ELECTRO-ACUPUNCTURE IMPROVES EPILEPTIC SEIZURES INDUCED BY KAINIC ACID IN TAURINE-DEPLETION RATS

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ABSTRACT:
Electro-acupuncture (EA) partially inhibited epilepsy with great success. The biological basis underlying EA anti-convulsion remained uncertain, which resulted in limited application and slow improvement of acupuncture. Our previous study indicated that taurine may play an inhibitory role against epilepsy as an inhibitory amino acid in the central nervous system and EA may inhibit epilepsy via up-regulating the expression of taurine transporter to increase the release of taurine. Involvement of taurine in kainic acid (KA)-induced epilepsy and anti-convulsion of EA was further addressed on taurine deficiency animal in the present work. We instituted endogenous taurine-deficiency model by supplementation of beta-alanine (3%) in drinking water for continuous 10 days initially, injected KA into lateral cerebral ventricle to induce epileptic seizure, and performed EA treatment on DU26 “RenZhong” and K1 “YongQuan” acupoints by an EA apparatus (Model G6805-2) using successive waves with the frequency 64Hz and the current intensity 0.8-1.0mA for 30 minutes in Sprague-Dawley (SD) rats.
Taurine levels markedly decreased in cortex, hippocampus, striatum and cerebellum of rats after beta-alanine administration by fluore-HPLC measurement. EA alleviated epileptic activity in rats at 3.5h time point after KA injection, whereas beta-alanine-induced taurine depletion rendered rats more susceptible to KA-induced epilepsy. Taurine transporter level increased after EA treatment. These results suggested that taurine participated in epileptogenesis and EA may be related to taurine in controlling epileptic seizure.

KEY WORDS: Taurine depletion; Epilepsy; Electro-acupuncture; Taurine transporter; Kainic acid; Rat

INTRODUCTION

Epilepsy is an episodic disorder of the central nervous system arising from the excessive synchronous and sustained discharge of a group of neurons. Inhibitory action from GABA neurotransmission quenched excitatory neurotoxicity partially but failed to improve symptoms of many intractable epilepsies. Acupuncture, as an alternative therapy, benefited a large number of patients with epileptic seizures clinically from two thousands years ago through nowadays. Experimental tests in our laboratory and others (He, 1989; Wang, 1994; Yang, 2000; Chao, 2001) also proved that EA had a significant anti-convulsive effect in rat epilepsy induced either by kainic acid or penicillin (13,14). Biological mechanism beneath acupuncture is still unclear.

One pathway of EA anti-convulsion referred to taurine, an essential inhibitory amino acid in recent reports. EA inhibited penicillin-induced epileptic activity in a synergistic manner with exogenous taurine at 40 mg/kg while both of them could reduce epileptic events partially in rat behavior and electro-encephalography and exogenous taurine enhanced the anti-convulsive effect of EA (Li, 2005). Electrical stimulation on the ear point increased the contents of taurine in hippocampus of penicillin- induced epileptic rat (Shu, 2004), and also EA enhanced taurine level on experimental epileptic animals (Wang, 1994) when it delivered the anti-epileptic effect.

Taurine (2-aminoethanesulfonic acid) is one of the most abundant free amino acids in the brain, with similar structure to glycine and GABA (Timothy, 1998, Saransaari, 2000, Pow, 2002). Two distinct Na+-dependent high-affinity taurine transporters (TAUT1 and TAUT2) regulate intracellular taurine levels in glial cells and neurons. During early neocortical development, nonsynaptically released taurine can activate Glycine receptors (Alexander, 1998, Renteria, 2004). In adult, taurine can activate GABA_A and Glycine receptors to mediate inhibitory synaptic transmission (Ye, 1997, Del Olmo, 2000, Louzada, 2004). Actually, taurine has been known to possess some mild anti-convulsive activities in both humans and experimental animal
models. Taurine suppressed epileptiform discharges induced by removal of Mg\(^{2+}\) in combined rat entorhinal cortex-hippocampus slices (Kirchner, 2003). *In vivo*, taurine was also found to have a significant anti-epileptic effect in the mouse model of KA-induced limbic seizures (El Idrissi, 2003). The data about taurine release came interesting. Taurine level increased after anti-convulsive treatment by ketogenic diet in cerebrospinal fluid of patients with refractory epilepsy (Dahlin, 2005) and by lamotrigine in rat hippocampus, frontal and parietal cortices (Hassel, 2001). Meanwhile, Taurine release was detected controversial, either with a notable increase in the epileptic hippocampus in a chronic kainate rat model (Wilson, 1996) and in a pentylentetrazol-kindled rat (Li, 2004) during the seizure period, or with no significant change in plasma and cerebrospinal fluid of patients with acute epilepsy, juvenile myoclonic epilepsy or refractory localization-related epilepsy (Rainesalo, 2004), or even a marked increase in surviving SSADH\(^{-}\) mice suffered from lethal tonic-clonic seizures in all brain regions except cerebellum (Gupta, 2004).

To further address the involvement of taurine in epileptic seizure and in EA anti-convulsion, in the present study, we would investigate seizure activity, neuronal cell death and taurine transporter expression in taurine depletion model during KA-induced epilepsy before and after EA administration.

**MATERIALS AND METHODS**

**Experimental taurine depletion (TD)**

Male SD rats (140-160g) were employed for the experiments (Shanghai Experimental Animal Center, Chinese Academy of Science). All rats were maintained 3 per cage at temperature (23-25°C), with a 12h light-dark cycle. Rats were randomly divided into two groups: one group received drinking water containing 3% beta-alanine (TD group; n=9) and the control group (n=9) only tap water. Food and drinking fluid were available *ad lib* throughout the study. All experimental procedures were approved by the Committee of Laboratory Animals, Fudan University. Ten days after initiation of TD regimen, rats (weighing 200-220g) were decapitated and the brain removed from each animal. The brain was dissected into cortex, hippocampus, striatum, and cerebellum and then placed in liquid nitrogen until use. The tissues were homogenized in 0.1M HClO\(_4\) solution and centrifuged at 12,000g for 30min at 4°C. The supernatants were transferred to another tube and re-spinned at 12,000×g for 30min at 4°C. The new resulting supernatants were then transferred to a third tube and placed at -70°C until assayed. O-Phthalaldehyde (OPA 27mg, Sigma) was dissolved in 5ml methanol and then the methanol solution was added 5ml 0.1M borate buffer (pH 9.5), and 40μl 2-mercaptoethanol, subsequently. The derivation was performed with the mixture of 20μl homogenate supernatant and 10μl OPA solution. After 2min, the sample was injected into the column. The concentration of taurine was measured by high performance liquid chromatography (HPLC) system with a fluorescence detector.
Epilepsy model and Electro-acupuncture treatment

Epilepsy model was induced by microinjection of 0.7\mu g Kainic acid (KA) in 0.7\mu l normal saline into right lateral cerebral ventricle by P0.2, R1.5, H4.5 (0.2mm posterior to bregma, 1.5mm lateral to the midline, 4.5mm below the surface of skull) according to the atlas of George Paxinos Stereotaxic Coordinates (1986).

EA was performed on a pair of acupoints (DU 26 “RenZhong” and K 1 “YongQuan”) (Fig. 1) using successive waves 30 min after KA injection and lasted for 30min. The acupuncture needles were stimulated with an electrical-stimulator (Model G 6805-2) made in Shanghai Medical Electronic Apparatus Company of China. The frequency was 64Hz and the current intensity 0.8-1.0mA.

Behavioral seizure score was classified single blindly according to Racine (Racine, 1972): stage 1, chewing, facial clonus (a series of rapid muscle contractions); stage 2, head nodding; stage 3, lateral forelimb clonus; stage 4, rearing and bilateral forelimb clonus; stage 5, rearing and falling.

![Stimulating wave form: 64Hz](image)

Fig. 1. Picture showing that EA was performed at DU 26 “RenZhong” and K 1 “YongQuan” with an EA apparatus (G6805-2)

Nissil Staining

Rats were anaesthetized with chloral hydrate (360mg/kg i.p.) at the time point of 24 hours after KA injection and perfused intracardially with 0.9% saline solution followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). The brains were removed, post-fixed, and immersed in 20% sucrose followed in 30% sucrose at 4°C until sunk. The coronal sections were sliced at 30 \mu m on a freezing microtome and were then dehydrated in 50%, 75%, 85%, 95%, and 100% ethanol sequentially for 5min, respectively. The staining was carried out in Nissil staining solution (0.1% cresyl violet containing 0.01% acetic acid) for 1h.

Immunohistochemistry

The sections were pre-treated with 0.3% H2O2 in 0.01M PBS (pH 7.2) for 30min
and blocked in PBS containing 10% normal goat serum and 0.5% Triton X-100 for 1 hour, and then incubated with primary taurine transporter antibody (Chemicon, diluted 1:200) for 1 hour at 37°C and 4°C overnight. After rinsed in PBS, the sections were incubated in biotinylated anti-rabbit IgG at a dilution of 1:200 for 1 h at 37°C, followed by incubated in 1:200 diluted avidin-biotin peroxidase complex for 1 h at 37°C. The peroxidase reaction was detected with diaminobenzidine staining kit (Vector). As negative controls, sections received identical treatment except for incubation with the primary antibody and showed no specific staining. Only one taurine transporter (TAUT1) antibody is commercial available.

Statistical analysis
Data were expressed as mean ± SEM. Significance was determined using Student’s t-test. Differences between groups were considered significant at P<0.05.

RESULTS

1. Taurine depletion by beta-alanine
Previous studies have shown that cardiac taurine pools can be significantly reduced by treating rats with the taurine transporter inhibitor, beta-alanine (Allo, 1997). Similar to those studies, we found that (Fig. 2) in the brain cortex taurine levels reduced from 3257.3 ± 78.3 to 2583.7 ± 135.7 nmol/g tissue (p<0.01), hippocampus taurine levels reduced from 3233.3 ± 149.0 to 2103.2 ± 135.8 nmol/g tissue (p<0.01), striatum taurine levels reduced from 3108.9 ± 523.5 to 730.5 ± 272.1 nmol/g tissue (p<0.01), and cerebellum taurine levels reduced from 2402.7 ± 64.3 to 1810.1 ± 246.0 nmol/g tissue (p<0.05).

2. Taurine depletion aggravated seizures
As shown in Fig. 3, in KA group (n=8), rats exhibited obvious epileptic activity 0.5h after KA injection and reached maximum seizure score (4-5 score) 1h-2h after KA injection, then the seizure score decreased gradually. In KA + EA group (n=8), rats exhibited lower seizure score at the time points of 1.5h, 2.5h, 3h, 3.5h, 4h and statistically significant (p<0.05) at time point of 3.5h after KA injection, compared with KA group. Whereas in beta-alanine + KA group (n=8), rats manifested higher seizure score at the time points of 1.5h, 2h, 2.5h, 3h, 3.5h, 4h and statistically significant (p<0.05) at time point of 3.5h after KA injection, compared with KA group. Furthermore, in beta-alanine + KA + EA group (n=10), rats manifested lower seizure score at the time points of 1h, 1.5h, 2h, 2.5h, 3h, 3.5h and statistically significant at time points of 1h (p<0.05), 1.5h (p<0.01), 2h (p<0.01), and 2.5h (p<0.01), compared with beta-alanine + KA group.
Fig. 2. Beta-alanine administration significantly decreased taurine levels in cortex, hippocampus, striatum, and cerebellum.

In control group (n=9), rats were available to tap water.
In beta-alanine group (n=9), rats were available to drinking water containing 3% beta-alanine.

* p<0.05; ** p<0.01 vs control.

Fig. 3. Seizure scores in different groups of rats.

- p<0.05 vs KA group;
- # p<0.05, ## p<0.01 vs Beta-alanine + KA group.

Seizure score was classified single blindly according to Racine described in the Materials and Methods

3. Taurine depletion aggravated cell death in CA3 area

In KA group, cell death in CA3 area was significant in both sides of hippocampus
(e, f), compared with control (a, b). Whereas, in KA + EA group, cell death in CA3 area was not significant in the contralateral side (i) and less in the ipsilateral side (j), compared with KA group. In beta-alanine + KA group, cell death in CA3 area was even worse in both sides (g, h), compared with KA group. On the contrary, in beta-alanine + KA + EA group, cell death in CA3 area was less in both sides (k, l) than those in beta-alanine + KA group (Fig. 4).

Fig. 4. Nissl staining of hippocampus
EA increased KA-induced decrease of taurine transporter expression in CA3 and dentate gyrus area

In KA group, positive staining of taurine transporter antibody significantly decreased in CA3 and dentate gyrus area in both lateral sides of hippocampus (Fig. 5. e, f), compared with control (a, b). While in KA + EA group, the positive staining in CA3 and dentate gyrus area enhanced in bilateral sides (i, j), compared with KA group. In beta-alanine + KA group, the positive staining in CA3 and dentate gyrus area even decreased (g, h), compared with KA group. Whereas, in beta-alanine + KA + EA group, the positive staining in CA3 and dentate gyrus area enhanced (k, l), compared with beta-alanine + KA group.
DISCUSSION

That endogenous taurine deficiency rendered animals susceptible to sub-acute seizure induced by kainic acid in the present study provided an evidence for the beneficial efficacy of exogenous taurine given (40 mg/Kg, i.p.) in penicillin-induced epileptic model in our previous study (Li, 2005). Both above works are consistent with documented reports from other labs (El Idrissi, 2003) about application of taurine clinically and experimentally both in vivo and in vitro. Taurine has been used with varying degrees of success in treating patients with epilepsy (Airaksinen, 1980, Birdsell, 1998). Acute injection of taurine (43 mg/Kg, s.c.) increased onset latency, reduced occurrence of tonic seizures and duration of tonic-clonic convulsions, and mortality rate in the mouse model of KA-induced limbic seizure (El Idrissi, 2003). Treatment of mutant mice with taurine could rescue lethal generalized seizures pharmacologically in murine succinate semialdehyde dehydrogenase deficiency, which otherwise would not survive from rapid death at postnatal day 16-22 (Hogema, 2001). In vitro data resembled in vivo investigation. Taurine suppressed seizure-like events in combined rat entorhinal cortex-hippocampal slices (400 micro m), of which epileptiform discharges were induced by reduced extracellular Mg²⁺ concentration (Kirchner, 2003). Although pooled findings ignited the minds to develop taurine into neuro-protective anti-convulsant, its application is still limited due to blood brain barrier. Taurine lipophilic derivatives like taltrimide (2-phthalimidoethanesulphon
-N-isopropylamide), which was designed against taurine limitations like restricted permeability, is already in the market as an anti-convulsant agent. Many other taurine analogues also have been reported in the literature with partial to marked activity in experimental models and they are undergoing clinical trials (Airaksinen, 1987, Gupta, 2005). Taurine depletion model here provided a perfect tool to explore the role of taurine in epilepsy. Reports further indicated that taurine significantly reduced neuronal cell death in the CA3 region of the hippocampus, the most susceptible region to KA in the limbic system (El Idrissi, 2003). Similarly, our present data showed that neuronal cell death increased in taurine depletion-aggravated epilepsy, compared with KA-induced seizure only.

The inhibitory effect of acupuncture on epileptic seizure has been well-documented through past two thousands years in China, starting from ‘Smart Pathway, Ling Su, Chapter Epilepsy and Madness’ of ‘The Yellow Emperor’s Classic of Internal Medicine, Huang Di Nei Jing’ before Christ. Imbalance between Yin and Yang was indicated underlying epileptic discharge by traditional Chinese medicine. Interestingly and correspondently, imbalance between inhibitory and excitatory neuro-transmission was elucidated as one of biological basis beneath seizure using modern techniques of biochemistry, molecular biology, and electro-physiology. Taurine, as an important inhibitory amino acid and a neurotransmission modulator, draw lots of focus in addition to GABA during last two decades. Synergistic improvement of EA and taurine in penicillin-induced epilepsy in our previous study led us explore whether EA suppresses seizures via taurine on taurine depletion model. EA modulated levels of taurine transporter in rat hippocampus and cortex as it improved seizures that were aggravated by endogenous taurine deficiency and decreased neuronal cell death in cortex and limbic system in the current report. Changes of taurine transporter expression gave us a hint that EA controlled seizure through mediating taurine transporter, ultimately, through the release of taurine. Additional studies will be done on taurine levels in the next step to better characterize the linkage between EA and taurine and between seizure susceptibility and taurine.

CONCLUSION

In conclusion, taurine levels markedly reduced in cortex, hippocampus, striatum, and cerebellum after beta-alanine supplementation in taurine depletion rats model, which rendered rats more susceptible to KA-induced epileptiform seizures and cell death in CA3 and dentate gyrus area of hippocampus. However, EA suppressed epileptic seizures and cell death partially, and enhanced taurine transporter expression in CA3 and dentate gyrus area.

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REFERENCES
