INVolVEMENT OF TAURINE IN PENICILLIN-INDUCED EPILEPSY AND ANTI-CONVULSION OF ACUPUNCTURE: A PRELIMINARY REPORT

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ABSTRACT:
The potential role of taurine on epilepsy and acupuncture anti-convulsion was addressed in the present study. Epilepsy was induced by micro-injection of penicillin into hippocampus of Wistar rats. Taurine was applied by intraperitoneal (i.p.) injection. Electro-acupuncture (EA) was performed on acupoints of DU 20 “Bai Hui” and DU 16 “Feng Fu” along DU channel. Epileptic grades were evaluated by electro-encephalography (EEG) and behavior score. We featured the dose-response relationship between taurine-treated epilepsy and epilepsy-only subjects, detected the effect of exogenous taurine on epilepsy and acupuncture treatment, and investigated taurine transporter immuno-activity in hippocampus using immunohistochemistry. It was found that: 1), taurine had a significant antiepileptic effect as applied at i.p. 20 mg/kg, 40mg/kg, 80mg/kg, especially at 40mg/kg in the rat model of penicillin-induced seizure. Animals were improved by one to three Racine grades in behavior and in frequency and amplitude of EEG. 2), Exogenous taurine enhanced the anti-convulsive effect of EA. Both behavior and EEG were improved in taurine-treated rats. EA inhibited
epilepsy. Exogenous taurine improved epilepsy in a synergistic manner to EA. 3), EA increased the concentration of taurine transporter in hippocampus by comparing EA-treated epilepsy with normal control and penicillin only, or EA-treated plus taurine-treated epilepsy with taurine-treated only epilepsy and penicillin only. The resulting data suggested 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 upregulating the concentration of taurine transporter to increase the release of taurine.

KEY WORDS: taurine, epilepsy, electroacupuncture, taurine transporter.

INTRODUCTION

Epilepsy is a common disease of the central nervous system characterized by excessive, episodic, synchronized activity of a group of neurons. The neurophysiologic disorder of cerebral function leads to paroxysmal derangement of epileptic seizure. The imbalance between GABAergic inhibitory and glutamatergic excitatory amino acid system contributes to epileptogenesis. Most of anticonvulsant drugs are developed to enhance Na⁺ channel inactivation or to augment inhibitory gamma-aminobutyric acid (GABA) transmission. However, some seizures are refractory to all antiepileptic drugs, which suggested that besides GABA there existed another biologic basis underlying the abnormal and spontaneous burst-in firing of neurons.

Acupuncture, as an effective and a safe treatment, has been served in inhibiting epileptic seizure in clinic for thousands of years. Mechanistic considerations of acupuncture on epilepsy are related to balance of GABA and glutamate, balance of enkephalin and dynorphin, receptors system and release of nitric oxide (He, 1989; Wang, 1994; Yang, 2000; Chao, 2001). To date, the potential neurobiological pathway of acupuncture remains uncertain.

Recent discoveries implicated that taurine (2-aminoethanesulfonic acid) suppressed the epileptic activity both in mouse model of kainic-acid-induced limbic seizure and in combined rat entorhinal cortex-hippocampal slices with reduced extracellular Mg²⁺ concentration (Kirchner, 2003; El Idrissi, 2003). It also implicated that electrical stimulation the ear point increased the contents of taurine in hippocampus of penicillin-induced epileptic rat as having its anti-epilepsy effects (Shu, 2004). Taurine, as another inhibitory amino acid besides GABA and a non-toxic endogenous antioxidant, is a potential neuro-protective factor in multiple neurological disorders, such as ischaemia-reperfusion injury (Kingston, 2004) and retinal development (Renteria, 2004). Clinically, taurine has been used with varying degrees of success in the treatment of epilepsy and other seizure disorders (Birdsall, 1998). In traditional Chinese medicine, a pharmacy named An-Gong-Niu-Huang-Wan is applied to treat patients with epilepsy in clinic. One of the major components of
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An-Gong-Niu-Huang-Wan is taurine. Taurine transporter is the main vector in plasma membrane for taking up of taurine from extracellular to intracellular.

The goal of the present work was to determine whether taurine might be an anti-epileptic factor and a target of acupuncture modulation in penicillin-induced epileptic rat.

MATERIALS AND METHODS

Epilepsy model and Electro-acupuncture treatment

Adult Wistar rats (200-220g) were provided by Experimental Animal Center of Shanghai Medical School of Fudan University.

Epilepsy model was induced by microinjection of penicillin at 350 units in 2μl normal saline into one side of hippocampus by P3, R/L2, H3.7 (3.0mm posterior to bregma, 2.0mm lateral to the midline, 3.7mm below the surface of skull) according to the atlas of George Paxinos Stereotaxic Coordinates (1986).

EA was performed on a pair of acupoints (DU 20 “Bai Hui” and DU 16 “Feng Fu”) (Fig 1) 25 min after injection of penicillin and retained for 15min and then pause for 15 min followed by additional 15min of treatment. The acupuncture needles were stimulated with an electrical-stimulator (Model G 6805-1A) made in Shanghai Medical Electronic Apparatus Company of China. The frequency was 100Hz and the current intensity was 6mA.

Fig 1. Picture showing that EA was performed at DU 20 “Bai Hui” and DU 16 “Feng Fu” with an EA apparatus (G6805 1A)
Taurine administration and animal grouping

Taurine was given 25 min after penicillin by intraperitoneal injection (i.p.). Animals were randomly grouped according to experiments. For the dose-response curve of taurine on epilepsy rats: 1), group 1 (n=7), as epilepsy only subjects, injected with penicillin. 2) 3) 4), group 2 (n=6), group 3 (n=13), group 4 (n=8), after penicillin i.p taurine at 20 mg/kg, 40mg/kg, 80mg/kg respectively. For the co-operation of taurine and EA and the detection of taurine transporter: A), group A (n=7), as epilepsy only subjects, injected with penicillin. B), group B (n=13), performed with EA after penicillin. C), group C (n=13), given 40mg/kg of taurine after penicillin. D), group D (n=10), added acupuncture plus 40mg/kg of taurine after penicillin. E), normal control without induction of seizures.

Behavior score and EEG recording

Behavioral seizure severity was classified according to 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.

EEG was recorded and analyzed by difference of frequency and amplitude.

Immuo-staining and hematoxilin-eosin (HE) restaining

For detection of taurine transporter in cortex and hippocampus, rat brains were removed after perfusion with paraformaldehyde and sliced into 30μm thick coronal sections using a frozen microtome. After blocked in 0.01M PBS containing 10% normal sheep serum and 0.2% Triton X-100, the brain sections were sequentially incubated with rabbit anti-rat taurine transporter antiserum (Chemicon, USA) for 1 hour at 37°C, followed by 48 hours at 4°C, biotinylated goat anti-rabbit secondary antibodies for 1 hour at 37°C, and the avidin biotin immunoperoxidase complex (Vector Labs, Burlingame, CA, USA) for 30 minutes. The immunoreactivity was demonstrated by diaminobenzidine tetrahydrochloride (Sigma, USA). After immunohistochemistry, the sections were counterstained with hematoxilin-eosin.

Statistical analysis

Significance was determined using Student’s t-test. Differences between groups were considered significant at P<0.05.

RESULTS

1, Epilepsy induced by penicillin.

Rats showed flattening and desynchronization in EEG and relative calmness in behavior at normal condition. Most of rats started epileptiform seizure evaluated by behavior and EEG 25 min after injection of penicillin and last for other 45min (p<0.05) (Fig 2.1 A, Fig 2.2 A). Spikes or sharp waves appeared in EEG with increased frequency and amplitude. In association with alterations of EEG, rats behavior presented chewing, head nodding, lateral forelimb clonus, rearing of tails, screaming and even rearing and falling.
2. The dose-response curve of taurine on epilepsy rats

By behavior, we found that group treated with 20mg/kg taurine improved by one or two Racine grades and groups treated with 40mg/kg or 80mg/kg taurine improved by two or three grades comparing to epileptic controls. We detected that all of the three groups treated with different doses of taurine improved in frequency and amplitude of EEG (Fig 2.1 B, C, D). The improvement of group treated with 40mg/kg taurine is the most significant among them. The dose-response curve was obtained upon EEG, as showed in Fig 2.2 B.

Fig 2.1. Representative EEG patterns without and with taurine treatment at 20mg/kg, 40mg/kg and 80mg/kg showed in A, B, C, and D, respectively. a, d, g, j, normal control. b, c, epilepsy 25 min and 70 min after injection of penicillin respectively; e, f, given 20mg/kg taurine 25 min and 70 min after penicillin respectively; h, i, given 40mg/kg taurine 25 min and 70 min after penicillin respectively; k, l, given 60mg/kg taurine 25 min and 70 min after penicillin respectively.
Fig 2. Changes of frequency and amplitude difference of EEG during penicillin treatment and taurine administration after penicillin.

Both differences were calculated by minus of the frequency or amplitude 70 min after penicillin injection from the one 20 min after penicillin injection. The frequency and amplitude difference was used to stand for the seizure severity.

The statistic result showed that penicillin induced severe seizure and taurine inhibited seizure discharge at 20mg/kg (b), 40mg/kg (c), and 80mg/kg (d) comparing to epileptic control (a). P<0.001, penicillin vs normal; b or c or d vs a; c or d vs b. P>0.05, d vs c.

3. Epilepsy suppressed by taurine and EA

EA reduced epileptic discharges in rat while inhibiting seizure in behavior (Fig 3.1.A). Exogenous taurine not only inhibited epilepsy but also enhanced the anti-convulsive effect of EA (Fig 3.1. B). Behavior was improved in taurine-treated or EA-treated rats from stage four to stage two and was also improved in taurine plus EA treated rats from stage four to stage two or one 70 min after penicillin injection. Frequency and amplitude difference of EEG were also improved by p<0.05 (Fig 3.2).

4. EA increased expression of taurine transporter

Taurine transporter immuno-activity in hippocampus and cortex was detected using immunohistochemistry (Fig 4.1 A, B, C). The taurine transporter level decreased during epilepsy by comparison with normal and increased after supplement of exogenous taurine or treatment of EA or both by comparison with epilepsy group (p<0.05). Similar signals of taurine transporter like immuno-reactivity were detected between taurine-treated and EA treated group (p>0.05). Some distinction existed between group treated with EA plus taurine and group treated with taurine only, but it was meaningless statistically (p>0.05). The difference of taurine transporter concentration between group double treated with EA plus taurine and group treated with EA only was significant (p<0.05) (Fig 4.2).
Fig 3.1. Representative EEG patterns without and with 40mg/kg taurine treatment in additional with EA treatment after penicillin showed in A and B, respectively.

a, d, normal control.
b, c, epilepsy 25 min and 70 min after injection of penicillin with EA treatment
e, f, epilepsy 25 min and 70 min after penicillin with both EA and taurine treatment,

Fig 3.2. Changes of frequency and amplitude difference of EEG with EA or/and taurine administration after penicillin.
Both differences were calculated by minus of the frequency or amplitude 70 min after penicillin injection from the one 20 min after penicillin injection.
The frequency and amplitude difference were used to stand for the seizure severity.
The statistic result showed that penicillin induced severe seizure (a) and either EA (b) or taurine (c) inhibited seizure, and EA plus taurine (d) inhibited seizure synergistically. P<0.001, b or c or d vs a; d vs b or c. P>0.05, b vs c.
Fig. 4.1. Taurine transporter immunostaining observed in CA1 area of rat hippocampus.

Brown signals refer to positive of taurine transporter. Counterstained with HE in purple signals specific for cell nucleus.

a, f, k, normal control. b, g, i, penicillin-induced epilepsy. c, h, m, EA treatment only after penicillin. d, i, n, taurine administration only after penicillin. e, j, o, EA plus taurine after penicillin. magnification x30 in a, b, c, d, e, f, g, h, i, j, k, l, and x200 in k, l, m, n, o.
Fig. 4.1. B. Taurine transporter immunostaining observed in CA4 area of rat hippocampus. The reference is the same as Fig. 4.1. A.
Fig. 4.1 C. Taurine transporter immunostaining observed in frontal cortex. The reference is the same as Fig. 4.1 A.
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Fig 4.2. Cell number of taurine transporter staining counted and compared in fixed same part of CA1, CA2 and CA3 area of hippocampus
(a) normal control
(b) penicillin-induced epilepsy
(c) EA treatment only after penicillin
(d) taurine administration only after penicillin
(e) EA plus taurine after penicillin
P<0.05, c or d or e vs a or b; c vs e.
P>0.05, c vs d, d vs e.

The result showed that both EA and taurine improved expression of taurine transporter, and EA + taurine may interact in a synergistic way.

DISCUSSION

The dose of taurine at 40mg/kg improving epileptic discharge in rat model of penicillin-induced seizure in the present study is in agreement with a recent report at 43mg/kg in the mouse model of KA-induced limbic seizures (El Idrissi, 2003). Taurine, as a semi-essential inhibitory amino acid, is present at high concentrations in the mammalian brain. The role of taurine is proposed to be involved in several physiological action, including neurotransmission, neuromodulation, osmoregulation, control of calcium influx, and cell excitability. Overactivation of glutamatergic transmission and imbalance between GABAergic inhibitory and glutamatergic excitatory amino acid mediate neuronal cell death and lead to seizure in epilepsy. Taurine could prevent the neurotoxicity of glutamate (Louzada, 2004) and activate GABA receptor (Del Olmo, 2000). Cellular depletion of taurine has been linked to cell damage. The neuroprotective effect of taurine has been shown in either in vitro experiments or in vivo animals as given exogenous taurine. Efficacy of taurine administration was reported on patients with epilepsy either in positive (Airaksinen, 1980) or in negative (Durelli, 1983) from clinic. Although taurine possess some mild anticonvulsant activity in both humans and experimental animal models during its application started forty years ago (Anyanwu, 1993), the use of taurine as a drug to treat epilepsy is still limited, which may due to its limitation to cross the blood brain barrier. However, a recent report in Nature Genetics casts a new light on taurine and epilepsy: Treatment of succinate semialdehyde dehydrogenase deficiency mice with taurine could rescue the mutant mice, which display ataxia and develop generalized seizures leading to rapid death at postnatal day 16-22 (Hogema, 2001).

Acupuncture is commonly practiced to inhibit epilepsy in present Chinese clinic. The first known document on epilepsy and acupuncture in ancient China appeared in 'Smart Pathway, Ling Su, Chapter Epilepsy and Madness' of 'The Yellow Emperor's
Classic of Internal Medicine, Huang Di Nei Jing', written by a group of physicians around 770-221 B.C. 'epileptic seizure, is from blockage of acupoint channels'. 'Over Yin leads to epilepsy and over Yang leads to madness.' The treatment of acupuncture on epilepsy is believed to make Yin and Yang balance and quench convulsion by making acupoint channels through in traditional Chinese medicine. The finding of synergistic anti-epileptic effect of EA and taurine in this work implicated taurine may be a potential biological basis of EA in additional to GABA, glutamate, endo-opioid and nitric oxide. Previous reports have also detected that EA could modulate the concentration of taurine during kainic acid-induced or penicillin-induced seizure (Shu, 2004). Experiments of taurine depletion using beta-alanine will be performed in the next step to investigate the effect of EA on epileptic seizure at deficiency of taurine for further elucidating the linkage between EA and taurine.

The demonstration of enhanced taurine transporter level by EA in our data also suggested preliminarily that taurine may contribute to EA anti-convulsion during epilepsy, especially the changes of taurine transporter level present in rat hippocampus and cortex, which regions are critical to epileptic seizure. Taurine transporter in plasma membrane controls cellular concentration of taurine at a ratio of 100-50 000:1 between inside and outside of cells with taurine biosynthetic enzymes cysteine dioxygenase and cysteine sulfinate decarboxylase, which is the foundation of many biological effects of taurine (Tappaz, 2004). Taurine transporter in blood brain barrier could even transport taurine from blood to cerebrospinal fluid. Previous study in our lab has showed that taurine level increased after EA on experimental epileptic animals (Wang, 1994), that is consistent with the change of taurine transporter in the present work. Altered expression of the cortical and hippocampal taurine transporter induced by EA may result in increased intracellular taurine content, which in turn may lead to neuronal protection, less of cell death and, ultimately, seizure improvement.

CONCLUSION

In conclusion, epileptic discharges induced by microinjection of penicillin in rat hippocampus could be suppressed by exogenous taurine at proper dose or/and EA treatment on Du channel. Both taurine and EA had an anti-epileptic effect on seizure and they interacted in a synergistic manner. Investigation of enhanced level of taurine transporter by EA during epilepsy provided evidence that the pathway between taurine and EA may be through taurine transporter.

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REFERENCES


14. Renteria R.C., Johnson J., Copenhagen D.R. Need rods? Get glycine receptors


