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Increased expression of DOC2A in human and rat temporal lobe epilepsy

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ABSTRACT

Temporal lobe epilepsy (TLE) is the most common form of intractable epilepsy. Currently, the molecular mechanisms underlying epileptogenesis in TLE remain elusive; however, synaptic transmission may play an important role in the pathogenesis of epilepsy. Synaptic transmission is regulated by diverse mechanisms, including presynaptic modulators of synaptic vesicle formation and release, modulators of neurotransmission and distinct Ca^{2+} sensors. DOC2A, a novel Ca^{2+} sensor, can regulate spontaneous synaptic transmission and has been implicated in Ca^{2+}-dependent neurotransmitter release. In this study, we demonstrate for the first time that DOC2A expression is significantly increased in human TLE and in two different rat models of TLE (pilocarpine- and kindling-induced) compared to the control groups. Localization of DOC2A in the human TLE patients and pilocarpine post-SE rat model was observed in neurons but not in astrocytes; DOC2A was also concentrated at the presynaptic terminals and colocalized with VMAT2. Our results suggest that the abnormal protein expression of DOC2A in epileptic brain tissue may play an important role in epilepsy.

1. Introduction

Epilepsy is a serious, disabling neurological disorder characterized by recurrent spontaneous seizures, and it affects approximately 50 million people worldwide. Epilepsy often requires lifelong medication and places an enormous burden on individuals and society (Ngugi et al., 2011). Despite the availability of a large number of antiepileptic drugs (AEDs) that have been clinically proven to suppress or prevent seizures, in 30–40% of patients, symptoms cannot be controlled (Baulac and Pitkanen, 2008). Temporal lobe epilepsy (TLE) is the most prevalent type of intractable epilepsy in adults; marked by partial complex seizures with a temporal-lobe origin, this disorder shows a progressive development (Baulac and Pitkanen, 2008). Although countless studies have attempted to understand the pathogenesis and progression of TLE, the biological basis of epileptogenesis remains unknown (O'Dell et al., 2012).

Epilepsy is characterized by spontaneous, recurrent seizures (SRS) that are caused by abnormal, synchronized, high-frequency neuronal discharges (Baulac and Pitkanen, 2008; O'Dell et al., 2012). Under physiological conditions, neuronal excitation and inhibition are maintained in a dynamic equilibrium. This balance can be disrupted by abnormal synaptic transmission, which can lead to inappropriate neuronal firing and ultimately the generation of spontaneous, recurrent seizures. Therefore, synaptic transmission may play a role in the pathogenesis of epilepsy.

The double C2 domain (DOC2) proteins, which constitute a novel protein family that may function in secretion, contain two C2 domains and include three isoforms: DOC2A, DOC2B, and DOC2C (Friedrich et al., 2010; Verhage et al., 1997). All three isoforms have a Mid (Munc13-interacting) domain and two C2 domains, C2A and C2B (Friedrich et al., 2010). DOC2B and DOC2C proteins are expressed in the brain and in other tissues, but DOC2A is virtually brain specific (Verhage et al., 1997) and may be involved in calcium-dependent neurotransmitter release and in dynein-dependent intracellular vesicle transport (Orita et al., 1995). Strikingly, recent studies have found that the knockout (KO) or knockdown (KD) of DOC2A in cultured neurons resulted in a large decrease in neurotransmitter release, suggesting that DOC2A might act as a Ca^{2+} sensor for synaptic transmission (Groffen et al., 2010; Pang et al., 2011; Sakaguchi et al., 1999; Yao et al., 2011). Moreover, DOC2A has been shown to interact with the secretory molecules of the mammalian uncoordinated (Munc) protein family, which have been proposed to regulate neurotransmitter release by modulating vesicle exocytosis (Mochida et al., 1998; Verhage et al., 1997).

Considering the specific localization of DOC2A in the brain and DOC2A's regulatory function in synaptic transmission, we hypothesized that the expression levels of DOC2A may change in the brain tissues of TLE patients and animal models and that such changes may be involved in epileptogenesis. In this study, our goal was to examine the expression

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Table 1
Clinical characteristics of TLE patients.

Patients	Gender (M/F)	Age (Year)	Course (Year)	AEDs before surgery	Resection tissue	Pathological diagnosis
1	M	29	28	VPA, CBZ, PHT, LTG, PB	TNr	NL
2	M	19	17	VPA, CBZ, LTG, OXC	TNr	NL
3	F	27	6	VPA, PB, TPM	TNr	NL, G
4	M	19	15	CBZ, TPM, LTG	TNl	NL
5	M	23	2	VPA, LTG, LEV	TNl	NL
6	F	40	20	CBZ, PHT, LTG, TPM,	TNl	NL, G
7	M	52	10	VPA, PHT, LTG	TNr	NL, G
8	M	19	18	VPA, TPM, LTG, LEV	TNl	NL
9	M	25	13	VPA, CBZ, LTG, PB	TNl	NL
10	M	8	7	VPA, TPM, OXC	TNr	NL, G
11	M	18	13	VPA, CBZ, LEV	TNl	NL, G
12	F	19	15	CBZ, VPA, LTG, OXC	TNr	NL, G
13	F	31	27	VPA, CBZ, LEV, CZP	TNr	NL, G
14	F	34	9	VPA, PHT, LTG	TNr	G
15	M	31	20	CBZ, PHT, TPM, LTG	TNl	G
16	F	21	10	CBZ, LTG, CZP	TNl	NL, G
17	F	25	10	CBZ, TPM, OXC	TNl	NL, G
18	M	21	14	VPA, CBZ, LTG	TNr	NL, G
19	F	13	12	CBZ, TPM, CZP	TNr	NL
20	F	32	19	VPA, PB, CBZ, TPM	TNl	NL, G
21	M	24	21	PHT, VPA, CBZ, TPM	TNr	NL
22	F	22	21	VPA, PHT, TPM, LEV	TNl	NL, G
23	F	33	9	VPA, OXC, LTG	TNl	NL
24	F	24	20	CBZ, OXC, LEV, PB	TNr	G
25	M	42	13	VPA, PHT, LTG	TNr	NL, G
26	M	24	12	OXC, LEV, PB	TNr	G
27	F	23	10	CBZ, OXC, LTG	TNr	NL, G
28	M	21	8	VPA, LTG, LEV	TNr	NL, G
29	F	24	17	VPA, CBZ, OXC, LEV	TNl	NL, G
30	M	16	13	VPA, OXC, LTG, PTH	TNr	G

M, male; F, female; AEDs, antiepileptic drugs; VPA, valproate; CBZ, carbamazepine; PHT, phenytoin; LTG, lamotrigine; PB, phenobarbital; OXC, oxcarbazepine; TPM, topiramate; LEV, Levetiracetam; CZP, clonazepam; TN, temporal neocortex; r, right; l, left; NL, neuron loss; G, Gliosis.

level of DOC2A in human TLE patients and two different experimental rat models of TLE. Furthermore, we investigated the cellular location of DOC2A in patients and in the chronic phase of the pilocarpine post-SE rat model.

2. Materials and methods

2.1. Human material

Thirty temporal neocortex samples that were surgically removed from patients with medically intractable TLE and ten histologically normal anterior temporal neocortex samples obtained from patients who were treated for post-trauma intracranial hypertension were randomly selected from our established brain tissue bank (Li et al., 2016, 2015; Table 1 and 2). The patient criteria, informed consent documentation, and brain tissue processing protocols are referenced in our previous publications (Li et al., 2016, 2015). Table 3 shows that there were no significant differences in age or sex between the TLE and control patients ($p > 0.05$, Table 3).

Table 2
Clinical characteristics of control patients.

Patients	Gender (F/M)	Age (Year)	Etiology diagnosis	Resection tissue	Pathology
1	M	36	Trauma	TNl	N
2	M	17	Trauma	TNr	N
3	M	23	Trauma	TNr	N
4	M	28	Trauma	TNl	N
5	M	25	Trauma	TNl	N
6	F	21	Trauma	TNr	N
7	F	47	Trauma	TNl	N
8	F	34	Trauma	TNl	N
9	F	26	Trauma	TNl	N
10	F	20	Trauma	TNr	N

M, male; F, female; TN, temporal neocortex; l, left; r, right; N, normal.

Table 3
Comparison of Clinical Data in the Patients with TLE and Controls.

Clinical variable	TLE group (n = 30)	Control group (n = 10)	P value*
Age (years)			
Mean ± SD	25.30 ± 8.91	27.7 ± 9.02	0.467
Range	8–52	17–47	
Gender (ratio)			
Male/female	16 : 14	5 : 5	0.85
Onset (years)			
Mean ± SD	11.0 ± 10.06	NA	NA
Range	1–42	NA	
Course (years) #			
Mean ± SD	14.30 ± 6.01	NA	NA
Range	2–28	NA	
Seizures frequency (No. /number)			
Mean ± SD	10.17 ± 8.30	NA	NA
Range	6–42	NA	

* P values were computed using unpaired Student's t-tests (age) or Chi-square test (male/female ratio). $P < 0.05$ was considered significant. # Course was calculated as the time between epilepsy onset (onset of habitual seizures) and surgery. NA, not applicable.

2.2. Animals

Adult male Sprague-Dawley rats weighing 210–250 g (6–8 weeks old) were obtained from the Experimental Animal Center of Chongqing Medical University. All the animals were housed in a temperature- and humidity-controlled (18–25 °C and 50–60%, respectively) room with a 12 h light/dark cycle. Water and food were available ad libitum. The experimental procedures were conducted in compliance with the Ethics of Experiments on Animals Commission of Chongqing Medical

University and were in complete compliance with international standards.

2.3. Pilocarpine-induced rat epilepsy model

The evoked behavioral seizures were classified according to Racine's standard criteria (Racine, 1972) as follows: class 1, facial clonus; class 2, head nodding; class 3, unilateral forelimb clonus; class 4, bilateral forelimb clonus and rearing; and class 5, rearing and falling. The modeling process was based on our previous study (Li et al., 2016). Briefly, the rats from the pilocarpine group were injected intraperitoneally with lithium chloride (127 mg/kg, Sigma-Aldrich) 18 h before the first pilocarpine administration (40 mg/kg, i.p., Sigma-Aldrich). Thirty minutes before the pilocarpine administration, the rats were pretreated with methylscopolamine (1 mg/kg, i.p., Sigma-Aldrich) to reduce the peripheral cholinergic effects of pilocarpine. Pilocarpine (10 mg/kg, i.p.) was administered repeatedly every 30 min until the rats developed seizures. Only those rats that exhibited convulsive seizures (class 4 or 5) according to Racine's scale were used for further analyses. One hour after the onset of status epilepticus, the animals were injected with diazepam (10 mg/kg, i.p.). Age-matched control rats (n = 6) received all the treatments (methylscopolamine and diazepam), but the same volume of sterile saline was injected instead of pilocarpine. Subsequently, to determine whether spontaneous seizures occurred in the rats during the chronic period, the behavior of the animals was recorded 24 h per day with a closed-circuit video system to measure the class 4 and class 5 seizures that occurred during the 30-day period. Only those rats exhibiting spontaneous seizures were included in the epilepsy group of this study (n = 8). The animals were sacrificed 1 month after SE onset, and the hippocampi and cortices of 6 animals from the epilepsy group and control group were removed for western blot analysis. The two remaining animals from the epilepsy group were subjected to double immunofluorescence labeling.

2.4. Amygdala-kindled rat epilepsy model

The surgery for the implantation of electrodes and the kindling procedure were performed according to a modified version of Madsen's protocol (Madsen et al., 2006). Briefly, twelve healthy adult rats were randomly assigned to the control or epilepsy groups (n = 6), and all the rats were deeply anesthetized with an intraperitoneal injection of 3.5% chloral hydrate (1 ml/100 g) and then fixed in a stereotaxic instrument (Stoelting Co. Ltd., USA). The skull was fully exposed, and the position of the left amygdala was located (2 mm posterior, 4.8 mm lateral, and 8 mm ventral to bregma). After drilling through the skull, an electrode was implanted and fixed with dental cement. The postoperative rats were reared under the same conditions as those used preoperatively. The initial afterdischarge threshold (ADT) was determined using an electric stimulator (SEN-7203; Nihon Kohden) 1 week after the surgical procedure with the following stimulation parameters: train stimulation; square wave; wave width, 1 ms; frequency, 50 Hz. The rats were stimulated once daily for 10 consecutive days. The model was considered to be successful when the rats exhibited seizures (≥ stage 4, according to Racine's scale). The control group underwent only stereotactic surgery.

2.5. Rat brain tissue preparation

For western blot analysis, animals were euthanized with an overdose of chloral hydrate, and the brains were frozen in liquid nitrogen and stored at −80 °C until protein extraction. For double immunofluorescence staining, rats were deeply anesthetized with chloral hydrate (0.35 g/kg, i.p.) and perfused via the ascending aorta with cold 0.9% NaCl followed by chilled 4% paraformaldehyde in 0.01 M PBS. The brain was then fixed in 4% paraformaldehyde, frozen, and sectioned at a thickness of 10 μm for staining.

2.6. Western blotting

Proteins were extracted from the entire hippocampus for the rats or from the temporal neocortex samples for the humans, subjected to SDS-PAGE, transferred to PVDF membranes and incubated with primary antibodies directed against the following: DOC2A (1:200, Abcam), GAPDH (1:1000, Beyotime). The primary antibodies were detected using a horseradish peroxidase (HRP)-conjugated secondary antibody from the appropriate species. Finally, the immunoreactive proteins were visualized using enhanced chemiluminescence (ECL, Beyotime, China), and the band intensities were quantified using Quantity One software (Bio-Rad, USA).

2.7. Immunohistochemistry and double immunofluorescence

These protocols were based on our previous study (Li et al., 2016). For immunohistochemistry, the tissue sections were sequentially incubated with the primary goat anti-DOC2A (1:100, Abcam), a biotinylated secondary antibody, and peroxidase-conjugated avidin. Diaminobenzidine was used to develop the color, and hematoxylin was used for counterstaining. A LEICA DM6000B automatic microscope (Leica Microsystems, Heidelberg GmbH, Germany) was used to collect the images. Five visual fields (200×) were randomly examined in each section. The Motic Med 6.0 CMIAS pathology image analysis system (Beihang Motic Inc., Beijing, China) was used for quantitative analysis of DOC2A expression. The mean optical density (OD) of each visualfield was automatically measured by a computer. The final OD values obtained by 2 independent researchers were averaged.

For the immunofluorescence studies, tissue sections were incubated with a mixture of primary antibodies at 4 °C overnight, and the sections were then incubated with Alexa-488 goat anti-rabbit or Alexa-594 goat anti-mouse secondary antibody. The sections were observed via laser scanning confocal microscopy (Leica). Primary antibodies: polyclonal rabbit anti-DOC2A (1:100, Abcam), mouse anti-GFAP (1:50, Zhongshan Golden Bridge, Beijing, China), mouse anti-MAP2 (1:50, Boster Biological Technology, Wuhan, China), mouse anti-NeuN (1:100, Millipore), mouse anti-PSD-95 (1:100, Abcam), and mouse anti-VMAT2 (1:50, Santa Cruz).

2.8. Statistical analysis

All the data are presented as the means ± standard deviation (SD), and all statistics were performed in SPSS 13.0. Comparisons of the clinical data from the patients with TLE and controls were performed using unpaired Student's t-tests (age) or Chi-square tests (male/female ratio). Significant differences in the levels of DOC2A expression in the TLE patients with respect to onset age, epilepsy course, or frequency of seizures were assessed using one-way analysis of variance (ANOVA). The data on DOC2A expression between the epilepsy and control groups in different models were compared by unpaired Student's t-tests, and the level of alpha was corrected with the Bonferroni-Holm adjustment for multiple testing. Then, the p value of each group was compared to its respective corrected alpha. Finally, $p < 0.05$ was considered indicative of statistical significance.

3. Results

3.1. Elevated DOC2A protein levels in the temporal cortices of epileptic humans

The immunohistochemical staining of sections from the 30 epileptic and 10 normal temporal cortex samples showed that DOC2A-positive cells were present in both the control and epileptic temporal cortices (Fig. 1A). In the TLE group, strong staining for DOC2A was observed in the cytoplasm and membrane of neurons in the temporal neocortices; the control group showed relatively weak DOC2A staining in the

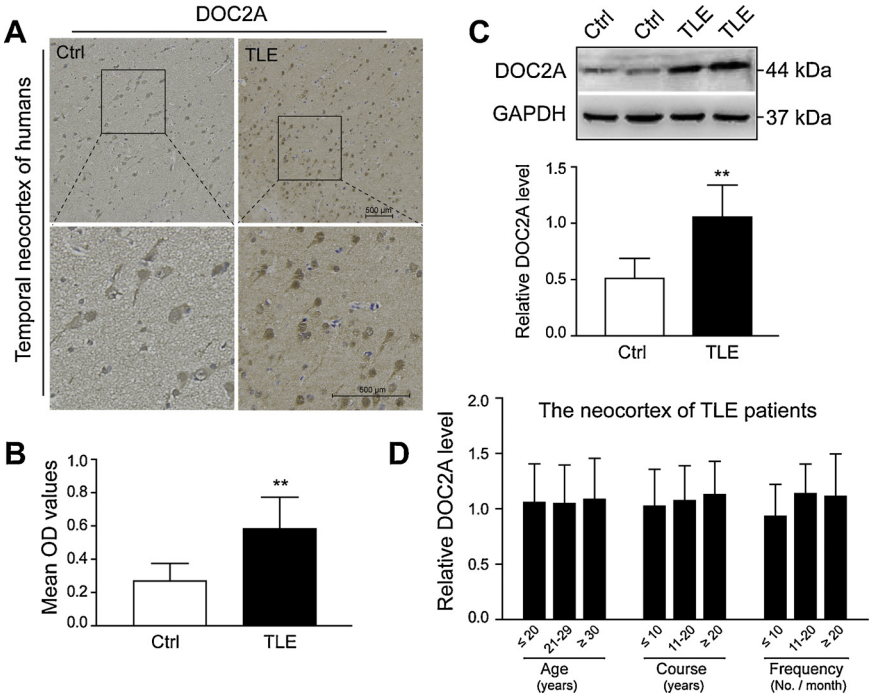

Fig. 1. DOC2A is upregulated in human temporal lobe epilepsy. **(A)** Representative photomicrographs of immunohistochemical staining for DOC2A in the temporal neocortex of humans. The scale bar is 500 μm. **(B)** A comparison of the mean OD values indicates significantly higher expression levels of DOC2A in the TLE group (n = 30) compared with the control group (n = 10) (**P < 0.01). **(C)** DOC2A expression shown by a representative western blot (top) and densitometry analysis (bottom). The levels of DOC2A were normalized to those of GAPDH. The data are expressed as the means ± SD; n = 30 in the TLE group; n = 10 in the control group; **P < 0.01 compared to the control, unpaired Student's t-tests. **(D)** The DOC2A expression levels showed no significant differences among patients with differences in age, disease course, and frequency of TLE seizures; P > 0.05, one-way ANOVA with a post hoc Dunnett's test.

corresponding regions. Immunoreactivity was significantly increased compared with the controls (0.59 ± 0.19 in the TLE patient samples vs. 0.27 ± 0.11 in the control samples, P < 0.01, Fig. 1B). Similarly, the western blot analysis showed that the level of the DOC2A protein was significantly increased in the epileptic temporal neocortices compared to the control group, with a strongly immunoreactive band at 44 kDa (1.07 ± 0.31 in the TLE group vs. 0.57 ± 0.18 in the controls, P < 0.01, Fig. 1C). Next, we investigated whether the increased levels of DOC2A in the epileptic group were caused by differences in underlying brain parameters. There were no significant differences in the levels of DOC2A expression in the patients with TLE with respect to onset age, epilepsy course, or frequency of seizures (P > 0.05, Fig. 1D).

3.2. Neuronal localization of DOC2A in the human temporal cortices

To evaluate the localization of DOC2A in the TLE patients, we performed double immunofluorescence staining with the dendritic marker MAP2 and the astrocytic marker GFAP. We found that DOC2A was expressed exclusively in the neurons of the temporal neocortex in the TLE patients. The DOC2A signal colocalized with the dendritic marker MAP2 but not with the astrocytic marker GFAP (Fig. 2A-B).

3.3. Increased expression of DOC2A immunoreactivity in the pilocarpine-induced rat epilepsy model

We detected DOC2A expression in the pilocarpine epilepsy model via western blot analysis. The results showed that, compared with the controls, a significant upregulation of the DOC2A protein level was observed in the samples from the epileptic group (n = 6 per group, *P < 0.05, **P < 0.01, Fig. 3A-B). This increase was observed in both the hippocampus and the neocortex.

3.4. Overexpression of DOC2A in the amygdala-kindled rat epilepsy model

We detected the expression of DOC2A in the rat hippocampus and cortex in the kindling-induced epileptic model. The western blot analysis revealed that the expression of DOC2A in the epilepsy group was significantly higher than that in the control group; similar trends were observed in both the hippocampus and the neocortex (n = 6 per group, **P < 0.01, Fig. 3C-D).

3.5. The location of DOC2A in the rat pilocarpine-induced epilepsy model

We investigated the location of DOC2A in the epileptic rat model in

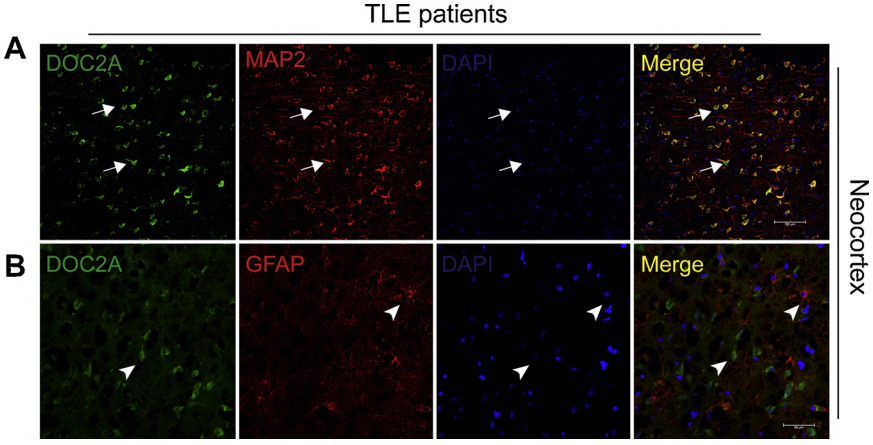

Fig. 2. DOC2A is located in neurons in human temporal lobe epilepsy. Double immunofluorescence for DOC2A (green), MAP-2 **(A)** or GFAP **(B)** (red), DAPI (blue), and the merged image (yellow). Arrows, neurons expressing DOC2A and MAP-2; arrowheads, neurons expressing DOC2A alone or astrocytes expressing GFAP alone. Scale bar indicates 100 μm **(A)** and 50 μm **(B)**.

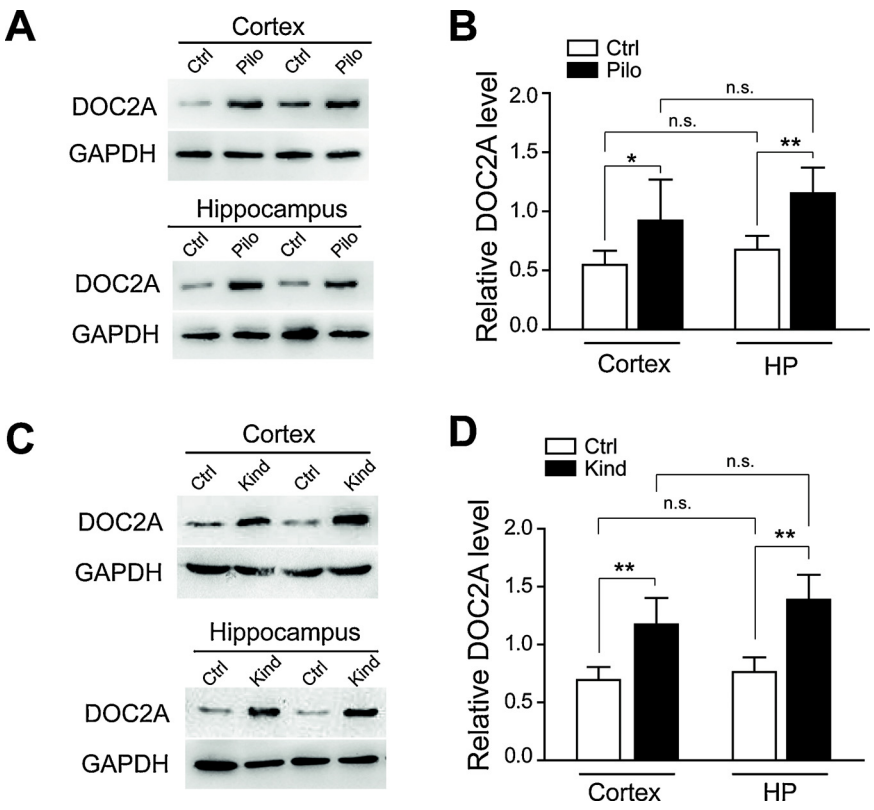

Fig. 3. The expression changes of DOC2A in animal models of epilepsy. **(A)** Representative western blot of DOC2A expression in the neocortex and the hippocampus of control individuals (Ctrl) or pilocarpine-induced rats (pilo) 30 days after SE. **(B)** Densitometry analysis shows that the level of DOC2A expression in both the neocortex and the hippocampus from the pilocarpine group increased significantly compared with the control group (*P < 0.05 for the cortex, ** P < 0.01 for the hippocampus). However, there was no significant difference in the DOC2A expression level between the neocortex and the hippocampus in individuals from the two groups (P > 0.05). **(C)** Representative western blot of DOC2A expression in the neocortex and the hippocampus of control individuals (Ctrl) or kindling-induced epileptic rats (kind). **(D)** Densitometry analysis in the kindled rats. All of the data are expressed as the means ± SD; n = 6 per group; *P < 0.05, **P < 0.01, Student's t-tests.

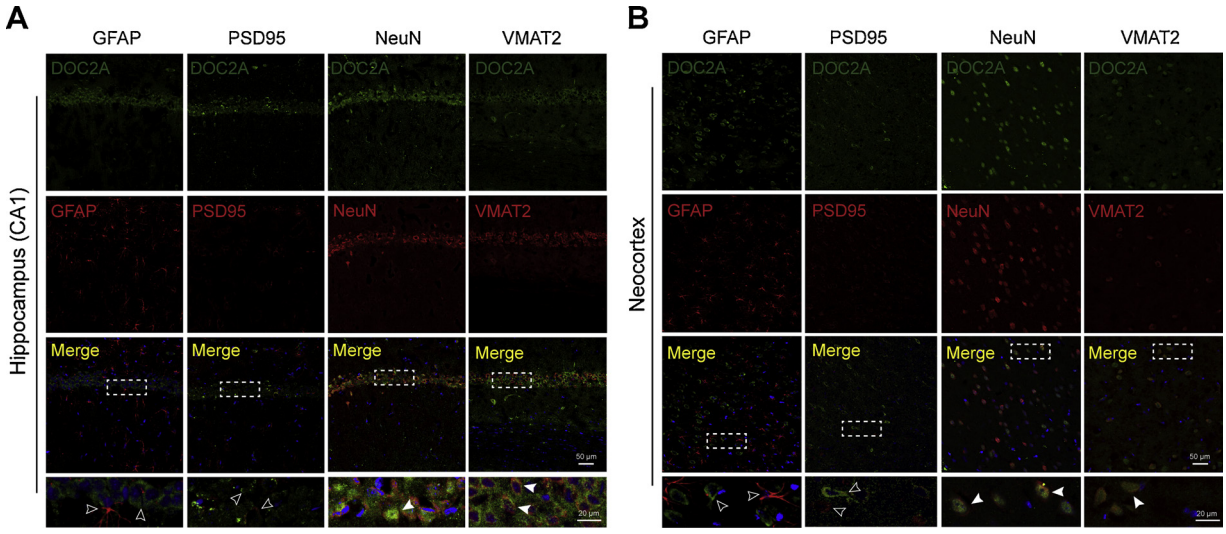

Fig. 4. DOC2A localization in a pilocarpine-induced rat model of epilepsy. DOC2A (green) co-localized with the neuronal marker NeuN (red) and presynaptic marker VMAT2 (red) in the hippocampus **(A)** and neocortex **(B)** but not with the astrocytic marker GFAP (red) or the postsynaptic marker PSD-95(red), both in the hippocampus **(A)** and neocortex **(B)**. Solid arrowheads, double-positive puncta; open arrowheads, puncta positive for DOC2A only. Scale bar indicates 50 μm (top) or 20 μm (bottom).

further detail. Immunofluorescence studies on sections from the pilocarpine-treated rat hippocampi and cortices showed DOC2A staining in the areas stained with the neuronal marker NeuN but not in regions stained with the astrocytic marker GFAP (Fig. 4A-B). For more detail regarding the location of DOC2A in the synaptic junction, we performed double immunofluorescence staining with the presynaptic marker VMAT2 and the postsynaptic marker PSD-95. DOC2A expression colocalized with VMAT2, whereas the immunoreactivity for DOC2A and PSD-95 overlapped to a lesser degree (Fig. 4A-B).

4. Discussion

Our study shows that DOC2A expression is increased in human and rat temporal lobe epilepsy. Neuronal localization of DOC2A in the human TLE patients and pilocarpine post-SE rat model was observed in neurons, but not in astrocytes, and it was concentrated in the presynaptic portion of the synapse. To our knowledge, this is the first demonstration of the relationship between DOC2A and temporal lobe epilepsy.

In our human tissue samples, we first investigated the expression of DOC2A via immunohistochemical and western blot analyses of the

temporal neocortices from TLE subjects and control individuals. Compared to the controls, a significant upregulation of the DOC2A protein level was observed using both methods in the samples from the TLE patients. Moreover, there were no significant differences in the DOC2A protein levels among the patients with respect to differences in age, disease course, and frequency of seizures. These data indicate that DOC2A expression was indeed increased in TLE patients and that the overexpression was not influenced by age, disease duration, or seizure frequency in TLE. Finally, our double immunofluorescence staining showed that DOC2A was co-expressed with MAP-2 in the cortices of the human TLE patients, which indicates that DOC2A is expressed specifically within neurons. DOC2 is a family of synaptic proteins first described by Orita et al. in 1995 (Orita et al., 1995), and Verhage et al. (Verhage et al., 1997) reported that in the normal rat brain, the DOC2A isoform was expressed specifically in neurons, not within astroglia, and co-purified with synaptic vesicles. Notably, in our study, we show the same characteristics of distribution in the patients' temporal neocortices and demonstrate an altered expression of DOC2A associated with epilepsy.

Practical and ethical reasons prevented us from performing equivalent comparisons of the hippocampal expression of DOC2A between the TLE patients and controls. In addition, there are some limitations when human brain tissue is used for investigation; most TLE patients have been treated with many specific AEDs, and these drugs may affect DOC2A expression. Therefore, we selected two different TLE rat models in which to observe the expression of DOC2A to exclude the influence of AEDs. In rodent models of TLE, a variety of different chemoconvulsants and intracerebral electrical stimulation patterns have been used to induce epilepsy; electrical kindling and pilocarpine have been well characterized with regard to seizure phenomenology, electroencephalographic features, and neuropathology, and they resemble the clinical characteristics of TLE (Morimoto et al., 2004; Stables et al., 2003). In our study, these two animal models provided direct evidence that epileptic activities lead to the upregulation of DOC2A expression both in the hippocampus and neocortex. These variations in DOC2A provide a preliminary explanation for how DOC2A may contribute to TLE.

The mechanism by which DOC2A is involved in epilepsy is unknown but may be related to synaptic transmission. Members of the Doc2-like protein family (Doc2A, Doc2B) have been recently identified as specific regulators of spontaneous (Groffen et al., 2010; Pang et al., 2011) or asynchronous (Sakaguchi et al., 1999; Yao et al., 2011) neurotransmitter release, which are two primary modes of neurotransmitter release in addition to synchronous release. In the cortex, high-frequency presynaptic activation of fast-spiking interneurons produces asynchronous release that lasts several seconds and that may prevent widespread synchronous firing and suppress epileptiform activity (Manseau et al., 2010). Asynchronous release from interneurons is also elevated in epileptiform tissue of rats and humans (Jiang et al., 2012). In addition, the summed spontaneous quantal release from many inputs onto a neuron can contribute to the excitability of neurons (Farrant and Nusser, 2005; Kombian et al., 2000). Finelli MJ et al. observed that protein TBC1D24 (Tre2/Bub2/Cdc16 (TBC)1 domain family member 24) knockdown resulted in decreased spontaneous neurotransmission and epilepsy (Finelli et al., 2018). These studies suggest that asynchronous and spontaneous release play an important role in epilepsy. Thus, the question is whether DOC2A is also involved in epilepsy through these synaptic transmission pathways. Recent research reports that excitatory miniature postsynaptic currents (mEPSCs), but not miniature inhibitory postsynaptic currents (mIPSCs), increase in amplitude after knockdown of Doc2 (Ramirez et al., 2017). N.A. Courtney et al. also found that DOC2A regulated mEPSCs but not mIPSCs (Courtney et al., 2018). These findings indicate that DOC2A may be involved in regulating the release of glutamate. In our study, we found that DOC2A and vesicular monoamine transporter 2 (VMAT2) (Tillinger et al., 2010) are abnormally expressed in epilepsy (Jiang et al., 2013) and are coexpressed in the neocortex and hippocampus of rats with pilocarpine-induced epilepsy. DOC2A may also be involved in epilepsy through regulating the release of monoamines.

In conclusion, this study shows that DOC2A expression was increased in human and rat temporal lobe epilepsy; subcellular localization studies showed that DOC2A was expressed specifically in neurons and was co-expressed with VMAT2. Based on these findings, we postulate that DOC2A may contribute to epileptogenesis by regulating the presynaptic effect on neurotransmission, possibly via the release of monoamine or other neurotransmitters. However, our findings focused on the association of DOC2A with epilepsy. Further studies are required to explore the role of DOC2A in epileptogenesis.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eplepsyres.2019.02.008.

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