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Increased EphA/ephrinA expression in hippocampus of pilocarpine treated mouse

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Summary
Purpose: EphA family receptor tyrosine kinases and their ephrinA ligands are involved in patterning axonal connections during brain development. Although it has been evidenced that these molecules continue to play a key role in synaptic reorganization and plasticity in normal and injured adult brains, their effect still remains unclear during epileptogenesis. Temporal lobe epilepsy (TLE) is the most common form of adult focal epilepsy and often associates with sclerosis of the hippocampus and mossy fiber sprouting (MFS). The purpose of this study is to evaluate the relationship between EphA/ephrinA molecules and epileptogenesis after status epilepticus (SE).
Method: A mouse model of chronic temporal lobe epilepsy was prepared by intraperitoneal administration of pilocarpine. EphAs/ephrinAs expression levels of the mouse hippocampus areas were detected at different time points after SE by PCR, in situ hybridization and immunohistochemistry. Mossy fiber sprouting was estimated by Neo-Timm staining.
Result: EphAs/ephrinAs were widely distributed in the hippocampus area. EphA10 and ephrinA4 were increased significantly following epileptogenesis, and mossy fiber sprouting appeared after SE.
Conclusion: The up-regulation of EphA/ephrinA expression after SE suggests that they are involved in the pilocarpine-induced epileptogenesis.

Introduction

Epilepsy is a prevalent neurological disorder, in which normal brain functions are disrupted as a consequence of intensive burst activity from neurons. Temporal lobe epilepsy (TLE), the most common type of epilepsy in adult humans,

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is characterized clinically by the progressive development of spontaneous recurrent seizures (SRS) of temporal lobe origin and pathologically by hippocampal neuronal loss and mossy fiber sprouting (MFS) (Cohen et al., 2002; Cendes, 2005; Andrade-Valenca et al., 2008). The pilocarpine status epilepticus (SE) model by a systemic injection of pilocarpine appears to be highly isomorphic with the human TLE, which is followed by hippocampal subfields damage, such as neuron loss and mossy fibers sprouting in the dentate gyrus (DG) and CA3 area (Sutula et al., 1989). In recent years, it has become a widely used rodent model of difficult-to-treat epilepsy type (Curia et al., 2008). Although TLE has been investigated both in human and animal models for years, the mechanisms underlying epileptogenesis remain largely unknown (Turski et al., 1983). Specifically, the mossy fiber sprouting has been accepted as a common neuropathological finding in patients with mesial temporal lobe epilepsy, but its role in epileptogenesis is unclear and controversial (Buckmaster and Lew, 2011).

Axon guidance molecules ephrin and their receptors Eph, the largest subfamily of receptor tyrosine kinase (RTK), are distributed widely during developing brain. They provide molecular guidance for axon targeting in the developing central nervous system (CNS) (Wilkinson, 2001). The Eph receptors comprise 14 members subdivided into an A-subclass (EphA1—A8, A10) and a B-subclass (EphB1—B4, B6). The ephrin ligands are also subdivided into an A-subclass (ephrinA1—A5) and a B-subclass (ephrinB1—B3) (Calo et al., 2006). Although the majority of researches on the Eph family focused on development, accumulating evidence indicated that the function of Eph receptors and their ligands ephrin are not limited to developing CNS. They may also modulate neuronal plasticity in the normal and damaged adult brains (Goldshmit et al., 2006). For example, ephrinAs have been reported to be up-regulated in adult zebrafish (Becker et al., 2000), adult mouse (Knoll et al., 2001) and in the retinal target after optic nerve transection of the adult goldfish (Rodger et al., 2000). In the hippocampus, it has been observed that an interaction between ephrinA3-expressing astrocytic processes that border the synapse and the EphA-expressing post-synaptic dendritic spines, and it might be critical for regulating synaptic functions and hippocampal remodeling and plasticity (Murai et al., 2003). Wang et al. (2003) observed an increase of the expression of mRNAs for ephrinA1, A2, A3, A5 and B1 in the deafferented rat hippocampus. The relatively high expression level of the Eph/ephrins in the hippocampal region implicates that they might be involved in reconstruction or modification of the neuronal network. This may eventually lead to an alteration of the circuit properties.

Although most members of the Eph/ephrins family are validated to be expressed in the adult CNS, their roles in adult brains, especially the relations between these members and epilepsy, are still largely unknown. Xu et al. (2003) found that EphA5/ephrinA3 regulated epileptogenesis and axonal sprouting in the hippocampus of a kindling rat model. Given the relatively high expression of Eph/ephrins in the hippocampal region (Yue et al., 2002) and their roles in axonal guidance (Knoll and Drescher, 2002), the present study examined the expression changes of EphA/ephrinAs in hippocampus of pilocarpine treated mice to explore the potential roles of EphA/ephrinAs in the epileptogenesis.

Methods

Animals preparing

All experiments were carried out in accordance with the Animal Care Guide for the care and use of experimental animals in the University of Electronic Science and Technology of China. A total of 151 adult male Kunming strain mice (body weighing 22—25 g), supplied by the Laboratory Animal Center in West China Animal Breeding Centre of Sichuan University, were maintained under standard laboratory conditions with a 12 h/12 h light/dark cycle (lights on at 7 a.m.), 22 ± 1 °C room temperature, 50—70% humidity and free access to food and water.

SE was induced by the administration of pilocarpine (290 mg/kg, Sigma, USA) intraperitoneal injection, preceded 30 min by using scopolamine methyl-nitrate (1 mg/kg, Sigma, USA) to limit peripheral cholinergic effects. Fifteen to thirty min after pilocarpine administration, the mice showed behavioral seizures, including continuous scratching, strong body tremor, mastication, clonic movements of forelimbs and head bobbing culminating. If it did not develop SE within 30 min, the mouse would receive an additional dose of pilocarpine (50 mg/kg) until SE started. The mice whose seizure severity score were up 3, 4 or 5 according to Racine (1972) were allowed to bring into the following study. Control group received the treatment of an equivalent volume of saline instead of pilocarpine. All mice received a single dose of diazepam (10 mg/kg) to stop seizures at 60 min after SE. The behavioral manifestation of SE and control group mice were monitored by video to estimate whether the mice showed SRS or not.

The semi-quantitative RT-PCR

Thirty mice ($n = 5$ for each time point) with SE as well as thirty control group mice ($n = 5$ for each time point) were decapitated under anesthesia with a 60 mg/kg dose of pentobarbital sodium at 1 h, 3 h, 7 h, 1 d, 3 d, 7 d after the experimental treatment. The hippocampus tissue were carefully dissected out, frozen in liquid nitrogen and stored at −70 °C until used for RNA extraction.

The total RNA of the hippocampus was isolated with Trizol reagent (Life Technology Inc., Invitrogen, USA) according to the manufacturer's protocol. To remove DNA contamination, the RNA samples were treated with RQ1 RNase-free DNase (Promega, Madison, WI, USA) and extracted with phenol: chloroform. The concentrations of the total RNA were measured with the biophotometer and the OD260/OD280 ratio of all RNA samples were up to 2.0 for reverse transcription. For multiple samples to be reverse-transcribed, we used a master mixture containing all the components as shown in the manufacturer's protocol except the RNA templates to minimize sample to sample variations. The master mixture was aliquoted into 0.5 ml Eppendorf tubes loaded with RNA. In the reaction above, reverse transcribed cDNAs were produced and used as the templates of PCR below.

Table 1 Primers of target genes and conditions used for the semi-quantitative PCR.

Target gene	Primer sequence (5′—3′)	Product size (bp)	Optimal cycle	Annealing temperature (°C)
GAPDH	F: TTCACCACCATGGAGAAGGC R: GGCATGGACTGTGGTCATGA	261	28	56 °C
EphA1	F: GCCTGGCCCTTTCTCCCCTG R: TCTCTGTCTCTGGCCTCTCC	240	35	60 °C
EphA2	F: TGGATGGCGAGTGGCTGGTG R: TTGGGGGCGAGGGTGGACG	250	35	59 °C
EphA3	F: AAGCAGGAGCAAGAGACGA R: CACCGGAGATGGAGAAAGAG	183	35	55 °C
EphA4	F: TCTTTTCGTTTCTCTTTGG R: GTTGTTCTGGCTGGCTTCC	222	28	51 °C
EphA5	F: AAGGGCAAAGAAGCGGGAC R: GGAAGGGGCGAGAGAGACG	244	32	57 °C
EphA6	F: ATTCTTCCTCTTTGGTTG R: GGTGGGTCTTTTTCTGCC	298	35	50 °C
EphA7	F: TCTGGCTGCTTGGCTTTGC R: TCTGTTTGTTGTGCTTTCG	252	32	55 °C
EphA8	F: TCACCACGAACCAGGCAG R: GAGAAGCAAGAGGAGCAC	386	30	54 °C
EphA10	F: GACCTTCAACGCATACTACCT R: CGCTCTATCCTGCCATCT	539	32	55 °C
EphrinA1	F: TGGGCAAGGAGTTCAAGG R: GCAGCAGTGGTAGGAGCA	255	30	54 °C
EphrinA2	F: CTCAAGGTTATGTGCGTCCA R: TGCGAGGGCTGAAGGGTT	142	30	56 °C
EphrinA3	F: CCACGCCCACTCACAACCTG R: CCTCAAAGTCTTCCAACACG	156	32	57 °C
EphrinA4	F: CAGCGCTACACACCCTTCCC R: GTGATGACCCGCTCTCCTTG	142	32	58 °C
EphrinA5	F: CCCAGACAACGGAAGAAG R: ACAGGCGGACGGGAGGAG	202	32	59 °C

F, forward; R: reverse.

The semi-quantitative PCR for EphA/ephrinA family members (except for ephrinA6 and EphA9 which was only distributed in fowl) was performed as described by the literature (Wang et al., 2007). The primer sequence, annealing temperature, and optimal cycle numbers for each target gene were listed in Table 1. PCR amplification of GAPDH transcript was used as an internal standard and to normalize the levels of target genes. The PCR products were run on a 1% agarose gel equilibrated in TAE buffer, and were then stained with ethidium bromide (0.5 μg/ml), photographed under UV light and analyzed by Quantity One software (Bio-Rad, Hercules, CA, USA).

In situ hybridization analysis

To validate the results of PCR, in situ hybridization (ISH) analyses for EphA10/ephrinA4 were performed at 1 d, 3 d, 7 d, 14 d, 21 d and 28 d. Twenty-four mice ($n = 4$ for each time point) with SE as well as eighteen control group mice ($n = 3$ for each time point) were rapidly perfused intracardially with 0.9% NaCl physiological solution 150 ml and 4% paraformaldehyde (PFA) with 0.1% diethylpyrocarbonate (DEPC) 300 ml under anesthesia with pentobarbital sodium (60 mg/kg). Brain tissue was quickly removed and stored for 30 min in 4% PFA. Coronal, serial, 5 μm sections were taken from dorsal hippocampus (−1.06 mm to −2.30 mm to bregma). Slides taken from approximately the same coordinates were separated into EphA10 and ephrinA4 groups. ISH procedures for EphA10 and ephrinA4 were performed on the same set of animals using digoxigenin-labeled riboprobes according to SABC reagent protocol (Wuhan Boster Biological Technology Co., Ltd., China). Pre-hybridization procedures were performed under RNase-free conditions for 4 h at room temperature. Hybridization was carried out at 42 °C overnight in hybridization buffer. After washing, slides were incubated for 20 min at 37 °C with Strept Avidin-Biotin Complex (SABC) and for 120 min at 37 °C with anti-DIG antibody coupling biotinylation. The sections were stained using Diaminobenzidine (DAB). Instead, the probe of control group was 0.1 M phosphate buffer solution (PBS).

Fluorescence immunohistochemistry

Further, twenty mice ($n = 4$ for each time point) with SE as well as fifteen mice with saline treatment ($n = 3$ for each

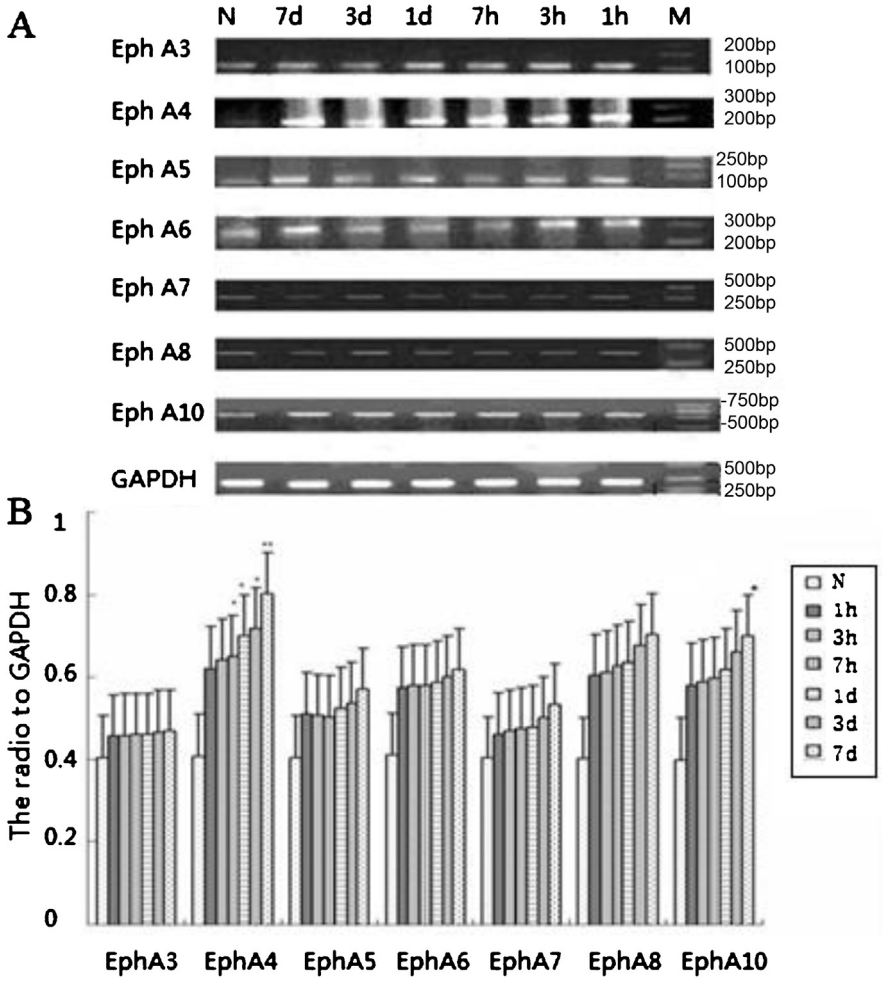

Figure 1 RT-PCR analysis of genes for EphAs mRNA in the hippocampus of normal and SE mice. (A) Photograph shows mRNAs expression for EphAs in hippocampus at different time point after SE. GAPDH is used as an internal standard to determine the amount of PCR template in each sample. The last lane on the right is DNA marker with bars indicative of molecular weight (bp). (B) The mRNA level is semi-quantified in each sample in relation to that of GAPDH. The levels of EphA4 and EphA10 mRNA increased significantly at post-SE. Each bar represents the means ± SD. *$P < 0.05$, **$P < 0.01$, vs normal group. N, normal group; M, DNA marker.

time point) were perfused intracardially at 3 d, 7 d, 14 d, 21 d and 28 d after SE using the above method except without 0.1% DEPC in PFA to estimate expression of EphA10 protein. The brain was quickly removed and fixed for 2 h in 4% PFA. Coronal, serial, 20 μm sections were taken from dorsal hippocampus. Immunostaining was performed using EphA10 antibody (diluted 1:100, Wuhan Boster Biological Technology Co., Ltd., China) overnight at 4°C. Antibody binding was visualized by incubating with biotinylated anti-rabbit IgG for 30 min, followed by the SABC-FITC reagent for 30 min (ABC System, Wuhan Boster Biological Technology Co., Ltd., China) at room temperature. Negative controls were carried out in which the primary antibody was omitted.

The images of ISH and fluorescence immunohistochemistry were acquired under DP71 digital color camera and analyzed by IPE6.0 microscope imaging software (Olympus, Japan).

Statistical analyses of the results were carried out using *t*-tests and repeated-measures ANOVAs using SPSS 16.0 statistical software. The results were expressed as mean ± SD. $P < 0.05$ was considered significant.

Neo-Timm staining for mossy fiber sprouting

To further evaluate the relativity between EphA10 and epileptogenesis, Neo-Timm staining was performed to measure mossy fiber sprouting at 14 and 28 d post-SE. Under anesthesia with pentobarbital sodium (60 mg/kg), eight mice with SE ($n = 4$ at every time point) and six mice with saline treatment ($n = 3$ at every time point) were perfused intracardially using 50 ml 0.1% sodium sulfide (Na_2S solution) in PBS and 200 ml 4% PEA. The brain was carefully dissected and transferred to 30% sucrose solution overnight for post-fixation. Sections of 30 μm thickness from dorsal hippocampus were stained using a modified Timm method for the detecting mossy fiber sprouting. To minimize the variability of Timm labeling, sections from different groups were stained simultaneously. The density of Timm granules

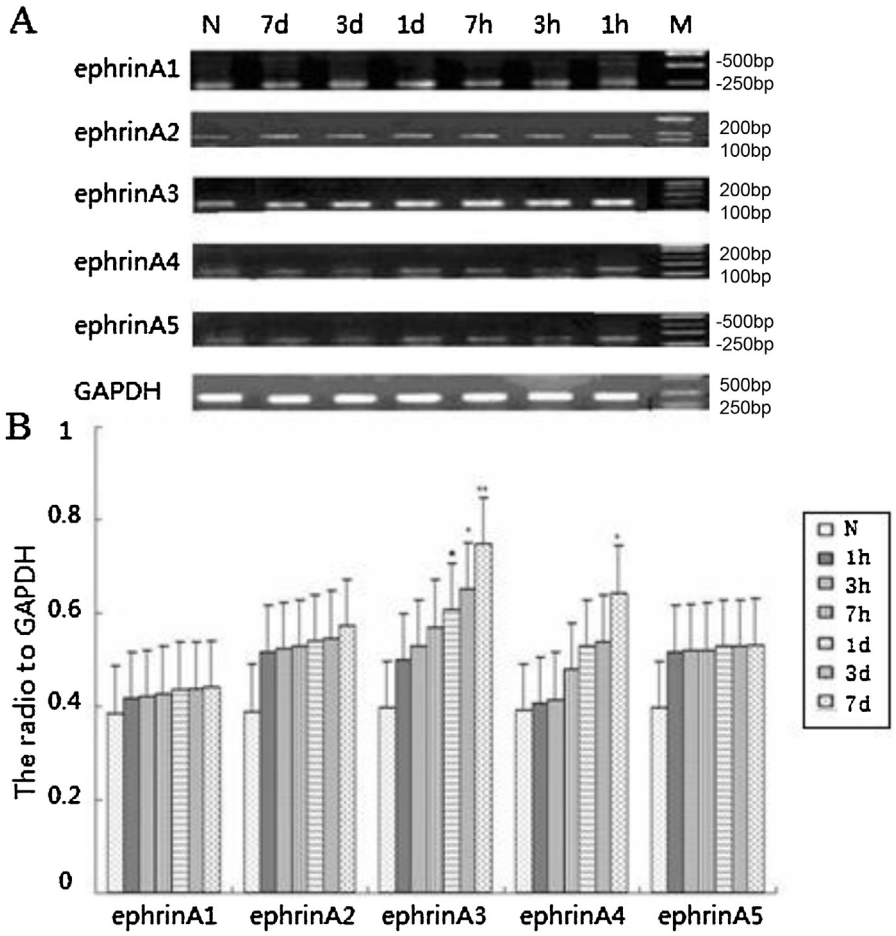

Figure 2 RT-PCR analysis of genes for ephrinAs mRNA in the hippocampus of normal and SE mice. (A) Photograph shows mRNAs expression for ephrinAs in hippocampus at different time point after SE. GAPDH is used as an internal standard to determine the amount of PCR template in each sample. The last lane on the right is DNA marker with bars indicative of molecular weight (bp). (B) The mRNA level is semi-quantified in each sample in relation to that of GAPDH. The levels of ephrinA3 and ephrinA4 mRNA increased significantly at post-SE. Each bar represents the means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, vs normal group. N, normal group; M, DNA marker.

in the CA3 region and dentate gyrus was measured based on the scale described by Holmes et al. (1999).

Results

Semi-quantitative PCR of EphAs/ephrinAs

PCR results showed that EphAs/ephrinAs molecules were widely distributed in the hippocampus of normal and epileptic mice. The expression levels seemed to be slightly up-regulated in the hippocampus at different time-points post-SE. The levels of EphA4, EphA10, ephrinA3 and ephrinA4 mRNA increased significantly at 7 d post-SE. Results were shown in Figs. 1 and 2.

Expression of EphA10/ephrinA4 mRNA in the hippocampus

The results showed the expression of EphA10 mRNA in the hippocampus of normal and epileptic mice. The expression level of EphA10 and its high affinity ligand ephrinA4 mRNA showed gradual up-regulation following SE. To further evaluate the result, we measured the expression of EphA10/ephrinA4 mRNA in hippocampus by ISH. Results showed that they were distributed mainly in pyramid cell layers of the hippocampal CA1, CA3 and granule cells of DG of both normal and epileptic mice. The expression level of ephrinA4 mRNA was up-regulated after SE and reached its maximum in CA3 and CA1 regions on the 7th and 14th day after SE ($P < 0.05$), respectively. There was no significant difference in DG between normal and epileptic mice (Fig. 3).

In contrast to ephrinA4 mRNAs, the expression level of EphA10 mRNA was consistently up-regulated in CA1, CA3 and DG region on the 7th or 14th day after SE ($P < 0.05$). Subsequently, their expression declined and recovered thereafter toward normal levels, even though there still appeared to be a small expression elevation on the 28th day after SE (Fig. 4).

Expression of EphA10 protein in the hippocampus

The results from the fluorescence immunohistochemistry method showed that EphA10 protein was widely distributed in neuronal cell layers of hippocampal CA1, CA3, DG and

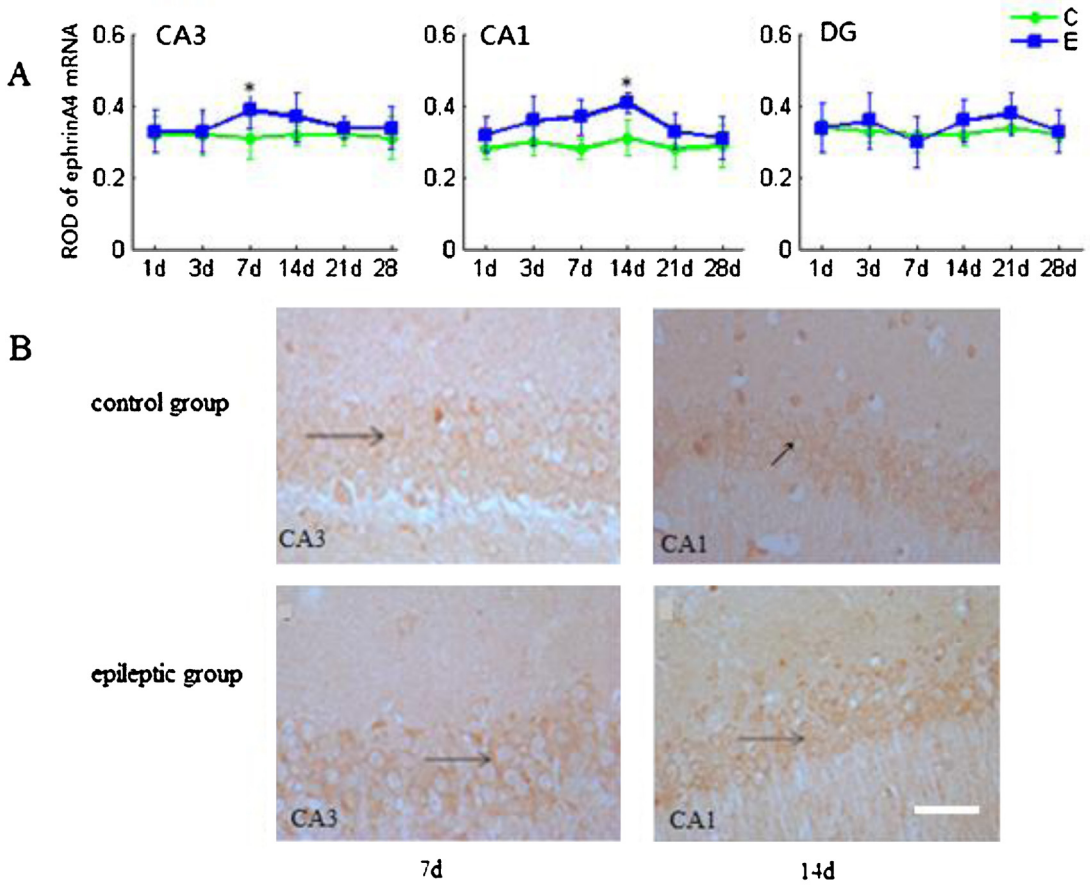

Figure 3 The in situ hybridization of ephrinA4 mRNA in the hippocampus. (A) The hybridization intensity (measured as relative optical density, ROD) in CA3, CA1 and DG of both normal and epileptic mice. The levels of ephrinA4 mRNA were up-regulated after SE and arrived at maximum at 7 d in CA3 region and at 14 d in CA1 region after SE. There was no significant difference in DG between normal and epileptic mice. Error bars represent standard error of the mean (SEM). (B) The photographs show the imaging of ephrinA4 mRNA at the significant time point in CA3 (7 d after SE), and CA1 (14 d after SE). The arrow represents positive reactivity cell. Scale bar: 200 μm. C, control group; E, epileptic group. $^{*}P < 0.05$ vs control group.

other brain regions (i.e. cortex and thalamus) of both normal and epileptic groups. Following epileptogenesis, EphA10 protein gradually increased and reached its maximum in CA1 and CA3 at 14 d after SE. However, no significant difference was observed in DG of control and epileptic mice (Fig. 5).

Timm staining of mossy fiber sprouting

On the 14th and 28th days after SE, mice were sacrificed and dorsal hippocampal slices were stained using a modified Timm staining for the analysis of mossy fiber sprouting. The results indicated that Timm granules increased gradually in stratum oriens of dorsal hippocampal CA3 area, but no mossy fiber sprouting was found in inner molecular layer (IML) of DG in SE mice with SRS.

Discussion

Effect of EphAs/ephrinAs in normal and injured adult brains

EphA receptors represent the largest subfamily of receptor tyrosine kinases and have been shown to play a role in determining patterns of synapse formation in development (Zhang et al., 1996). EphA receptors interact with ephrinA ligands to mediate axonal guidance and influence cell behavior, such as attraction/repulsion, adhesion/de-adhesion and migration during neural system development (Kullander and Klein, 2002).

Although the majority of researches on the Eph family focused on development, a continuous low level expression of Ephs/ephrins were found with both normal and injured adult CNS (Gould et al., 1999). And the Eph receptors and their ligands may play an important role in the underlying maintenance of neuronal connections in adult CNS, such as synapse formation, regulation of synaptic function and plasticity (Takasu et al., 2002; Murai et al., 2003; Battaglia et al., 2003). Most members of the Eph receptor family, including EphA2 to A5 and EphB1 to B6, have been demonstrated to be expressed in adult CNS (Gerlai, 2001). In goldfish and rats, ephrinA2 expression gradients persist in the adult tectum and superior colliculus respectively, although at lower levels than during development (Rodger et al., 2004). EphrinA concentration gradients would repel new axons of granule cells with EphA in DG from the inner molecular layer to hillus and CA3 area (Hunter et al., 2006). Gao and Phillips

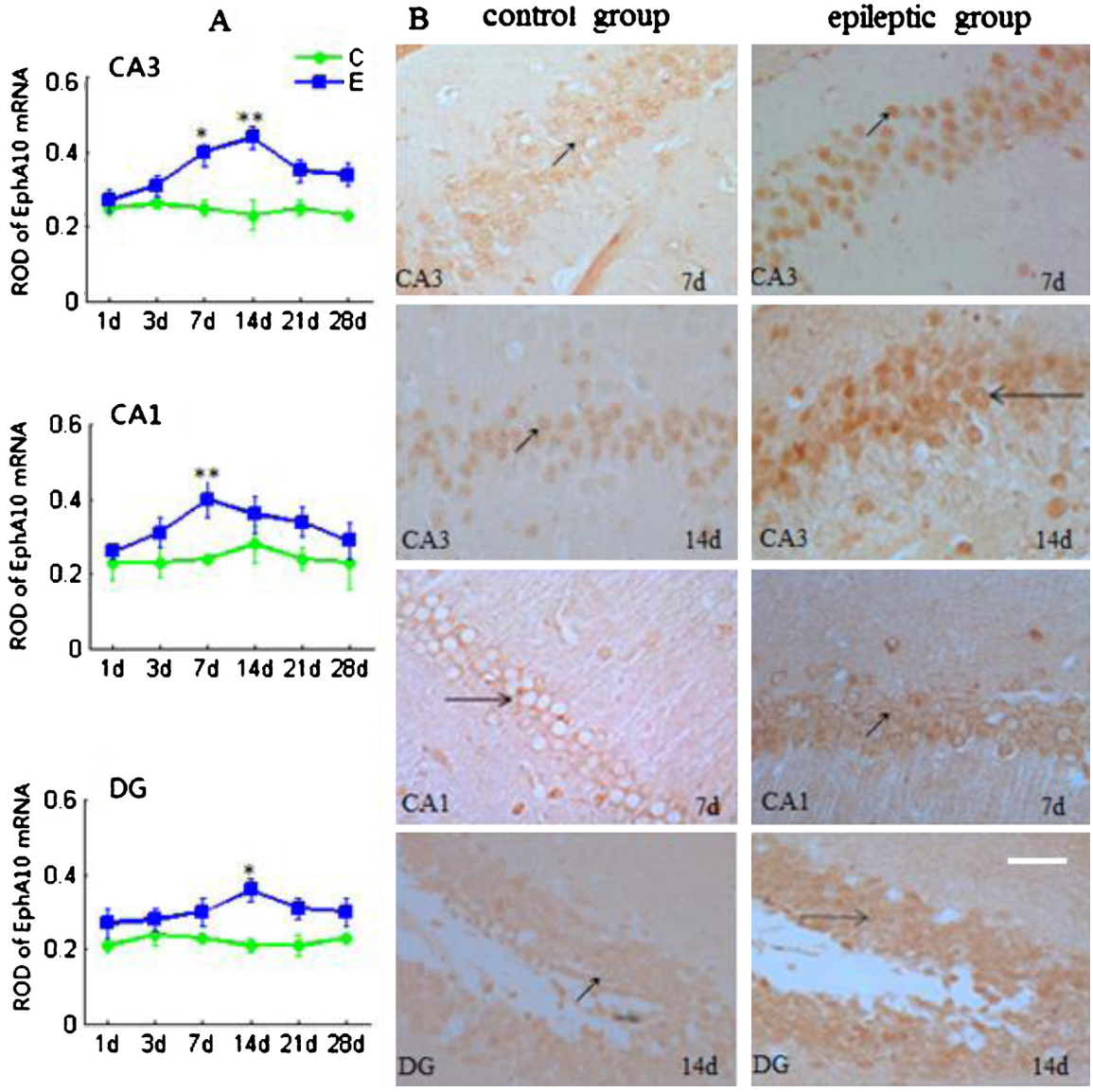

Figure 4 The in situ hybridization of EphA10 mRNA in the hippocampus. (A) The hybridization intensity (measured as relative optical density, ROD) in CA3, CA1 and DG of both normal and epileptic mice. The levels of EphA10 mRNA were consistently up-regulated in CA1 region, CA3 region and DG region after SE. Error bars represent standard error of the mean (SEM). (B) The photographs show the imaging of EphA10 mRNA at the significant time point in CA3, CA1 and DG under 20× amplification. The arrow represents positive reactivity cell. Scale bar: 200 μm. C, control group; E, epileptic group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control group.

(1999) detected the expression of EphA5/ephrinA2, A5 in adult mouse hippocampus, piriform cortex and structures that exhibit a high degree of neuronal plasticity and activity-dependent axonal reorganization. It is notable that the up-regulation of ephrin mRNAs (i.e. A1, A2, A3, A5 and B1) occurs during the reorganization in the deafferented hippocampus, suggesting that ephrins may be involved in the plasticity events of the adult brain after lesion (Wang et al., 2003).

Thus, the up-regulation of Eph receptors and ephrins following neural injury on different cell types during adulthood may suggest that Eph family members also play a key role in regeneration following lesion, neuronal plasticity and cognitive function of adult CNS (Zhou, 1998; Klein, 2004). Our primary results showed that EphA10 was expressed widely in different brain regions, such as cortex, thalamus and hippocampus of both normal and epileptic mice. This suggests that, similar to other Eph/ephrin family members, EphA10 and its high affinity ligand may also play an important role in the adult brain.

EphAs/ephrinAs and epilepsy

In adult CNS, increased neurogenesis may follow many physiological status and pathological disorders, such as learning (Gould et al., 1999), seizures (Parent, 2002), and ischemia (Kokaia and Lindvall, 2003). Sprouting and reorganization of

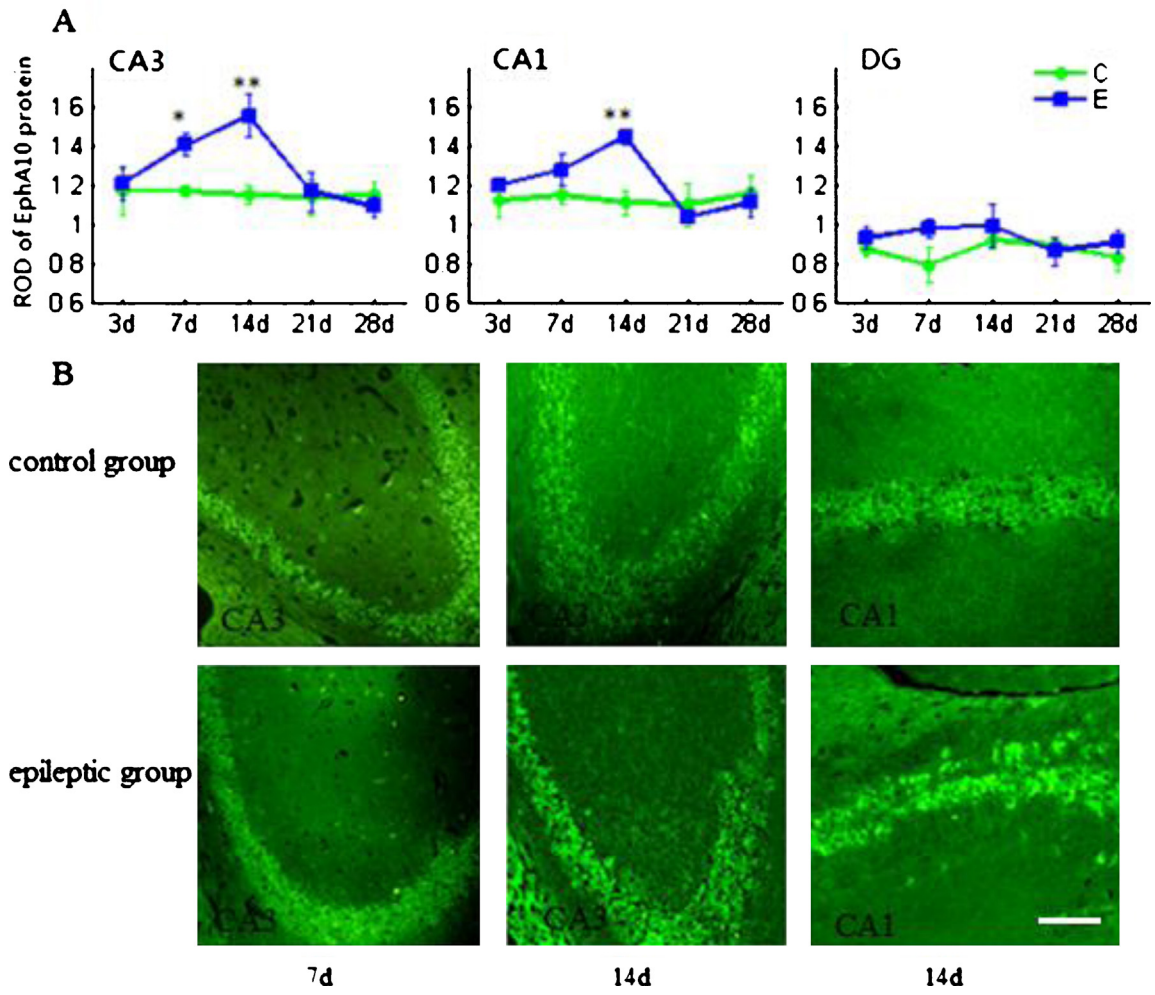

Figure 5 Expression of EphA10 protein in different hippocampus regions. (A) The drawings show relative optical density (ROD) of Eph A10 protein in hippocampus CA3, CA1 and DG, respectively. Eph A10 protein gradually increased and arrived at top at 14 d after SE in CA1 and CA3. However, there was no significant difference in DG between normal and epileptic mice. Error bars represent SEM. (B) The photographs present section of Eph A10 protein at the significant time points (7 d and 14 d after SE) in normal and epileptic hippocampus. Scale bar: 200 μm. C, control group; E, epileptic group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control group.

the mossy fiber system are induced by high levels of neuronal activation (Parent, 2002). In most animal models and autopsy from refractory TLE patient hippocampus, it has been suggested that there are MFS in the inner molecular layer (IML) of DG and stratum oriens of CA3 area, and that fiber sprouting time is consistent with spontaneous seizures. Thus, axonal sprouting may result in reconstruction or modulation of the neuronal network in epileptogenesis (Kullander and Klein, 2002).

Synaptic sprouting and circuit reconstruction might be modulated when Eph and its high affinity ligands show high expression level (Henderson et al., 2001; Murai et al., 2003; Moeller et al., 2006). The role of the Eph/ephrins system extends to the development of kindling, an activity-dependent form of neuronal plasticity that underlies epileptogenesis in limbic regions, such as the hippocampus and amygdala. For example, Xu et al. discovered that continuous intracerebroventricular infusion of clustered EphA5/Fc, which activates the cognate ligands ephrinA3/A5 but disrupts forward signaling at endogenous EphA5 receptors, retards the development of kindling induced by electrical stimulation of the perforant pathways. Whereas clustered ephrinA5/Fc infusion accelerates the development of kindling. Interestingly, ephrinA5 infusion alters the distribution of sprouted mossy fiber collaterals along the dorso—ventral and anterior—posterior axis in kindled animals (Xu et al., 2003). These studies suggest that EphAs/ephrinAs can control axon growth in the hippocampus circuit of the electric kindling epilepsy rats. The up-regulated of Eph/ephrin may also activate glia cells to supply mossy fiber sprouting environment after SE (Otal et al., 2006). At present, only a few Eph/ephrin family members, including EphA4, EphA5, ephrinA3 and ephrinA5, are considered to be involved in epileptic activity (Xu et al., 2003). However, whether other members are associated with neural circuit reorganization after seizure still remains unknown.

EphA10, a new member of Eph receptor subfamily, was first found to express in testis tissue and the best binding ligands of EphA10-Fc were to ephrinA3, A4 and A5. The gene

encoding EphA10 is located on chromosome 1p34 (Aasheim et al., 2005). But it has not been reported if EphA10 is distributed in adult CNS. In our experiment, we first find the expression of EphA10 in normal and epileptic mouse hippocampus and other brain regions, such as cortex and thalamus. Meanwhile, the present work also confirmed up-regulation of EphA10 expression level in the hippocampus of epileptic mice.

The adult mossy fiber system is subject to sprouting and rewiring following brain activation or lesion. The sprouted mossy fibers could either abnormally innervate the dentate IML (Buckmaster et al., 2002), or form new synapses with apical dendrites of CA3 pyramidal cells (Muramatsu et al., 2008). Most studies on experimental models of partial-onset epilepsy have described the sprouting of mossy fiber axons in the IML of the DG (Adams et al., 1997a). However, some studies demonstrated that CA3 pyramidal neurons are vulnerable to cellular injury and thus synaptic reorganization (Cilio et al., 2003). For example, the rats exposed to KA-SE on P15 showed synaptic reorganization of distal mossy fiber axons into the stratum oriens of CA3. However, reorganization was absent in the proximal mossy fiber pathway (IML of the DG) and the proximal CA1 axons (CA1 stratum molecular) (Cross and Cavazos, 2007). Synaptic reorganization induced by excitotoxic injury was shown in the distal projection of the mossy fibers to the stratum oriens of the CA3 pyramidal region in the immature experimental models (Holmes et al., 1999) and in models of adult seizures (Represa et al., 1989a). The sprouting in the IML is usually more evident in the ventral portion of the hippocampus (Li et al., 2002), while the sprouting in the stratum oriens of CA3 is usually more prominent in the dorsal portion of the hippocampus (Ramirez-Amaya et al., 2001; Rakhade and Jensen, 2009). These results demonstrated that different patterns of mossy fiber sprouting arise in the dorsal—ventral hippocampus. The present analysis is based on the dorsal hippocampus at 28 d stage after SE, the results showed that increased mossy fiber projection occurred only in CA3 not DG. To confirm whether similar fiber sprouting occurs in IML of DG in our model, we may need to check the ventral hippocampus at even the later stage than the current 28 d in the future.

The fact that EphA10 protein and its high affinity ligand ephrinA4 mRNA increased in CA3 may imply that the expression pattern of EphA10/ephrinA4 is related to mossy fiber sprouting of pilocarpin-induced epileptogenesis. This indicates that EphA10 might regulate the formation of hippocampal neural circuitry post-SE. However, the significance of EphA10 expression throughout adult brain is not clear so far, and the exact effect of EphA10 in normal and epileptic brain still needs to be clarified in the future.

In summary, the observed up-regulation of EphA10/ephrinA4 expression following epileptogenesis in the present study suggests that they may play an important role in TLE by modulating MFS and synaptic circuit regeneration. It is not clear at present whether expression up-regulation of the Ephs/ephrins molecules would lead to a cascade of events after SE. However, as previous studies have shown that Ephs/ephrins expression and activation may modulate neuronal regeneration after injury by multiple mechanisms, understanding the effect of Ephs/ephrins following CNS injury may be helpful to further clarify the underlying mechanisms of epilepsy and enable effective treatment strategies for such CNS diseases.

Acknowledgements

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