Inhibition of convulsive status epilepticus-induced abnormal neurogenesis by sodium valproate.

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Abstract

Background: Status Epilepticus (SE) is a life-threatening neurological emergency with high morbidity and mortality. The mechanism underlying the preventive effect of sodium valproate (VPA) on SE remains unclear.

Objectives: The current study aims to explore the potential effects of VPA on Neural Stem Cells (NSCs) proliferation, migration and neurogenesis in hippocampus of Wistar rats after Convulsive Status Epilepticus (CSE).

Materials and methods: A rat model of CSE was initially established. Effective dosage of VPA was screened out. Then, all test rats were divided into control group (Normal rats), CSE group (Model rats) and CSE+VPA group (Model rats treated with VPA). The induced success rate of Long-Term Potentiation (LTP) in hippocampus was detected. New-born NSCs and neurons in hippocampus were analysed by immunohistochemical staining.

Results: The effective dosage of VPA in controlling CSE for 35 d old (D35) rats was 300 mg/kg 6 times per day. CSE significantly promoted neurogenesis with obviously increasing new-born NSCs and neurons in Subgranular Zone (SGZ) and Subventricular Zone (SVZ), which showed a migration tendency to the CA1 area. VPA intervention could effectively prevent the effect of CSE.

Conclusion: VPA is effective to inhibit the abnormal proliferation and migration of NSCs and new-born neurons induced by CSE in SGZ and SVZ. Neurogenesis proves a pivotal regulatory element during CSE in rats.

Introduction

Status Epilepticus (SE) is one of the most common neurological emergencies in childhood, which requires immediate and effective treatment [1]. The incidence rate accounts for 0.16% among 2-year-old children and the mortality rate reaches 7% [2]. Aborting ongoing symptoms of SE and preventing the recurrence were the main goals of the treatment [3]. Though the first-line drugs, including benzodiazepines and phenytoin/fosphenytoin, were effective in about 60% of all episodes [4], there was still a great portion of patients suffering from SE. It has become a hot topic in the neurological, pediatric, and rehabilitation medical communities to effectively prevent convulsive brain injury and reduce cognitive impairment in children after SE.

Sodium valproate (VPA) has become a competitive drug widely used in SE treatment [5]. Intravenous VPA could reach high drug level in the brain and exhibit a rapid anticonvulsant activity through efficient central nervous system penetration [6]. Both adults and children have shown the efficacy and safety of intravenous VPA in controlling SE [7]. No potential major cardiovascular adverse symptoms, such as cardiac arrhythmia or hypotension, have been reported [8]. VPA can be used as the first-line antiepileptic drugs in SE with a good seizure control.

Several studies demonstrated that dentate granule cell neurogenesis was induced by SE using the rodent models of mesial temporal lobe epilepsy [9-11]. Dentate gyrus cell proliferation was increased by 5- to 10-fold within 3 d after drug-induced SE, respectively [9,11]. In addition, it was reported that neurogenesis was correlated with hippocampus-

dependent learning and memory [12]. In our previous study, the effect of VPA on cognitive function of rats after Convulsive Status Epilepticus (CSE) has been confirmed [13]. Whether neurogenesis plays a role in the antiepileptic effect of VPA remains unknown. Here, the current study aims to investigate the function of neurogenesis in SE treatment with VPA using a rat model.

Methods and Materials

Animals

All Wistar rats aged 35 d old (D35) weighing 60-80 g (Animals Certificate Number: SYXK (Yu) 20040001) were purchased from Chongqing Medical University Animal Center. All animal procedures were approved by the local ethics committee of Chongqing Medical University Animal Center and conformed to the National Institutes of Health (NIH) guidelines. Animals were housed in standard laboratory with the conditions, at room temperature $25 \pm 2°C$ 12 h light/dark cycle, humidity $60 \pm 5\%$ and had free access to water and food.

Establishment of CSE model

All tested rats for CSE model establishment [14] were given 3 mEq/kg lithium chloride intraperitoneally and after 18-20 h with pilocarpine by intraperitoneal injection (40 mg/kg body weight, Sigma-Aldrich, MO, USA). All CSE rats reached Class IV were treated with atropine sulfate (10 mg/kg body weight, Southwest Pharmaceutical Co., Ltd, Chongqing, China) and Diazepam (10 mg/kg body weight, Tianjin Jinyao Amino Acid Co., Ltd, Tianjin, China) by intraperitoneal injection 15 min and 30 min after onset of CSE, respectively. If no CSE happened after pilocarpine injection within 30 min, additional pilocarpine (10 mg/kg body weight) was injected. If Class IV CSE still not occurred, rats were dropped.

Sodium valproate (VPA) administration and grouping

To obtain the optimal treatment paradigm, plasma concentrations of VPA were measured after 5 d of VPA administration. D35 rats include normal rats (n=36) and CSE rats (n=36) were evenly divided into 12 groups (n=6), which were all orally administered sodium valproate (VPA) (Shanghai pharmaceutical group co., LTD, Shanghai, China) at a series doses (100 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg, 600 mg/kg), 6 times per day for 5 d.

To establish a CSE model, the other rats were further divided into Control group (Normal rats treated with 0.9% saline, n=6), CSE group (Model rats, n=6) and CSE+VPA group (Model rats treated with VPA, n=6). After 5 d of CSE model establishment, all rats were sacrificed by intraperitoneal injection of 10% chloral hydrate (Meilunbio Company, Dalian, China), and the brain tissues were obtained.

Electrophysiological recording of LTP

The brain tissues extracted were put into oxygenated slice liquid (0-4°C) for 1-2 min. The bilateral hippocampus was rapidly separated from the inside and 400 μm hippocampal slices were placed in the recording solution containing 95% O_2 and 5% CO_2 and incubated at 35°C for 30-45 min. Then the temperature was decreased to 24°C and hippocampal slices were incubated again for at least one hour. Hippocampal slices of rats were directly incubated at 24°C. When the hippocampal slices were moved to the recording bath, with continuous perfusion of oxygenated recording solution (124 mmol/L NaCl, 2.8 mmol/L KCl, 1.5 mmol/L NaH_2PO_4, 24 mmol/L $NaHCO_3$, 2 mmol/L $CaCl_2$, 2 mmol/L $MgSO_4$, 2 mmol/L ATP-Mg, 0.4 mmol/L vitamin C, 2 mmol/L sodium pyruvate, 10 mmol/L glucose, pH 7.3-7.4, 1.5 ml/min, 35°C), and the bipolar stimulation tungsten electrode was placed on the Schaffer collateral pathway in the hippocampal CA3 zone with a stimulus intensity of 0.1-0.25 mA , the data of field Excitatory Postsynaptic Potential (fEPSP) began to be recorded using glass microelectrode placed in CA1 zone (filled with 2 M NaCl, 10 mM HEPES and 10 mM EGTA, impedance 1-2 MΩ, pH 7.3-7.4). The data was collected and analysed by pCLAMP 9.2 software. The 50% stimulated intensity of the maximum response value of fEPSP was selected as the basic stimulus, and LTP was induced by High Frequency Stimulation (HFS) after baseline stabilization for 30 min. If fEPSP slope increases by no less than 20% and maintains more than 30 min, LTP is considered to be induced successfully.

Immunohistochemical staining

BrdU (5-bromo-2-deoxyuridine, 50 mg/kg body weight, Sigma-Aldrich, USA) was intraperitoneally injected twice per day on the 5th d of VPA administration. After 24 h of intraperitoneal injection of BrdU, rats were killed. For immunohistochemical staining, brain tissues were fixed by 4% paraformaldehyde and dehydration by sucrose. The brain tissues were sectioned in a coronal plane (6 um, 1-in-5 series) from 2 mm after visual acuity to posterior ventricle angle on a cryostat. Staining for BrdU and DCX (doublecortin) was performed according to standard protocol using primary antibodies against BrdU (mouse monoclonal anti-BrdU, Sigma-Aldrich, 1:250) and DCX (mouse monoclonal anti-DCX, Sigma-Aldrich, 1:250), respectively. Fluorescein Isothiocyanate (FITC) labeled rabbit anti-mouse secondary antibodies (Sigma-Aldrich, 1:250) were used. Images were collected on a confocal microscope. Under light microscope (100X), five fields were randomly selected from each slice, and the number of positive cells was counted. The average value was calculated. The number of BrdU-positive cells and DCX-positive cells were counted.

Statistical analysis

All data were analysed with SPSS 19.0 and presented by mean ± SEM. Two-way ANOVA was used for analysis of the induced success rate of LTP and BrdU-positive cell number among rats with three different treatments. P<0.05 was considered as statistically significant.

Results

A treatment paradigm for VPA to maintain adequate drug level

CSE model was established by intraperitoneally injection of pilocarpine. The evaluation of convulsions was based on the previous methods [14]. According to previous study, the effective plasma concentration of VPA in rat was 50-100 μg/ml [15]. To obtain the optimal treatment paradigm of VPA, we detected the plasma concentration of VPA in rats after various doses of VPA treatment.

As shown in Table 1, normal rats showed the effective VPA levels of 64.98 ± 10.69 (μg/ml) and 96.91 ± 20.36 (μg/ml) when treated with 300 mg/kg or 400 mg/kg of VPA, respectively. CSE rats treated with 300 mg/kg of VPA demonstrated an effective VPA level of 82.26 ± 11.23 (μg/ml), as well. Thus, the paradigm for VPA used to treat CSE rats was 300 mg/kg 6 times per day in our study.

VPA intervention can significantly improve the induced success rate of LTP after CSE

The induced success rate of LTP was analysed after the hippocampal slices stimulated by high frequency stimulation (HFS) for five minutes (Figure 1). Compared with the rats in Control group, CSE significantly inhibits the induced success rate of LTP in the rats of CSE group ($P<0.05$). On the contrary, VPA significantly promoted the induced success rate in the D35 rats of CSE+VPA group ($P<0.05$).

Moreover, the sustainable period of LTP was analysed. In Control group, the fEPSP slope showed no change from 60 min. However, the fEPSP slope was decreased to beneath baseline of 120% after 90 min in CSE group. In CSE+VPA group, VPA prolonged the sustainable time of LTP to 140 min. VPA significantly improved the fEPSPS amplitude in rats of CSE+VPA group, compared with CSE group ($P<0.05$).
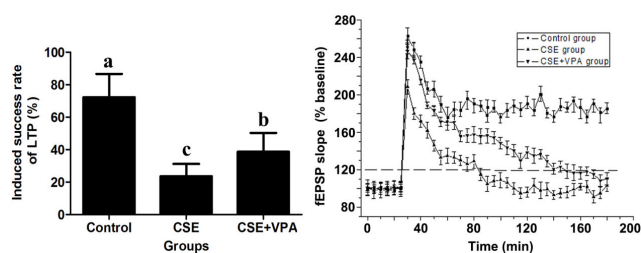

Figure 1. Induced success rate of LTP and fEPSP slopes in each group. LTP: Long-Term Potentiation; fEPSP: Field Excitatory Postsynaptic Potential. Different letters above the bar indicate significant different at a P value, P<0.05.

Effect of VPA on nerve regeneration in hippocampus after CSE

Neural Stem Cells (NSCs) were mainly existed in Subgranular Zone (SGZ) of hippocampus. In Subgranular Zone (SGZ), the average number of BrdU-positive cells was 18.21 ± 16.25 in Control group, 172.33 ± 32.13 in CSE group and $123.25 \pm$

25.26 in CSE+VPA group (Figure 2). CSE induced the regenerated NSCs number, which was significantly higher in CSE and CSE+VPA groups than Control group ($P<0.05$). However, VPA significantly blocked the effect of CSE, and the number of BrdU-positive cells was significantly decreased after VPA application in CSE+VPA group than CSE group ($P<0.05$).

Meanwhile, the number of NSCs in Subventricular Zone (SVZ) also significantly increased after CSE (Figure 2A). Compared with control group (5.63 ± 2.11), BrdU-positive cell number in CSE group and CSE+VPA group were significantly increased to 29.63 ± 5.55 and 19.21 ± 9.38, respectively ($P<0.05$, Figure 2B). Compared with CSE group, VPA significantly inhibited the NSCs regeneration in CSE+VPA group.
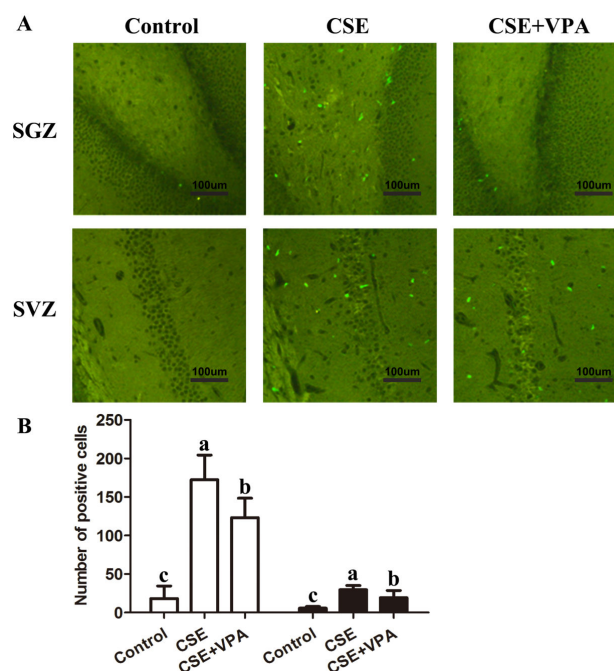

Figure 2. The number of BrdU-positive cells in D35 rats after CSE was analysed by immunohistochemistry (100X). A Subgranular Zone (SGZ) of the dentate gyrus and Subventricular Zone (SVZ); Sections were counterstained with Fluorescein Isothiocyanate (FITC) after BrdU immunostaining. Bright green indicated BrdU-positive cells. B Analysis of number of BrdU-positive cells in three groups. Under light microscope (100X), five fields were randomly selected from each slice, and the number of positive cells was counted. The average value was calculated. Different letters above the bar indicate significant different at a P value, P<0.05.

Effect of VPA on migration of new-born neurons after CSE

The new-born cells presented normal functions only after migrating to the specific location and constructing relationship with neighbouring cells. DCX was a vital microtubule-associated related protein in neural cells and expressed in plasma and axons, which was used as a marker for mature or migrating cells [16]. To investigate the effect of VPA on new-born neurons after CSE, the new-born neurons were analysed

by immune-staining, which represent DCX-positive cells (Figures 3 and 4). The results demonstrated that new-born neurons in SGZ increased obviously along with NSCs propagation after CSE (Figure 3). The axon number and length of the new-born neurons were obviously increased after CSE, as well. In CSE+VPA group, the VPA intervention alleviated the changes, such as the increased axonal number and length. Normally, the new-born neurons in SGZ migrated to granular cell layers to form granular cells. Our results showed that a migration trend of new-born neurons to dentate gyrus was detected after CSE. Interestingly, some new-born neurons stretched no axons but with shorter and thick pseudo-yl dendritic structure in CSE rats (Figure 4A). In addition, the new-born neurons in SVZ was also significantly increased, and showed a migration tendency to the CA1 area in rats after CSE (Figure 4B).

Table 1. Plasma concentrations of VPA in control and CSE group.

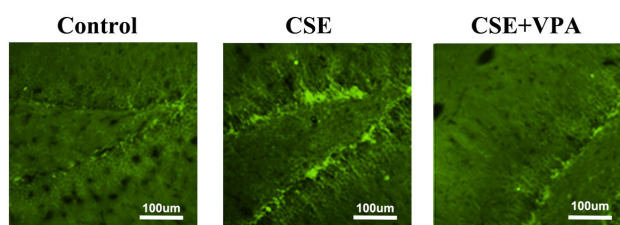| Dosage	Normal rats		CSE rats	
	Plasma concentration	n	Plasma concentration	n
100 (mg/kg, q6h)	13.52 ± 6.58 (µg/ml)	6	26.83 ± 8.47 (µg/ml)	6
200 (mg/kg, q6h)	35.26 ± 10.11 (µg/ml)	6	46.64 ± 9.64 (µg/ml)	6
300 (mg/kg, q6h)	64.98 ± 10.69 (µg/ml)	6	82.26 ± 11.23 (µg/ml)	6
400 (mg/kg, q6h)	96.91 ± 20.36 (µg/ml)	6	129.65 ± 28.33 (µg/ml)	6
500 (mg/kg, q6h)	2 rats drowsiness	4	3 rats drowsiness	3
600 (mg/kg, q6h)	2 rats drowsiness	4	1 rat died	5

Figure 3. Distribution of DCX-positive cells in Subgranular Zone (SGZ) of D35 rats after CSE (100X). Sections were counterstained with FITC after DCX immunostaining. Bright green indicated DCX-positive cells. A: Control group; B: CSE group, C CSE+VPA group.
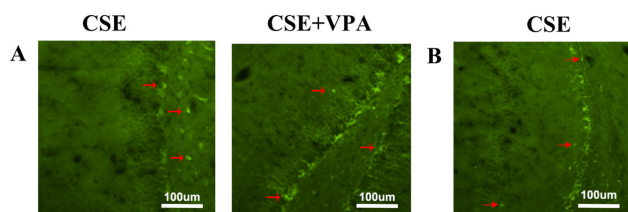

Figure 4. Migration of new-born neurons in D35 rats after CSE (100X). A: Abnormal migration in Subgranular Zone (SGZ); B: Migration in Subventricular Zone (SVZ). Sections were counterstained with FITC after DCX immunostaining. Bright green indicated DCX-positive cells. Arrows represent new-born neurons in migration.

Discussion

In the current study, a model of CSE was established using 35 d old rats. CSE increased the number of new-born cells, but induced the abnormal migration and morphology of new-born neurons. VPA intervention prevented effectively neurogenesis induced by CSE in Subgranular Zone (SGZ) and Subventricular Zone (SVZ). Our findings suggest adult neurogenesis appears to be a potential mechanism underlying VPA treatment.

Hippocampus plays an important role in learning and memory and lesions in hippocampus lead to dysfunction of cognition [17]. Choy et al. revealed that inflammatory pathological changes in hippocampus after a single CSE lasted at least 7 d [18]. Studies have demonstrated that neurogenesis involved in hippocampus-dependent learning and memory [12]. Adult neurogenesis mainly occurs in SGZ of hippocampus [19]. New-born neurons replace the dead ones to maintain the specific function. The changes in number or function of the new-born neurons may result in neurological disease. After brain damage, including epileptogenic brain insults and seizures, neurogenesis increases in the SGZ of adult rodent. The new-born neurons in response to such stimuli have been displayed abnormal function in connecting with neighbouring cells [20-22]. It is consistent with our results that CSE induced Neural Stem Cells (NSCs) proliferation with the increasing number of new-born cells in SGZ and SVZ. However, the migration and morphology of new-born neurons were different from normal ones. Additionally, our previous study demonstrated that hippocampus-dependent spatial learning and memory ability was damaged in CSE rats [13]. These suggest adult neurogenesis in hippocampus is an alternative participator in CSE. Cognitive impairment not only correlates with the nerve regeneration. How to control the normal migration of new-born neurons after nerve regeneration to form a normal neural circuit in hippocampal brain regions is also one of the keys to improving cognitive function.

As a short-chain fatty acid, VPA possesses competitive anticonvulsant activity and lower incidence rate of adverse events, which has been used to treat SE [8,23]. Our previous study showed that VPA significantly improved spatial cognitive dysfunction in CSE rats. LTP is an information storage method at the synaptic level, which mainly manifested as the enhancement of fEPSP after synaptic links receive a certain amount of intensive stimulation [24]. In the current study, the effect of VPA on neurogenesis was further investigated. CSE obviously reduced induced success rate of LTP and fEPSP slope. VPA could improve the induced success rate of LTP and alleviated the effects of neurogenesis induced by CSE. fEPSP slope was increased after VPA administration. VPA may play a role in epilepsy by regulating the synaptic transmission between excitatory signals.

In conclusion, neurogenesis proves a pivotal regulatory element during CSE in rats. VPA is effective to inhibit the abnormal proliferation and migration of NSCs and new-born

neurons induced by CSE in hippocampus, especially SGZ and SVZ.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Study design: P Wu, data collection: P Wu and XJ Li, statistical analysis: P Wu, M Cheng and L Jiang, data interpretation: P Wu, M Cheng and L Jiang. Manuscript preparation: All co-authors. Funds collection: L Jiang.

Consent for Publication

The authors declare that they consent for publication.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

All animal procedures conformed to the National Institutes of Health (NIH) guidelines and were approved by the local ethics committee of Chongqing Medical University Animal Center.

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