Inhibiting the soluble epoxide hydrolase increases the EpFAs and ERK1/2 expression in the hippocampus of LiCl-pilocarpine post-status epilepticus rat model

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Research Article

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Abstract

Purpose

This study aimed to investigate the enzyme activity of soluble epoxide hydrolase (sEH) and quantify metabolic substrates i.e. epoxygenated fatty acids (EpFAs) and products of sEH in the hippocampus after administering TPPU [1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl)urea], the inhibitor of sEH, and further explored whether the extracellular signal-activated protein kinase 1/2 (ERK1/2) was involved in the anti-seizure effect of TPPU in the lithium chloride (LiCl)-pilocarpine induced post-status epilepticus (SE) rat model.

Methods

The rats were intraperitoneally (I.P.) injected with LiCl and pilocarpine to induce SE and then spontaneous recurrent seizures (SRS) were observed. Rats were randomly assigned into SRS + 0.1 TPPU group (intragastrically administering 0.1 mg/kg/d TPPU), SRS + PEG 400 group (administering the vehicle instead), and Control group. Enzyme-linked immunosorbent assay, Western-blot analysis, and ultra-high-performance liquid chromatography/mass spectrometry (LC/MS) were performed to measure the enzyme activity of sEH, the protein level of sEH and ERK1/2, and the concentration of TPPU and polyunsaturated fatty acids (PUFAs) metabolisms in the hippocampus.

Results

The frequency of SRS that equal to or greater than Racine 3 degree ranged from 0 to 19 every week in the SRS + PEG 400 group comparing to 0 to 5 every week in the SRS + 0.1 TPPU group. The enzyme activity and protein level of sEH was significantly increased in the SRS + PEG400 group compared with the Control group. After administering TPPU, the concentration of TPPU in the hippocampus was 10.94 ± 4.37 nmol/kg; the enzyme level of sEH was significantly decreased in the LiCl-pilocarpine-induced post-SE rat model, however, the protein level of sEH did not decrease significantly; the regioisomers 8,9-, 11,12-, and 14,15-EETs, the sums of EETs, the ratio of EETs/DHETs, and other EpFAs including 16(17) EpDPA and the ratio of 19(20)-EpDPA/19,20-DiHDPA in the hippocampus were significantly increased. In addition, the ratio of p-ERK1/2 to ERK1/2 in the hippocampus was significantly increased after TPPU administration either.

Conclusion

We demonstrated that inhibiting sEH with TPPU increased the levels of EETs and some other EpFAs and expression of ERK1/2 in the hippocampus of LiCl-pilocarpine-induced post-SE rat model, indicating the

cellular mechanism of EETs through ERK1/2 pathway might be responsible for the anti-seizure effect of TPPU.

1 Introduction

Epilepsy is a chronic brain disease which is characterized by recurrent seizures and neuropsychiatric comorbidities that cause poor quality of life in patients with epilepsy[1]. However, the mechanisms of epileptogenesis are still unclear. Mounting evidence from humans with epilepsy and animal models of epilepsy indicates that neuroinflammation makes a great contribution to epileptogenesis[2, 3]. In the process of epileptogenesis, proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumour necrosis factor alpha (TNF-α), and prostaglandin E2 (PGE2) are produced excessively [4, 5]. Anti-inflammatory treatment might be an optimal therapeutic strategy for patients with epilepsy [6].

Epoxide hydrolases (EHs) are proteins which have a catalytic mechanism and open epoxides to diols by the addition of water[7]. There are several types of EHs including microsomal epoxide hydrolase (mEH), soluble epoxide hydrolase (sEH), and cholesterol EH etc.in organisms, of which sEH has been found to be widely expressed in multiple human tissues and may take part in the inflammatory process[8]. Epoxygenated fatty acids (EpFAs) including epoxyeicosatrienoid acids (EETs) are endogeneous substrates for sEH which are derived from polyunsaturated fatty acids (PUFAs) such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), linoleic acid (LA), and docosahexaenoic acid (DHA). ARA, LA, EPA, and DHA are abundantly stored in membrane phospholipids that are metabolized into active intermediate substrates by cyclooxygenases (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) epoxygenases, of which CYP450 epoxygenases metabolize ARA etc. into different subtypes of EETs and other EpFAs[9]. Studies demonstrate that EETs have anti-inflammatory and neuroprotective effects[10]. However, EETs are hydrolyzed by sEH and converted to their respective diols such as dihydroxyeicostetraenoic acids (DHETs) which are reported to be mostly inactive or even have harmful effects in regulating inflammation[11].

In mammals, the sEH protein is a 125 kDa dimer composed of two identical 62 kDa monomers[8], which is highly expressed and distributed in central nervous system and across various brain areas such as cerebral cortex, hippocampus, amygdala, and striatum[12]. From a micro-structural level, it shows that sEH has cell type-specific localizations including the soma and processes of neuronal cells, astrocytes, oligodendrocytes, and microvascular endothelial cells in the brain[13]. Studies indicate that sEH has been involved in the pathological process of neurological diseases[14]. In a study of 20 patients underwent anterior temporal lobe resection due to temporal lobe epilepsy, the level of sEH is significantly higher in the temporal cortex of patients with epilepsy than control group[15]. The inhibitors of sEH have been demonstrated to have anticonvulsant effects on spontaneous recurrent seizures (SRS) and anti-depressant effects on epilepsy-associated depression in the pilocarpine induced rodent epilepsy models[16, 17]. Although these studies have found that the protein level of sEH is increased in the hippocampus of pilocarpine rodent epilepsy models, the enzyme activity of sEH and how the levels of its substrates and products change in the brain have not been determined. In this study, we aimed to

investigate the enzyme activity of sEH and quantify the polyunsaturated fatty acids (PUFAs) metabolic substrates i.e. epoxygenated fatty acids (EpFAs) and products of sEH in the hippocampus after administering TPPU [1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl)urea], the inhibitor of sEH, and further explore whether the extracellular signal-activated protein kinase 1/2 (ERK1/2) pathway is involved in the anti-seizure effect of TPPU in the LiCl-pilocarpine induced post-status epilepticus rat model.

2 Methods

2.1 Animals

Male adult Sprague-Dawley rats aged 6 to 8 weeks and weighing 200 to 250 g (Shanghai Charles River Laboratory) were used in this study. They were raised 4/cage at 22–25℃ and under a 12h day-night cycle. The experiment was approved by the Committee of Animal Care and Use in Zhongshan Hospital of Fudan University (Shanghai, China) and conformed to the guidelines of the National Institutes of Health. Measures were taken to reduce the number of animals used, and efforts were made to minimize animal suffering.

2.2 Establishment of the LiCl-pilocarpine-induced post-SE rat model and grouping

As described previously[18], rats were intraperitoneally injected (I.P.) LiCl (127 mg/kg, dissolved in water, Sigma, St. Louis, MO, USA), scopolamine methyl bromide (1 mg/kg, Sigma-Aldrich, USA), and muscarinic agonist pilocarpine (40 mg/kg, Sigma-Aldrich, USA) sequentially at intervals of 24h and 30min respectively. The seizure severity was evaluated by the modified Racine scale [19]. The standard of SE in this study was defined as sustained recurrent seizures greater than or equal to Racine stage 4 for 30min. At 30min after seizure onset, rats meeting the standard of SE were treated with diazepam (10 mg/kg, Tianjin, China) to terminate seizures. One week after SE induction, the survived rats were monitored with a video surveillance system (a CCD camera, JVC, Japan) to observe SRS. Six-weeks monitoring period (from the onset of the 2nd week to the end of the 7th week after SE induction) with 6h/d was conducted. The frequency of SRS reaching a Racine stage 3 to 5 (rearing and/or rearing and falling) were counted.

The rats of LiCl-pilocarpine induced post-SE model were divided into two groups randomly according to administration of TPPU (dissolved in a saline solution containing 40% polyethylene glycol 400, PEG 400, at 0.1 mg/kg/d) or not: the SRS + 0.1 TPPU group and the SRS + PEG 400 group. TPPU was given for 4 weeks from the 21st day to 49th day after SE induction by gastric gavage at 8am every morning[20]. The SRS + PEG 400 group was given the vehicle (PEG 400) instead of TPPU and the Control group was simply given LiCl and PEG 400. At 7w after SE induction, the brain tissues were harvested.

2.3 Tissue preparation and protein extraction

The brain tissues were taken out after rats were deeply anesthetized with 4% chloral hydrate and euthanized by cervical dislocation. The hippocampi of rats were carefully dissected out and put into 4℃

phosphate-buffered saline (PBS). The tissue protein extraction reagent (Beyotime Institute of Biotechnology, China) containing EDTA-free complete protease inhibitors (Beyotime, China) was used to extract total protein from the hippocampi of rats, and the Bio-Rad protein assay kit (Beyotime, China) was adopted to measure total protein concentration.

2.4 Enzyme-linked immunosorbent assay (ELISA)

The enzyme activity of sEH in the hippocampi of rats was measured using the Soluble Epoxide Hydrolase Inhibitor Screening Assay Kit (Cayman Chemical). The procedures were as follows: 1) adding 190μL of assay buffer and 5μL cell lysis buffer to a well for the background; 2) Five concentration gradients of the standard sEH sample were set, 0.5, 1, 2, 4, 8μL standard samples were placed into the well A1-5, add 5μL cell lysis buffer, and add some assay buffer to make the volume into 195μL; 3) other wells for samples were added into 10μL samples and 185μL assay buffer; 4) incubating for 2 hours at room temperature (RT) on a shaker at 800 rpm; 5) the liquid was removed and then 100μL wash buffer were put into wells and washed for three times; 6) covering, and incubating for 1h at RT on the shaker at 800 rpm, washing was repeated as before; 7) adding 50μL of diluted streptavidin-PE to each well, incubating for 30 minutes at RT on the shaker at 800 rpm, and repeating the wash again; 8)100μL of wash buffer was added to each well, covered, and incubated for 2min at RT on the shaker at 800 rpm. A Luminex analyser was used to read the results within 90min.

2.5 Western blot analysis

The expression level of ERK1/2 in the hippocampi of rats was measured by the Western-blot analysis. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate total proteins, which was transferred to cellulose acetate membranes. After that, the membranes were blocked and incubated with primary antibodies i.e. rabbit anti-ERK1/2 (42/44kDa, 1:1000, CST) and rabbit anti-sEH (63 kDa, 1:500, ABclonal) at 4°C. The rabbit anti-β-actin primary antibody (40kDa, 1:1000, Beyotime) was set as the internal reference. The membrane was washed with 4°C PBS (10 mM, pH 7.4) after incubation for 24h, and then it was incubated with the goat anti-rabbit IgG secondary antibody (1:1000, Beyotime) for 2h at RT. Quantitative analysis of target proteins bands was conducted by Tanon Image software (version 4100, Shanghai, China). The optical density (OD) value of each sample was normalized by the corresponding amount of β-actin.

2.6 The ultra-high-performance liquid chromatography/mass spectrometry (LC/MS) method

The homogenate of rat hippocampus was prepared by using a solid phase extraction (SPE) method. The sample mixed with SPE solution etc. and pass through the cartridges by gravity. The cartridges were washed with the 2ml SPE solution, dried under vacuum for 5min, and then eluted with ethyl acetate (EtOAc, 1.5 ml) into 2ml tubes each containing 30% glycerol in MeOH (5μL) on the bottom as a trap solution. The volatile solvents were removed from the tubes by using a SpeedVac concentrator (Thermo Scientific) until the glycerol remained on the bottom. The residue was formulated with IS II solution (50μL), strongly mixed on a vortex mixer for 5min, and then centrifuged at 11200 × g for 5min under 4ºC.

The entire supernatant was transferred into an ultra-free centrifugal filter. After centrifuging at 11200 × g for 5min under 4ºC, the filtrate was then transferred into a 150μL insert fixed in a 2ml vial and stored at -20°C until analysis.

The concentrations of TPPU and PUFAs metabolisms were analysed by established liquid chromatography electrospray ionization tandem mass spectrometry method reported by Luo et al. [21,22]. Specifically, chromatographic separation was performed on an Agilent 1260 Infinity liquid chromatography instrument equipped with a 2.1 X 150 mm ZORBAX Eclipse Plus C18 1.8 μm column held at 50 ºC. The solvent system consisted of water/AA (999/1 v/v, solvent A) and ACN /MeOH/AA (840/159/1 v/v; solvent B). The injection volume was 10 μl. The samples were kept at 4 ºC in the autosampler. Analytes were monitored by negative mode electrospray ionization tandem mass spectrometry in MRM mode on an AB Sciex QTrap6500 Mass Spectrometer (AB Sciex, Framingham, MA). The gas flow rate was fixed for curtain gas, ion source gas 1 and ion source gas 2 as 35, 50, 50 L/h, respectively. The collision gas was set as medium. Electrospray ionization was performed with an IonSpray Voltage set at -4500 V and the source temperature was set at 500 ºC.

2.7 Statistical analysis

The Graphpad Prism 7 software was used to conduct the statistical analysis in this study. The Student t test and one-way analysis of variance (ANOVA) test were used to do comparisons between groups. When using the one-way ANOVA test, a post-hoc Tukey test was adopted for comparisons between two groups. A *P* value of less than 0.05 was considered to be statistically significant. The data were expressed as mean ± standard deviation (SD).

3 Results

3.1 SRS observation in the LiCl-pilocarpine-induced post-SE rat model

As described in the earlier article[16], rats induced SE by I.P. administering LiCl-pilocarpine were video-monitored for 7w to observe SRS. Fourteen rats having observed SRS at 2w after SE were included, of which 7 rats each were randomly assigned to SRS + PEG 400 and SRS + 0.1 TPPU groups. There was no difference in the latency of inducing SE between the two groups (14.25 ± 3.3min SRS + PEG 400 group vs. 14.5 ± 2.38min in SRS + 0.1 TPPU group, P > 0.05). The seizure frequency of SRS that equal to or greater than Racine 3 degree ranged from 0 to 19 (median, 3) every week in SRS + PEG 400 group comparing to 0 to 5 (median, 1) every week in SRS + 0.1 TPPU group.

3.2 The levels of sEH protein and sEH enzyme activity in the hippocampus were compared between the LiCl-pilocarpine-induced post-SE rat model and Control group

Quantitative measurement of sEH protein was performed by the Western-blot method. The level of sEH protein in the hippocampus was significantly increased in the LiCl-pilocarpine-induced post-SE rat model (SRS + PEG400 group) compared with Control group (Fig. 1, *P < 0.05).

The enzyme activity of sEH in the hippocampus measured by ELISA was compared between the SRS + PEG400 and Control groups. The result showed that the enzyme activity of sEH was significantly increased in the SRS + PEG400 group compared with Control group (see Fig. 2).

3.3 TPPU administration increased EpFAs and the ratios of sEH substrates and products in the hippocampus of LiCl-pilocarpine-induced post-SE rat model

The concentration of TPPU was measured by the LC/MS method in the hippocampus, which was 10.94 ± 4.37 nmol/kg in the SRS + 0.1TPPU group compared with blank in the SRS + PEG400 and Control groups. Simultaneously, the enzyme level of sEH was significantly decreased after TPPU administration in the SRS + 0.1TPPU group compared with the SRS + PEG400 group (*P < 0.05, see Fig. 2), however, the protein level of sEH did not decrease significantly (see Fig. 1).

Next, the PUFAs metabolic substrates and products of sEH in the hippocampus were measured through the LC/MS method in this study either. As shown in Fig. 3, the diagram depicts the metabolic profiles of PUFAs including ARA, LA, and DHA in this study. The PUFAs are metabolized by CYP450 epoxygenases into different subtypes of EETs, epoxyoctadecamonoeneoic acid (EpOMEs), and epoxydocosapentaenoic acid (EpDPAs), which are the substrates of sEH. They are further metabolized by sEH into the products including dihydroxyeicosatrienoic acid (DHETs), dihydroxy octadecamonoeneoic acid (DHOMEs), and dihydroxydocosapentaenoic acid (DiHDPAs), respectively. As listed in Table 1, the concentrations of PUFAs metabolic substrates and products of sEH measured in this study were compared among the SRS + PEG400, SRS + 0.1TPPU, and Control groups (*P < 0.05, **P < 0.01). The regioisomers 8,9-, 11,12-, and 14,15-EETs, the sums of EETs, and the ratio of EETs/DHETs in the hippocampus was significantly increased in the SRS + 0.1TPPU group compared with SRS + PEG400 group (*P < 0.05, **P < 0.01, Fig. 4A). In addition, the level of other EpFAs including EpOMEs and EpDPA in the hippocampus could also be detected in this study, and it showed that 16(17) EpDPA and the ratio of 19(20)-EpDPA/19,20-DiHDPA were both significantly increased in the SRS + 0.1TPPU group compared with SRS + PEG400 group (*P < 0.05, **P < 0.01, Fig. 4B), while no difference in the ratio of EpOMEs/DHOMEs was found after administering TPPU.

Table 1
The levels of TPPU and metabolic substrates and products of sEH in the hippocampus of LiCl-pilocarpine post-SE rat model

Measuring items (nmol/kg)	Control group (n = 7)	SRS + PEG400 group (n = 7)	SRS + 0.1TPPU group (n = 7)	P value (ANOVA test post-hoc analysis, SRS + PEG400 vs. SRS + 0.1TPPU)
TPPU	NA	NA	10.94 ± 4.37	NA
14(15) EET	32.72 ± 7.73	31.01 ± 9.03	60.79 ± 31.49	**0.026**
11(12) EET	26.53 ± 6.94	22.41 ± 9.07	40.97 ± 15.65	**0.016**
8(9) EET	20.83 ± 4.83	15.76 ± 4.40	31.86 ± 12.60	**0.005**
5(6) EET	47.16 ± 11.09	39.14 ± 12.25	57.23 ± 15.91	0.05
9(10)-EpOME	8.44 ± 7.13	4.81 ± 1.59	6.72 ± 1.63	0.692
12(13)-EpOME	19.42 ± 13.72	12.42 ± 3.92	17.47 ± 3.09	0.514
Sum (EETs)	127.24 ± 29.88	108.32 ± 33.44	190.85 ± 73.63	**0.016**
Sum (DHETs)	3.10 ± 0.82	3.34 ± 1.85	2.69 ± 0.72	0.598
EETs/DHETs	43.23 ± 14.64	38.25 ± 15.86	71.70 ± 21.38	**0.006**
Sum (EpOMEs)	27.86 ± 20.71	17.23 ± 5.37	24.19 ± 4.08	0.047
Sum (DHOMEs)	7.41 ± 2.89	5.34 ± 3.40	4.91 ± 2.35	0.96
EpOMEs/DHOMEs	3.51 ± 1.75	5.10 ± 3.92	5.86 ± 2.67	0.879
16(17) EpDPA	2.52 ± 0.65	2.18 ± 0.80	4.93 ± 2.13	**0.004**

DHET: dihydroxyeicosatrienoic acid; DHOME: dihydroxy octadecamonoeneoic acid; DiHDPA: dihydroxydocosapentaenoic acid; EET: epoxyeicosatrienoic acid; EpDPA: epoxydocosapentaenoic acid; EpOME: epoxyoctadecamonoeneoic acid; NA: not applicable; SE: status epilepticus; sEH: soluble epoxide hydrolase; SRS: spontaneous recurrent seizure; TPPU: 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea

Measuring items (nmol/kg)	Control group (n = 7)	SRS + PEG400 group (n = 7)	SRS + 0.1TPPU group (n = 7)	P value (ANOVA test post-hoc analysis, SRS + PEG400 vs. SRS + 0.1TPPU)
19(20)-EpDPA/19,20-DiHDPA	1.75 ± 1.02	0.31 ± 0.11	2.43 ± 1.73	**0.008**

DHET: dihydroxyeicosatrienoic acid; DHOME: dihydroxy octadecamonoeneoic acid; DiHDPA: dihydroxydocosapentaenoic acid; EET: epoxyeicosatrienoic acid; EpDPA: epoxydocosapentaenoic acid; EpOME: epoxyoctadecamonoeneoic acid; NA: not applicable; SE: status epilepticus; sEH: soluble epoxide hydrolase; SRS: spontaneous recurrent seizure; TPPU: 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea

3.4 The expression of ERK1/2 was significantly increased in the hippocmapus of LiCl-pilocarpine-induced post-SE rat model after TPPU administration

The expression level of ERK1/2 and its phosphorylated form was measured by the Western-blot method. As shown in Fig. 5, the result showed that the ratio of p-ERK1/2 to ERK1/2 was significantly increased in the SRS + 0.1TPPU group compared with SRS + PEG400 group (*P < 0.05).

4 Discussion

In this study, we found that the expression of sEH protein and the enzyme activity of sEH were both increased in the hippocampus of LiCl-pilocarpine-induced post-SE rat model compared with Control group. After administering TPPU, the enzyme activity of sEH was decreased and the PUFAs metabolic substrates EpFAs of sEH and ratios of substrates to products were increased in the hippocampus of LiCl-pilocarpine-induced post-SE rat model. This finding indicated that TPPU might take anti-seizure effect through the cellular mechanism of PUFAs metabolic substrates of sEH including EETs. The increased expression of ERK1/2 in the hippocampus indicated it might take part in the cellular mechanism of EETs in the LiCl-pilocarpine-induced post-SE rat model.

In our previous study, we have found that administering TPPU in the LiCl-pilocarpine-induced post-SE rat model reduced the frequency of SRS and alleviated epilepsy-associated depressive behaviors of rats[16]. Another study by Hung et al. also verified that TPPU, the sEH inhibitor, had anti-seizure effect in two mouse models of temporal lobe epilepsy induced by either pilocarpine or electrical amygdala kindling[17]. In addition, Vito et al. demonstrated that the sEH inhibitor had anti-inflammatory effect and prevented tetramethylenedisulfotetramine (a potent convulsant poison) induced mortality in mice by combined treatment with diazepam[23]. The behavioral observation in this study found the frequency of SRS was decreased after administering TPPU, which was consistent with the previous studies.

The sEH is one of the key enzymes for hydrolysing EETs etc. which are metabolisms from PUFAs that are highly stored in membrane phospholipids. The study shows that sEH is extensively expressed in the central nervous system[12]. The expression of sEH protein has been found to be increased in neurological and psychiatric diseases including epilepsy, Parkinson's disease, Alzheimer's disease, depression, bipolar disorder, schizophrenia, and autism-like spectrum disease[15, 24–26, 9]. However, the enzyme activity of sEH sometimes showed controversial results[27]. In our previous study, we found the expression of sEH protein was significantly increased in the prefrontal cortex and hippocampus of the LiCl-pilocarpine-induced post-SE rat model[16]. In this study, we demonstrated that the expression of sEH and the enzyme activity of sEH were both significantly increased in the hippocampus of LiCl-pilocarpine-induced post-SE rat model. After administering TPPU, the enzyme activity of sEH significantly decreased while the no significant change was observed for the expression of sEH protein. This result indicated that the action of TPPU mainly contributed to decrease the enzyme activity of sEH but not the expression level of sEH protein. The expression level of sEH protein might be affected by many other pathological factors.

There are several sEH inhibitors developed in recent years, including 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA), 1-adamantan-1-yl-3-(5-(2-(2-ethoxyethoxy) ethoxy) pentyl) urea (AEPU), trans-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid (t-AUCB), and TPPU etc.[9]. The ideal sEH inhibitors should have maximal bioavailability and longer half-lives, higher maximum drug concentrations in the blood (C_{max}), and larger area under the curve (AUC)[26]. Except for that, for drugs targeting the central nervous system, it's important for them to take effects by successfully passing the blood-brain-barrier (BBB). Studies have demonstrated that TPPU is a potent sEH inhibitor with sufficient solubility in water, vast systemic distribution to tissues, and crosses BBB effectively either after intraperitoneal injection or oral administration[28, 27]. In this study, TPPU was given to the rats via intragastric administration. We measured the concentration of TPPU in the hippocampus by the LC/MS method and showed the concentration was elevated significantly compared with the blank controls, indicating the excellent gastric absorption and BBB permeability of TPPU either.

The anti-seizure effect of TPPU might be contributed by the elevation of EETs and other EpFAs. As demonstrated in this study, we found the sum EETs, the ratio of EETs/DHETs, 14(15) EET, 11(12) EET, and 8(9) EET were all increased significantly in the hippocampus of LiCl-pilocarpine-induced post-SE rat model after TPPU administration. However, there was no significant difference in 5(6) EET between SRS + 0.1TPPU and SRS + PEG400 group. Moreover, other EpFAs such as EpOMEs, 16(17) EpDPA, and 19(20) EpDPA derived from LA and DHA were also elevated significantly in the hippocampus of LiCl-pilocarpine-induced post-SE rat model after TPPU administration. A study showed that combined injection of the sEH inhibitor with EETs but not with epoxy-DHA or epoxy-EPA into the brains of mice delayed the onset of pentylenetetrazol-induced seizures, which might support the major role of EETs in the anti-seizure effect[29]. In the physiological state, the endogenous EETs have important roles in cellular actions, regulation of cerebral blood flow, neurohormone release, and synaptic transmission in the brain[10]. How the level of EETs change in the pathophysiological state of neurological and psychiatric diseases have not been fully elucidated. In this study, we didn't find any difference of EETs and other EpFAs levels in the

hippocampus of LiCl-pilocarpine-induced post-SE rat model compared with Control group. The membrane phospholipids might be damaged under pathological states, PUFAs released from the membrane lipids and were metabolized through COX, LOX, CYP hydroxylases, and CYP epoxygenases pathways[30], which implied that the levels of EETs and other EpFAs might be influenced by multiple factors but not solely the activity of sEH.

The functions of EETs are complex and have not been very clear yet. Studies indicate that EETs activate K^+ channels and have anti-inflammatory effects[31, 32]. In addition, EETs may also have membrane receptor mechanism, which is initiated by EET binding to a plasma membrane EET receptor, and then the signal transduction pathways such as Mitogen-activated protein kinase (MAPK) will be activated[33, 34]. Moreover, EETs block the pathological endoplasmic reticulum (ER) stress response and attenuate oxidative stress[35]. ERK1/2 has been demonstrated to be one of responsive down-stream molecules to ER stress and involved in the defensive effects against ER stress[36, 37]. In this study, the expression level of ERK1/2 was significantly increased after TPPU administration, indicating the cellular mechanism of EETs through ERK1/2 pathway might be responsible for the anti-seizure effect of TPPU.

5 Conclusion

In this study, we demonstrated that inhibiting sEH with TPPU increased the levels of EETs and some other EpFAs and expression of ERK1/2 in the hippocampus of LiCl-pilocarpine-induced post-SE rat model, indicating the cellular mechanism of EETs through ERK1/2 pathway might be responsible for the anti-seizure effect of TPPU.

Declarations

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Author Contributions

WP designed the experiment, completed reanalysis of article data, and wrote the manuscript. YS performed major parts of the animal experiment. QW instructed the process of animal experiment. JD and XW conceptualized, designed, and sponsored the study.

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Ethical declaration

All procedures were compliant with the requirements from the Animal Ethics Committee of Zhongshan Hospital, Fudan University.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Figures

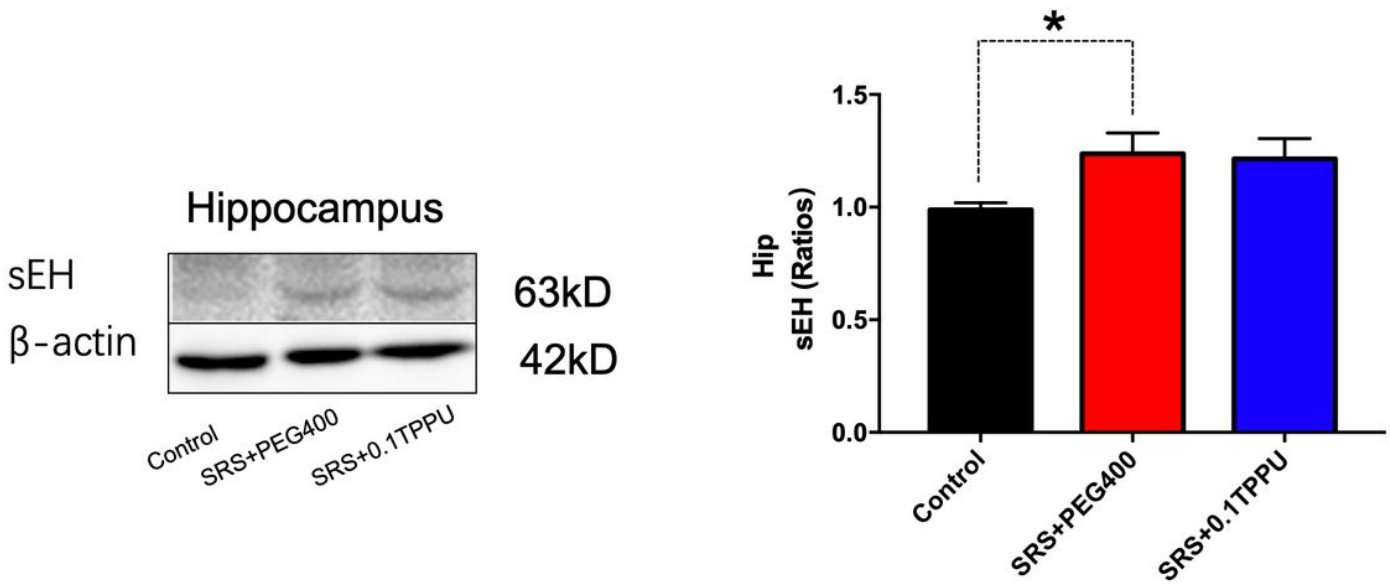

Figure 1

The expression level of sEH protein in the hippocampus was significantly increased in the LiCl-pilocarpine-induced post-SE rat model (SRS + PEG400 group) compared with Control group, n = 7 in every group, *P<0.05.

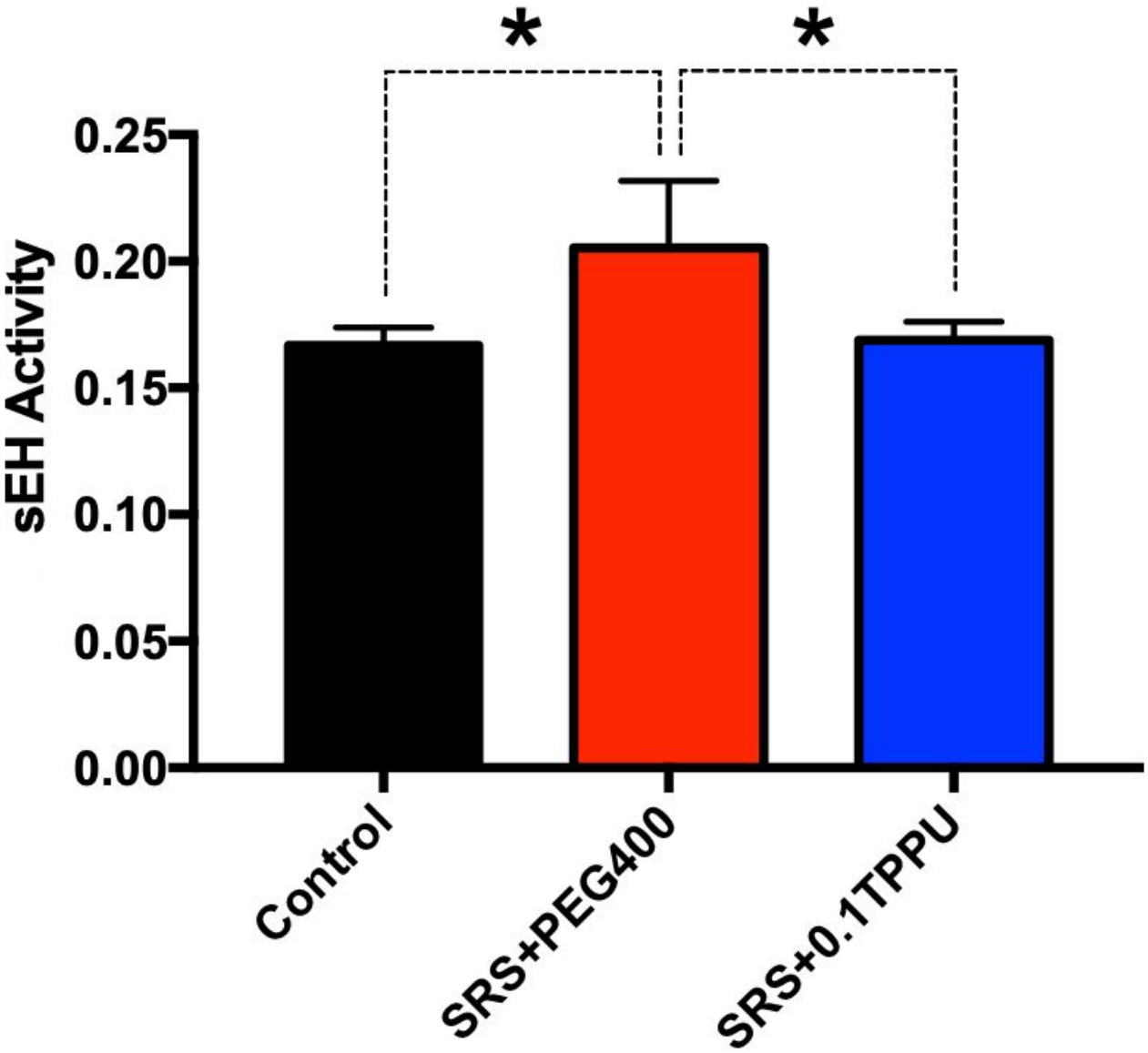

Figure 2

The enzyme activity of sEH was significantly increased in the SRS + PEG400 group compared with Control group, and it was significantly decreased in the SRS + 0.1TPPU group compared with SRS + PEG400 group, n = 7 in every group, *P<0.05.

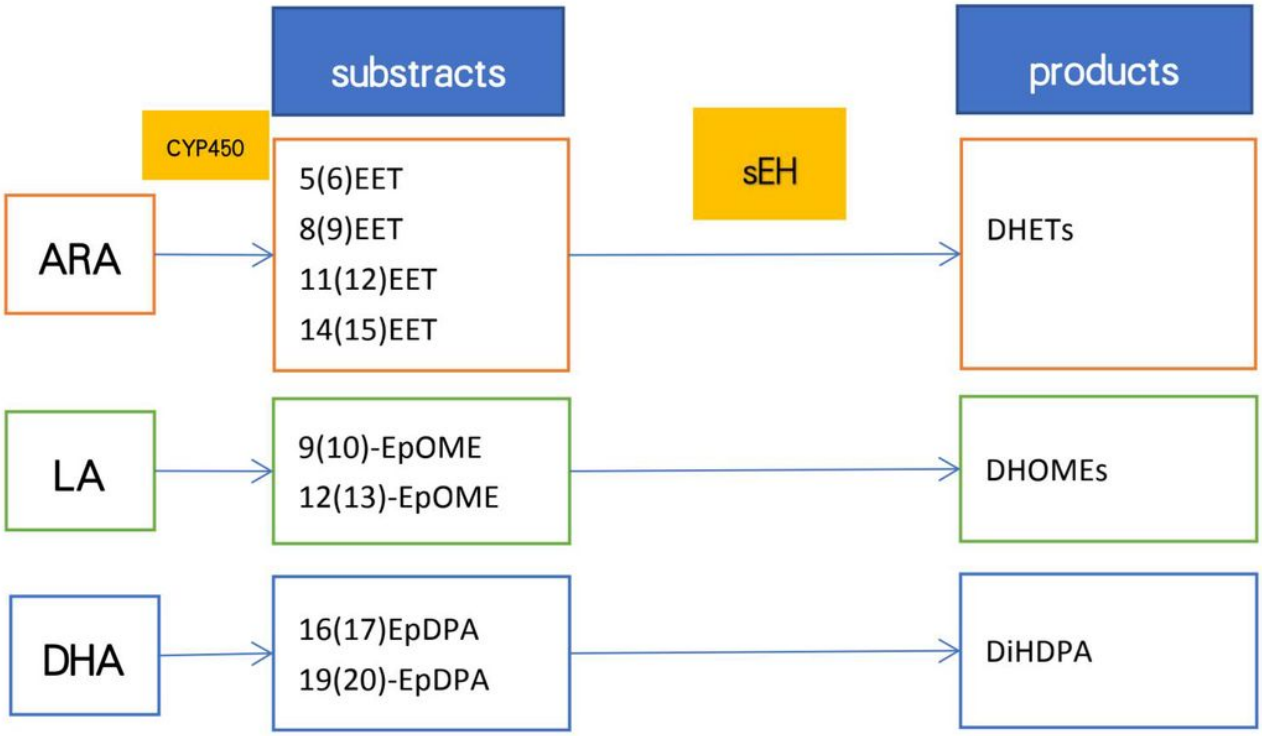

Figure 3

A simplified cascade of the metabolism of polyunsaturated fatty acids (PUFAs) including ARA, LA, and DHA via cytochrome P450 epoxygenase and sEH pathway.

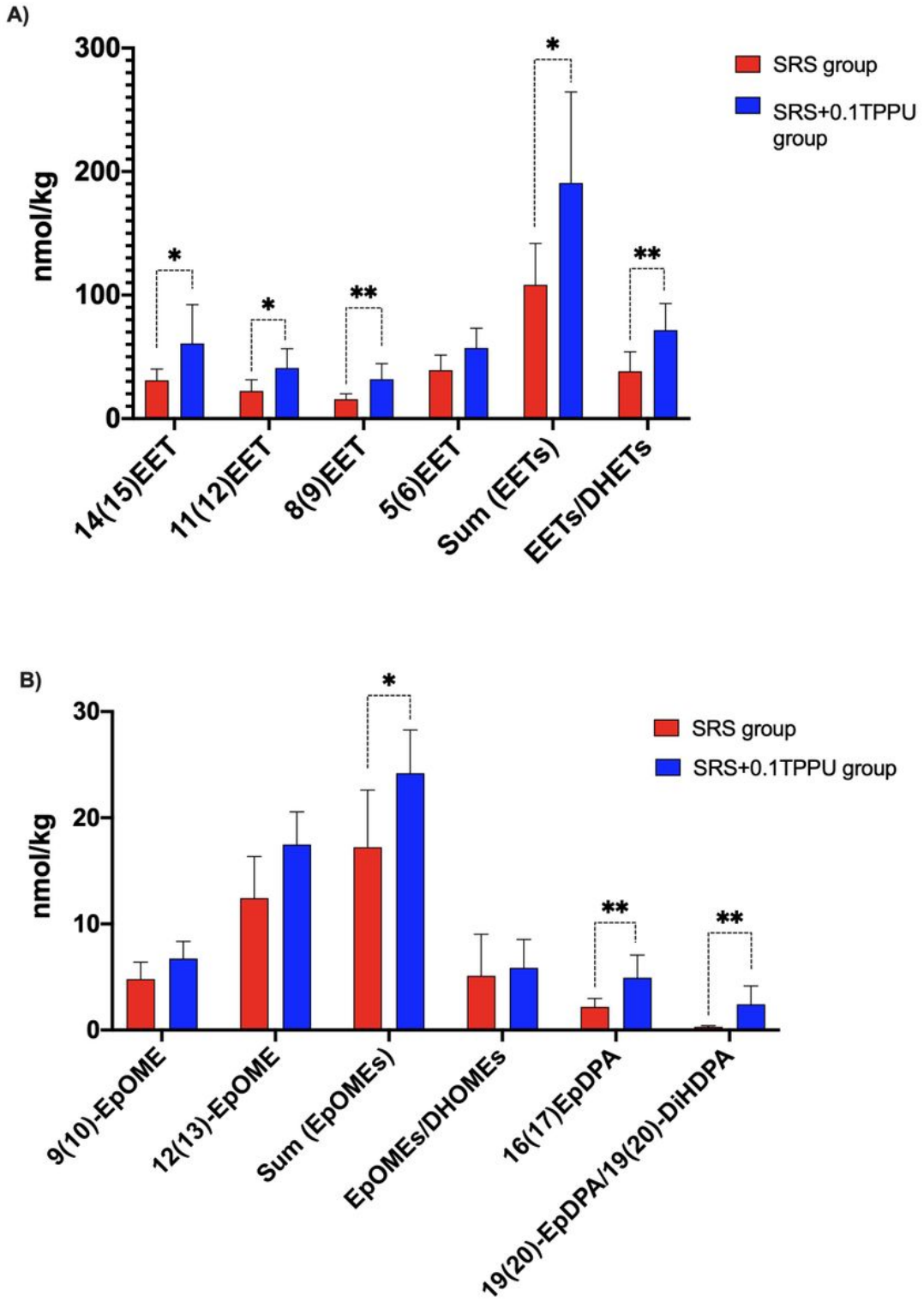

Figure 4

A) The regioisomers 8,9-, 11,12-, and 14,15-EETs, the sums of EETs, and the ratio of EETs/DHETs in the hippocampus was significantly increased in the SRS+0.1TPPU group compared with SRS+PEG400 group, *P<0.05, **P<0.01; B) The level of other EpFAs including EpOMEs, 16(17) EpDPA, and the ratio of 19(20)-EpDPA/19,20-DiHDPA were both significantly increased in the SRS+0.1TPPU group compared with SRS+PEG400 group , n=7 in each group, *P<0.05, **P<0.01.

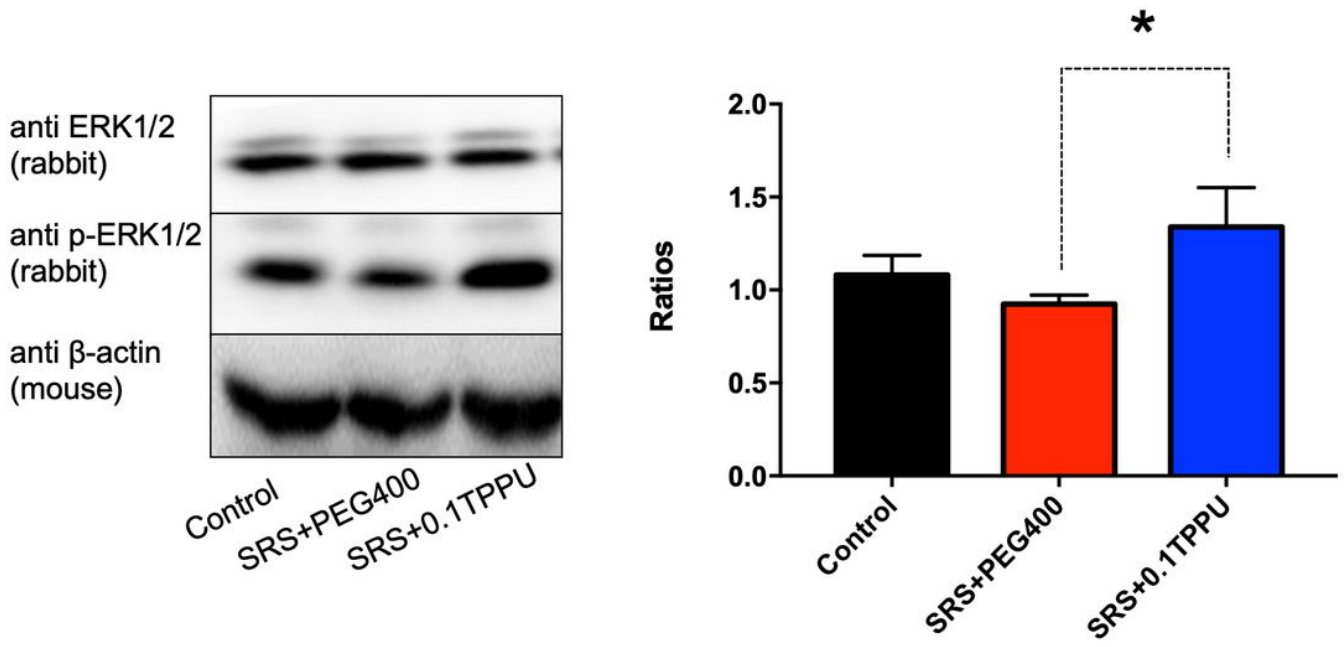

Figure 5

The ratio of p-ERK1/2 to ERK1/2 was significantly increased in the SRS+0.1TPPU group compared with SRS+PEG400 group, n=7 in every group, *P<0.05.