression of GAT-1 in TRMs hippocampus was significantly higher than that in control groups (0.64 ± 0.06 in TRMs vs. 0.32 ± 0.10 in Wistar rats, $p < 0.01$; Fig. 1g). Our finding also illustrated that GAT-3 protein was detectable as 74-kDa-immunostained polypeptide in experimental and control groups (Fig. 1e). In comparison with normal Wistar rats, increased expression of GAT-3 was apparently observed in TRMs hippocampus (0.94 ± 0.06 in TRMs vs. 0.51 ± 0.15 in Wistar rats, $p < 0.01$, Fig. 1h). It was noteworthy that the Western blot with GABAR α1 antibody exhibited an anticipated single band of 51 kDa (Fig. 1f) and the increased expression of GABAR α1 protein was obviously found in TRMs hippocampus compared with Wistar rats (0.75 ± 0.08 in TRMs vs. 0.44 ± 0.05 in control rats, $p < 0.01$, Fig. 1i). Thus, these results by Western blot analysis further confirmed the distinct expressions of GAT-1, GAT-3 and GABAR α1 at protein level in TRMs hippocampus compared with Wistar rats, which were in accordance with our real time PCR results.

Immunohistochemical analysis was employed in TRMs and Wistar rats hippocampus by using the anti-GAT-1, anti-GAT-3 and anti-GABAR α1. The positive reactions appeared brown on the membrane. It was obviously observed that GAT-1 and GABAR α1 proteins in TRMs and Wistar rats were both localized widely in hippocampus including CA1, CA3 and DG regions (Fig. 2(A) and (C) for GAT-1 and GABAR α1, respectively). However, in terms of GAT-3, a weak immunoreaction appeared in CA3 and DG regions except for the evident observation in CA1 region (Fig. 2(B)). With the help of the resultant analysis of immunohistochemistry, the integrated optical density values of GAT-1 in TRMs hippocampus were much higher than those in Wistar rats including CA1, CA3 and DG regions (Fig. 3(A): CA1 and DG regions, $p < 0.05$; CA3 region, $p < 0.01$). Meanwhile, it was quite evident for the integrated optical density of GAT-3 in the CA1 region of TRMs hippocampus compared with Wistar rats (Fig. 3(B): $p < 0.01$), however no significant changes were shown in CA3 and DG regions. Besides, the integrated optical density of GABAR α1 in the CA1 region of TRMs hippocampus was more pronounced than that in control groups (Fig. 3(C): $p < 0.01$).

Extracellular GABA was decreased in cerebrospinal fluid (CSF) obtained from patients during seizures [22]. Similarly, low levels of GABA were found in several animal models following induction of seizures [8,14]. In this study, we have employed the synaptosomal sample to measure the concentration of GABA in TRMs hippocampus. The synaptosome has the advantage of maintaining original synaptic function under proper conditions and thus it is a suitable subcellular fraction for investigating the mechanism of epileptogenesis. Several reports support the notion that alterations of synaptosomal neurotransmitter levels are more neuroactive than those of whole brain tissue in seizure-susceptibility or -severity

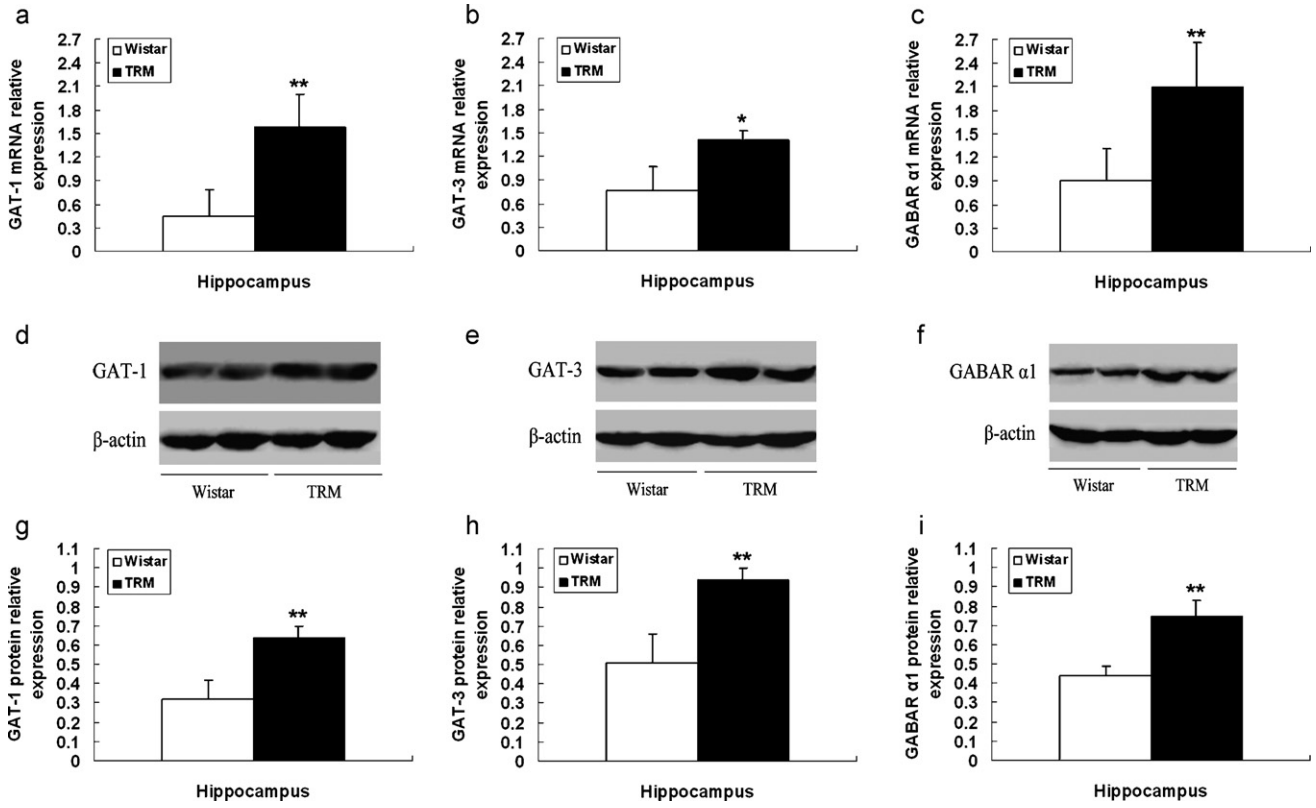

Fig. 1. mRNA and protein expressions of GAT-1, GAT-3 and GABAR α1 in TRMs and Wistar rats hippocampus by real time PCR and Western blot (mean ± S.D., $n = 5–7$). (a)–(c) Represented mRNA relative expressions of GAT-1, GAT-3 and GABAR α1, respectively. $^{*}p < 0.05$ vs. Wistar rats. $^{**}p < 0.01$ vs. Wistar rats; (d)–(f) showed representative bands of GAT-1, GAT-3 and GABAR α1, respectively. GAT-1: 67 kDa; GAT-3: 74 kDa; GABAR α1: 51 kDa; β-actin: 43 kDa; (g)–(i) represented protein relative expressions of GAT-1, GAT-3 and GABAR α1, respectively. $^{**}p < 0.01$ vs. Wistar rats.

[5,25]. A sharp reduction of total synaptosomal GABA content was observed in TRMs hippocampus implicating that GABA might be responsible for the hippocampal hypoinhibition of TRMs. Furthermore, we speculated that GATs and GABARs might be responsible for the hippocampal GABAergic inhibition of TRM. Therefore, the following study was to examine the expressions of GAT-1, GAT-3 and GABAR α1 in TRMs hippocampus.

GAT-1 has a wide distribution in neurons and astrocytes in hippocampal and limbic regions [17]. It is the most abundant GAT in the brain where it is responsible for about 85% of the total GABA re-uptake [4]. Persuasive evidence for the potential importance of GATs came from the GAT-1 deficient mouse, resulting in a large increase in a tonic postsynaptic hippocampal $GABA_A$ receptor-mediated conductance [10]. Indeed, the suppression of GATs can greatly inhibit the generation of seizures, an excellent example being a well-known inhibitor of GAT-1, NNC 711, which has produced a profound inhibitory effect on the repetitive seizures induced by pentylenetetrazol.

Several previous reports have been associated with the GAT-1 expression in various animal models of epilepsy and human epileptic patients. Mathern et al. reported regional variation in alteration of GAT-1 expression in human hippocampal tissue [18]. In animal models, several reports were consistent with our investigation. Suarez et al. found that the number of detectable GAT-1 immunoreactive cell bodies was increased in hippocampal granule and pyramidal cells including DG and CA1 regions in the corticotropin-releasing hormone (CRH)-induced seizures [20]. The result that GAT-1 protein was increased bilaterally (from 150% to 250%) at 5–15 days after $FeCl_3$-injected amygdala was demonstrated by Ueda and Willmore [29]. On the contrary, in pentylenetetrazol-kindling model, the expression level of GAT-1 protein in easily-kindled rats was decreased by 30% when compared to kindling resistant animals at 30 days after the last PTZ injection [30]. At the mRNA level, the results also appeared contradictory. Hirao et al. previously noticed a transient but significant increase of GAT-1 level in the hippocampus of the amygdaloid kindled rats [9]. In contrast, dramatic reductions in GAT-1 mRNA were found in genetically epileptic-prone rats in all brain regions including hippocampus [2]. Interestingly, 30% increase appeared 9 h after kainate injection at GAT-1 mRNA level but was reduced by about 25% at later intervals [27].

The present study illustrated that GAT-1 protein immunoreaction was observed widely in hippocampus including CA1, CA3 and DG regions in TRMs and Wistar rats, especially for the regions of granule cells and pyramidal cells due to the neuronal transporter itself. We found more noticeable immunoreactions for GAT-1 protein in TRMs hippocampal regions. Besides, elevated expressions of GAT-1 at protein and mRNA levels were indicated by Western blot and real time PCR analysis, respectively, suggesting that the elevation of GAT-1 might contribute to epileptogenesis and hypoinhibition. There is a minor difference between the result of our investigation and some previous reports possibly due to the sorts of model application.

GAT-3 has a lower affinity for GABA than does GAT-1 [15]. The result that GAT-3 mRNA was very weak in the granule cells and molecular layer of DG was reported by Sperk et al. [27]. In contrast, an increased immunoreaction of GAT-3 was also observed in the granule cells of hippocampus after kainic acid injection [29]. In human epilepsy, Mathern et al. found increased GAT-3 expression in hilus, CA1 and CA3 regions in patients with non-hippocampal sclerosis and decreased GAT-3 expression in patients with hippocampal sclerosis present [18].

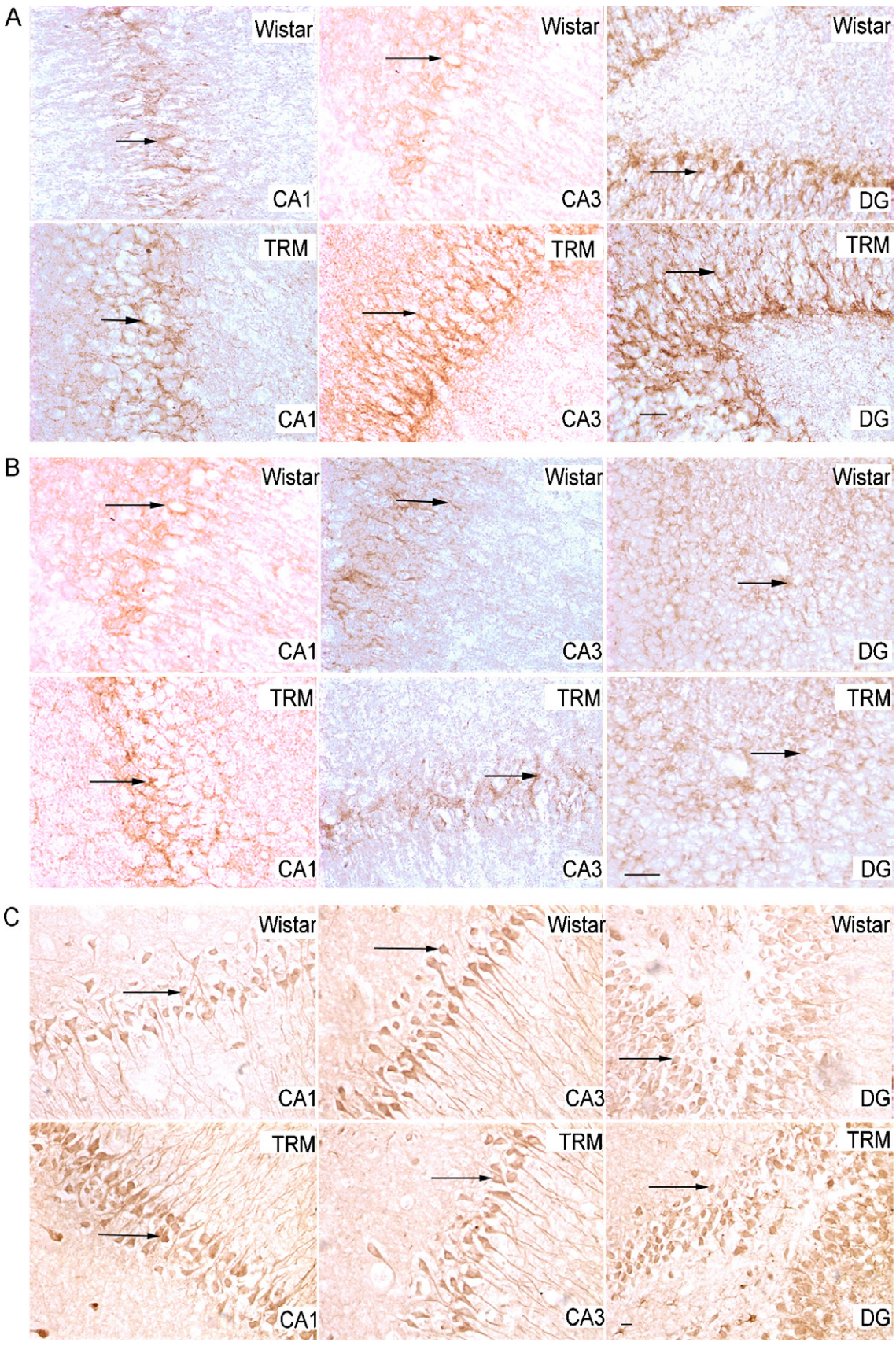

Fig. 2. Protein distributions of GAT-1, GAT-3 and GABAR α1 in TRMs and Wistar rats hippocampus by immunohistochemistry. The arrows indicated the positive reactions. (A) and (B) Represented protein distributions of GAT-1 and GAT-3 in TRM's and Wistar rat's hippocampus CA1, CA3 and DG regions, respectively. Scale bars: 30 μm; (C) represented protein distribution of GABAR α1 in TRM's and Wistar rat's hippocampus CA1, CA3 and DG regions. Scale bars: 10 μm.

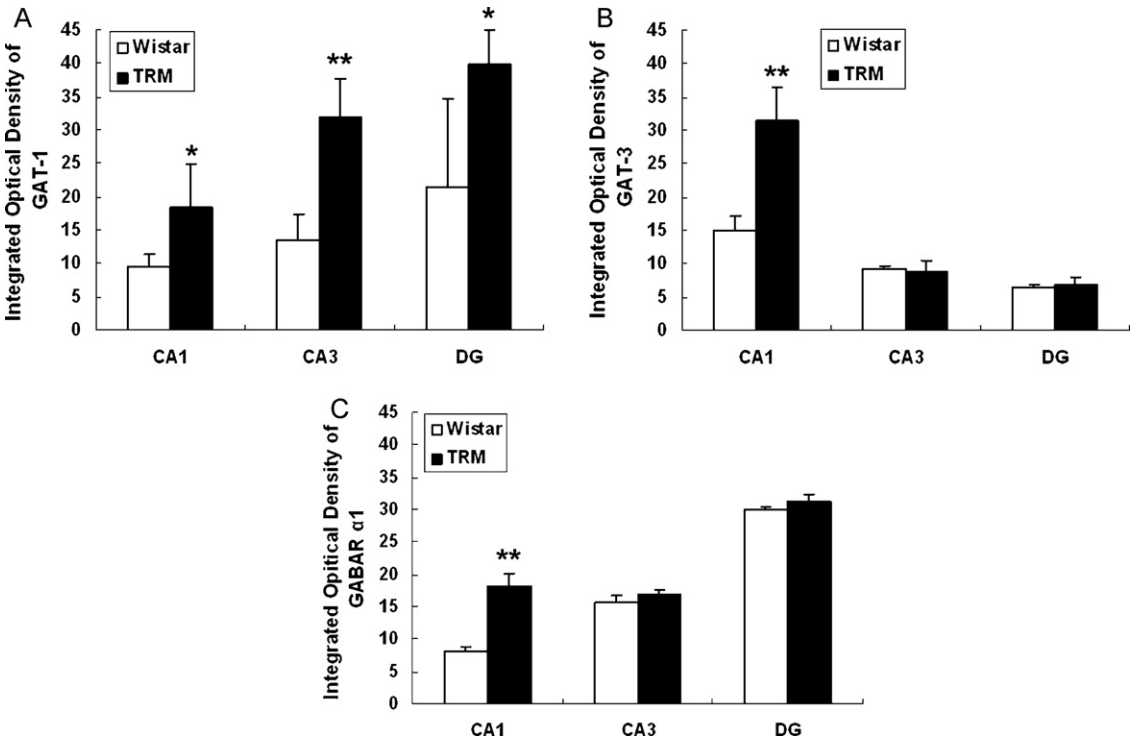

Fig. 3. Statistical analyses of the integrated optical density stained with GAT-1, GAT-3 and GABAR α1 antibody in TRMs and Wistar rats hippocampus (mean ± S.D., $n = 5$). (A)–(C) Showed the integrated optical density stained with GAT-1, GAT-3 and GABAR α1 antibody in TRMs and Wistar rats hippocampus, respectively. $^{*}p < 0.05$ vs. Wistar rats. $^{**}p < 0.01$ vs. Wistar rats.

In agreement with several previous reports, we noticed a very weak immunoreaction of GAT-3 in the granule cells of DG and CA3 regions. However, it was noteworthy that in CA1 region, the GAT-3 had a significant distribution and its immunoreaction was increased in TRM. Furthermore, the expression of GAT-3 at protein level was dramatically elevated in TRMs hippocampus compared with Wistar rats, which was in line with our real time PCR results, indicating that the increased expression of GAT-3 might also facilitate the generation of TRM seizure. Further work will be essential to elucidate the regulating mechanism of it.

One of the major findings in the present study was that the expression levels of GABAR α1 mRNA and protein were both up-regulated and positive reactions were widely distributed in CA1, CA3 and DG regions of TRMs hippocampus. Generally speaking, decreased GABAR α1 level can increase the occurrence of developing epileptic seizure. Consistent with our study, up-regulation of GABAR α1 has been reported in brain regions of kindled rats with temporal lobe epilepsy [24], suggesting that increased GABAR α1 subunit level could augment GABAergic inhibition and reduce the frequency and severity of seizure activity propagating in the brain. In addition, lasting increases in GABAR α1 mRNA level and IR have been also shown in hippocampal molecular layer and granule cells after kainic acid-induced seizures in the rat, respectively; conversely, there was a significant reduction of GABAR α1 mRNA in CA1 7 days after acute kainic acid-induced seizures in the kainic acid-treated rats [28]. However, the marked reduction was observed in the hippocampal sclerotic specimens of epileptic patients [21]. The variability in findings from the same epileptic model may result from the heterogeneity of cell types sampled when the whole hippocampus is examined. The present study demonstrated that the up-regulation of GABAR α1 subunit at mRNA and protein levels was in line with most of the previous studies. Consequently, we assume that in a state of hypoinhibition in TRM hippocampus, up-regulation of GABAR α1 subunit might act as an adaptive mechanism to reduce the occurrence or severity of epilepsy.

In conclusion, for the first time, the present investigation illustrated the up-regulation of GAT-1, GAT-3 and GABAR α1 in TRM hippocampus, which might represent a modulation of hippocampal inhibition in genetic epilepsy and underlie the observed seizure phenotype of TRM. The hippocampus seems to be the most vulnerable structure in epilepsy, in light of the observation of up-regulation of GAT-1, GAT-3 and GABAR α1. Further work is essential to clarify the detailed mechanism by which GAT-1, GAT-3 and GABAR α1 are changed in TRM. However, TRM can be used as an important animal model to screen effective GATs and GABAR α1 drugs for medicine and gene therapy of epilepsy. Altering the expression of GAT-1, GAT-3 and GABAR α1 via some means might provide therapeutic target for genetic epilepsy.

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References

[1] N.J. Allen, R. Káradóttir, D. Attwell, Reversal or reduction of glutamate and GABA transport in CNS pathology and therapy, Pflugers Arch. 449 (2004) 132–142.
[2] M.T. Akbar, M. Rattray, R.J. Williams, N.W.S. Chong, B.S. Meldrum, Reduction of GABA and glutamate transporter messenger RNAs in the severe-seizure genetically epilepsy-prone rat, Neuroscience 85 (1998) 1235–1251.
[3] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.

[4] F. Conti, A. Minelli, M. Melone, GABA transporters in the mammalian cerebral cortex: localization, development and pathological implications, Brain Res. Rev. 45 (2004) 196–212.
[5] L. Ciesielski, S. Simler, J. Clement, P. Mandel, Effect of repeated convulsive seizures on brain gamma-aminobutyric acid metabolism in three sublines of mice differing by their response to acoustic stimulations, J. Neurochem. 49 (1987) 220–226.
[6] N.O. Dalby, Inhibition of g-aminobutyric acid uptake: anatomy, physiology and effects against epileptic seizures, Eur. J. Pharmacol. 479 (2003) 127–137.
[7] P.R. Dunkley, P.E. Jarvie, J.W. Heath, G.J. Kidd, J.A. Rostas, A rapid method for isolation of synaptosomes on Percoll gradients, Brain Res. 372 (1986) 115–129.
[8] V. Fuentes-Santamaría, J. Alvarado, A. Herranz, N. García-Atarés, D. López, Decreased levels of GABA in the inferior colliculus of the epilepsy-prone hamster (GPG/Vall), Epilepsy Res. 79 (2008) 224–227.
[9] T. Hirao, K. Morimoto, Y. Yamamoto, T. Watanabe, H. Sato, K. Sato, S. Sato, N. Yamada, K. Tanaka, H. Suwaki, Time-dependent and regional expression of GABA transporter mRNAs following amygdala kindled seizures in rats, Brain Res. Mol. Brain Res. 54 (1998) 49–55.
[10] K. Jensen, C.S. Chiu, I. Sokolova, H.A. Lester, I. Mody, GABA transporter-1 (GAT1)-deficient mice: differential tonic activation of $GABA_A$ versus $GABA_B$ receptors in the hippocampus, J. Neurophysiol. 90 (2003) 2690–2701.
[11] K. Kulig, M. Szwaczkiewicz, The role of structure activity relationship studies in the search for new GABA uptake inhibitors, Mini. Rev. Med. Chem. 8 (2008) 1214–1223.
[12] E.R. Korpi, G. Grunder, H. Lüddens, Drug interactions at $GABA_A$ receptors, Prog. Neurobiol. 67 (2002) 113–159.
[13] T.C. Kang, H.S. Kim, M.O. Seo, S.K. Park, H.Y. Kwon, J.H. Kang, M.H. Won, The changes in the expression of γ-aminobutyric acid transporters in the gerbil hippocampal complex following spontaneous seizure, Neurosci. Lett. 310 (2001) 29–32.
[14] S.M. Lasley, Roles of neurotransmitter amino acids in seizure severity and experience in the genetically epilepsy-prone rat, Brain Res. 560 (1991) 63–70.
[15] B. Lopez-Corcuera, Q.R. Liu, S. Mandiyan, H. Nelson, N. Nelson, Expression of a mouse brain cDNA encoding novel gamma-aminobutyric acid transporter, J. Biol. Chem. 267 (1992) 17491–17493.
[16] B.S. Meldrum, M.A. Rogawski, Molecular targets for antiepileptic drug development, Neurotherapeutics 4 (2007) 18–61.
[17] A. Minelli, N.C. Brecha, C. Karschin, S. DeBiasi, F. Conti, GAT-1, a high affinity GABA plasma membrane transporter is localized to neurons and astroglia in the cerebral cortex, J. Neurosci. 15 (1995) 7734–7746.
[18] G.W. Mathern, D.M. endoza, A. Lozada, J.K. Pretorius, Y. Dehnes, N.C. Danbolt, N. Nelson, J.P. Leite, L. Chimelli, D.E. Born, A.C. Sakamoto, J.A. Assirati, I. Fried, W.J. Peacock, G.A. Ojemann, P.D. Adelson, Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy, Neurology 52 (1999) 453–472.
[19] R.M. McKernan, P.J. Whiting, Which GABAA-receptor subtypes really occur in the brain? Trends Neurosci. 19 (1996) 139–143.
[20] S. Orozco-Suarez, K.L. Brunson, A. Feria-Velasco, C.R. Ribak, Increased expression of gamma-aminobutyric acid transporter-1 in the forebrain of infant rats with corticotropin-releasing hormone-induced seizures but not in those with hyperthermia-induced seizures, Epilepsy Res. 42 (2000) 141–157.
[21] S. Pirker, C. Schwarzer, T. Czech, C. Baumgartner, H. Pockberger, H. Maier, B. Hauer, W. Sieghart, S. Furtinger, G. Sperk, Increased expression of $GABA_A$ receptor β-subunits in the hippocampus of patients with temporal lobe epilepsy, J. Neuropathol. Exp. Neurol. 62 (2003) 820–834.
[22] D. Rating, H. Siemes, W. Löscher, Low CSF GABA concentration in children with febrile convulsions, untreated epilepsy, and meningitis, J. Neurol. 230 (1983) 217–225.
[23] U. Rudolph, F. Crestani, D. Benke, I. Brunig, J.A. Benson, J.M. Fritschy, J.R. Martin, H. Bluethmann, H. Mohler, Benzodiapine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes, Nature 401 (1999) 796–800.
[24] Y.S.H. Raol, G.J. Zhang, I.V. Lund, B.E. Porter, M.A. Maronski, A.R.B. Kayal, Increased $GABA_A$-receptor α1-subunit expression in hippocampal dentate gyrus after early-life status epilepticus, Epilepsia 47 (2006) 1665–1673.
[25] S. Simler, L. Ciesielski, J. Clement, P. Mandel, Amino acid neurotransmitter alterations in three sublines of Rb mice differing by their susceptibility to audiogenic seizures, Neurochem. Res. 15 (1990) 687–693.
[26] T. Serikawa, Y. Ohno, M. Sasa, J. Yamada, S. Takaori, A new model of petit mal epilepsy: spontaneous spike and wave discharges in tremor rats, Lab. Anim. 21 (1987) 68–71.
[27] G. Sperk, C. Schwarzer, J. Heilman, S. Furtinger, R.J. Reimer, R.H. Edwards, N. Nelson, Expression of plasma membrane GABA transporters but not of the vesicular GABA transporter in dentate granule cells after kainic acid seizures, Hippocampus 13 (2003) 806–815.
[28] K. Tsunashima, C. Schwarzer, E. Kirchmair, W. Sieghart, G. Sperk, $GABA_A$ receptor subunits in the rat hippocampus III: altered messenger RNA expression in kainic acid-induced epilepsy, Neuroscience 80 (1997) 1019–1032.
[29] Y. Ueda, L.J. Willmore, Hippocampal γ-aminobutyric acid transporter alterations following focal epileptogenesis induced in rat amygdale, Brain Res. Bull. 52 (2000) 357–361.
[30] Y. Ueda, T. Doi, K. Nagatomo, L.J. Willmore, Role of glutamate and GABA transporters in development of pentylenetetrazol-kindling, Neurochem. Res. 34 (2009) 1324–1331.
[31] H.D. Yan, K. Ishihara, T. Serikawa, M. Sasa, Activation by N-acetyl-L-aspartate of acutely dissociated hippocampal neurons in rats via metabotropic glutamate receptors, Epilepsia 44 (2003) 1153–1159.