The Effects of Ear-Point Stimulation on the Contents of Somatostatin and Amino Acid Neurotransmitters in Brain of Rat with Experimental Seizure

Jia Shu, M.D., Lecturer, Department of Anatomy
Rong-Yu Liu, M.D., Lecturer, Department of Neurobiology
Xian-Fen Huang, M.D., Professor, Department of Neurobiology
Institute of Acupuncture Research
WHO Collaborating Center for Traditional Medicine
Fudan University Shanghai Medical College (Formerly Shanghai Medical University), Shanghai 200032, P.R China

Correspondence: Xian-Fen Huang, M.D., Department of Neurobiology, Fudan University Shanghai Medical College, 138 Yi Xue Yuan Road, Shanghai 200032, P.R. China
Tel: 86-21-54237611. Email: wmzhang8@sh.cnuninet.net

(Received April 22, 2004; Accepted with revisions April 26, 2004)

ABSTRACT:
The goal of this study was to elucidate the anti-convulsion mechanisms of ear-point stimulation in rat with experimental seizure. We prepared the epilepsy rats by intrahippocampal injection of penicillin. One hour later the lower 1/2 auricular lobules of seizure rats, containing ear-points Pizhixia and Shenmen etc., was electrically stimulated, which was imitated as ear-point electrical acupuncture in humans. Radioimmunoassay and biochemical techniques were used to determine the contents of somatostatin and amino acid neurotransmitters in hippocampus of rats. The outcomes revealed epileptiform behaviors of rat were appeared after penicillin-injected. The contents of somatostatin, aspartic acid, glutamine and GABA were increased. When these rats were subsequently given the ear-point electrical stimulation, the convulsion behaviors were definitely improved. At the same time the contents of somatostatin, aspartic acid and glutamine in hippocampus of seizure rat were significantly decreased correspondingly. The contents of glycine, taurine and GABA had increased. Based on the results above, it was suggestive that ear-point electrical stimulation had anti-epilepsy effects, which might be involved in the
decreases of the contents of the somatostatin, aspartic acid and glutamine, and increases of the contents of glycine, taurine and GABA in hippocampus of seizure rat.

KEY WORDS: Penicillin-Induced Seizure Rat; Ear-Point Electrical Stimulation; Somatostatin; Amino Acid Neurotransmitters; HPLC System; Push-Pull Perfusion

INTRODUCTION

Temporal lobe epilepsy is the most common type of medically intractable epilepsy in human, which is frequently resistant to anticonvulsant medication. The pathological mechanism of epilepsy had not been understood completely up to now. Some studies in epileptics indicated that it was commonly associated with characteristic neuropathological abnormalities in forebrain, particularly true in the hippocampal dentate gyrus. The contents of various neuropeptides and amino acidic neurotransmitters in the hippocampus neurons are responsive to changes in seizure activity. Somatostatin (somatotropin release-inhibiting factor, SRIF) is one of the neuropeptides in the central nervous system, and relative with the epilepsy. However, what did the central functions of somatostatin on seizures is still not determined. Acupuncture ear-points, one of Chinese traditional medicine, had been used to treating diseases for a long time, which had the certain curative effects with minimal or no adverse events. Some ear-points such as Pizhixia and Nao, etc., lying at the lower third of the auricular lobe innervated by anterior branch of great auricular nerve, were often employed in treatment for epilepsy. Experimental model of epilepsy induced by penicillin-injected had some characteristics of steady and virtual seizure symptoms. Which were easy to be observed in study. We had previously observed the epileptiform waves recorded in sensorimotor cortex and behaviors of epilepsy rats were distinctively repressed by ear-point or great auricular nerve electrical stimulation (in press). In this study, we focus on the content changes of both somatostatin and amino acidic neurotransmitters in hippocampus by electrical stimulating ear-points of rats with experimental seizure.

MATERIALS AND METHODS

Experimental model of epilepsy

Adult male wistar albino rats, weighting between 150～200 g, were used in the experiment. The rats were anaesthetized with an intraperitoneal (i.p.) dose of 40 mg / kg sodium pentobarbitone. One injecting tube was inserted in the unilateral hippocampus of rat according to Konig and Klippel’s atlas (1963). Indices of hippocampus site were P 3.3, L / R 2.0, H 3.7. Diameter of outside tube was 0.8 mm, inside tube 0.4 mm. When the rats awake, 200 u / μl sodium penicillin was slowly injected through the microinjection into hippocampus in 5 minutes (see Fig. 1). The elicited epileptiform behaviors of rats were assigned to five classes according to Racine. The epileptiform behaviors of rats ranging from class IV to V were selected as experimental model of epilepsy.
Ear-point stimulation

Bilateral lower 1/2 of rat auricles, being equal to human ear containing ear-points Pizhixia, Shenmen, Zeng and Nao, etc., were clipped by froggy-heart electrodes besmeared electrode grease, which was imitated as ear-point electrical acupuncture in humans. Electrode ends were connected with electrical therapeutic apparatus (type G6805-H, Shanghai Medical Equipment Inc, China). Stimulating parameters were as follows: continuous wave with electric current about 0.2 milli-ampere, pulse width generally 0.6 millisecond, frequency 80 Hz. 60 minutes after rat treated with penicillin-injected, twice of electrical stimulation were totally submitted in the experiment. Each went on 30 minutes and the interval between two stimulations was also 30 minutes (see Fig. 1, 2, 3).

Quantity measurement of somatostatin and amino acidic neurotransmitters

Sample collections: The outer tube of push-pull perfusion was implanted in unilateral hippocampus opposite to the injecting tube. 30 ml artificial cerebral-spinal fluid was infused into hippocampus of rats through the inner tube of perfusion in the flow speed of 0.075 ml / min. 20 minutes, 180 minutes, 240 and 300 minutes after rat treated with penicillin-injected, 750 μl sample was obtained from collected tube every time. 400 μl of which was boiled 10 minutes for measuring content of somatostatin; 350 μl, for amino acid (see Fig. 1, 3, 4).

Fig. 1. Schematic diagram of experimental rat
Perfusion pump for infusing artificial cerebral-spinal fluid warmed in 37°C water into hippocampus, collecting sample passing through 4°C water from hippocampus. Stimulator for ear-point stimulation with electrodes on rat ears.
Fig. 2. Schematic diagram of typical waveform induced by electrical stimulator. Continuous wave with electric current about 0.2 milliampere; pulse width 0.6 millisecond; frequency 80 Hz. Horizontal axis is millisecond (ms) and vertical axis milliampere (mA).

Fig. 3. Schematic diagram of push-pull perfusion in hippocampus of experimental rat. In: infusing artificial cerebral-spinal fluid; Out: collecting sample; •: The needle points of push-pull perfusions in hippocampus in the experiment.

**Quantity examination of somatostatin:** The content of somatostatin was determined by radioimmunoassay (RIA). The concentration of $^{125}$I-somatostatin was 7000cpm / 100ul and that of anti-somatostatin was 1:1500. 400ul samples were of frost and aridity, mixed together with 300ul PELH dilution and 100ul anti-somatostatin serum, incubated in 4 °C for 24 hours, then adding in 100ul $^{125}$I-somatostatin and hatched for 24 hours. The combinative antigen was separated using activated charcoal. The difference value between total radiograph count and sediments radio count in each tube was the connected value.
Collecting sample from hippocampus for measuring the content of somatostatin and amino acids; Injecting penicillin in hippocampus; Electrical stimulation of ear-points.

Quantity examination of amino acids neurotransmitters: The contents of amino acid neurotransmitters were determined using the HPLC system and type 157-fluorescence measure. The peak values of measured samples were compared with standard curve. Area integral was analysed with chromatogram control system (Gold System) and calculated by outer standard according to standard curve.

Statistical analysis
Results were expressed as the M ± S.E.M. Differences among experimental groups were analyzed using the Student's t-test. Compared with those obtained from the normal or seizure group, P< 0.05 was considered statistical significant.

RESULTS

The effects of ear-point electrical stimulation on behaviors of seizure rats
A half-hour after penicillin-injected, over 95 percent of rats appeared a phenomenon of myoclonic seizure-like episodes. One hour later the convulsive actions of tonic-clonic nature ranged from class IV to V and persisted over 5 hours. When electrical stimulating the ear-points of these rats, the number of behavioral tonic-clonic seizures were decreased. The inhibitory role was enhanced as long as the time protraction of electrical stimulation. This indicated that ear-point stimulation could certainly ameliorate the epileptiform behaviors of seizure rats.

The influences of ear-point electrical stimulation on somatostatin content
Somatostatin in perfusate of hippocampus was collected, a half-hour before and after rat penicillin-injected, and 0.5, 1.5, 2.5 hours after seizure rats given the secondary ear-point stimulation. There was a marked decrease of somatostatin contents in hippocampus of rats with ear-point stimulation compared with that of rats with penicillin-injected in the same time. That meant the anti-epilepsy role of ear-point electrical stimulation was dealt with the decrease of somatostatin contents in hippocampus (see Table).
Table The variety of somatostatin contents in hippocampus of seizure rats treated with ear-point stimulation (mean ± S.E., n=8)

<table>
<thead>
<tr>
<th></th>
<th>Before PIS</th>
<th>0.5 h after PIS</th>
<th>3 h after PIS</th>
<th>4 h after PIS</th>
<th>5 h after PIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PISg</td>
<td>72.13±5.07</td>
<td>147.56±7.63</td>
<td>224.74±11.56</td>
<td>108.74±7.58</td>
<td>75.93±7.57</td>
</tr>
<tr>
<td>ESG</td>
<td>75.56±6.94</td>
<td>169.8±15.31</td>
<td>72.18±7.09**</td>
<td>50.78±6.64*</td>
<td>60.75±7.80</td>
</tr>
</tbody>
</table>

Somatostatin content (pg /ml). PISg, penicillin-induced seizure group; ESG, ear-point stimulation group, * P< 0.05, ** P<0.01 vs the result of the PIS group.

The influences of ear-point stimulation on concentration of amino acid neurotransmitters

0.5, 4 hour after penicillin-injected (here we chose to measure 4 hours later for hereinafter compared with the group of ear-point electrical stimulation), the concentrations of amino acid neurotransmitters were selectively changed in hippocampus compared with normal rats (Control group). The contents of aspartic acid and glutamine were more increased with a statistically significance. The contents of glycine, taurine and GABA were variety, but had no difference in statistics. Compared with the seizure rats 4 hour after penicillin-injected (the time is equal to one and a half hours after secondary ear-point electrical stimulation), the contents of aspartic acid and glutamine were significantly reduced in hippocampus of rats treated with ear-point electrical stimulation. The contents of glycine, taurine and GABA had increased with a difference in statistics (see Fig. 5).

Fig. 5 The influences of ear-point stimulation on contents of amino acid neurotransmitters in hippocampus of seizure rats (mean ± S.E., n=7)
Asp, aspartic acid; Glu, glutamine; Gly, glycine; Tau, taurine; GABA, gamma-amino butyric acid; PISg, penicillin induced seizure group; ESg, ear-point stimulation group. *: P<0.05 vs. the contents in hippocampus of normal rats. Q: P<0.05 vs. the contents in hippocampus of PIS group in 4 hours.

DISCUSSION

We have previously investigated that the ear-point electrical stimulation had a remission of seizures, whereas these effects of anti-seizure were blocked after cutting the great auricular nerve off [in press]. It had been known there was an interneuronal dysfunction in the hippocampal dentate gyrus of patients or models with epilepsy. The inhibitory interneurons were decreased and excitatory interneurons increased [2-3]. It was known that somatostatin was involved in the epilepsy activity. What a role did the somatostatin play in epilepsy? Some studies showed the effects of somatostatin were predominantly excitatory, but they varied and were dose-dependent. Excessive somatostatin in hippocampus induced epilepsy and hippocampal CA1 area neuronic excitability [19]. A study in vitro indicated application of exogenous somatostatin are consistent with the concept that somatostatin acts as an excitatory neurotransmitter / neuromodulator in hippocampus [19]. There had significantly changes in the hippocampal somatostatin system in experimental models of epilepsy. Somatostatin biosynthesis and release are increased in the kindled rat hippocampus. In contrast, the hippocampal somatostatin system undergoes damage in the dentate gyrus following experimental epileptics [6,11]. In situ hybridization study showed there had a higher expression of somatostatin mRNA in granule cell layer and polymorphical layer of rat hippocampus with penicillin-induced seizure compared with normal rat [12]. Electrical acupuncture decreased epileptiform discharge in cortex and suppressed the epileptiform behaviors of rat. What did the relationship between ear-point stimulation and somatostatin during anti-seizure? It had understood cysteamine could lower the somatostatin content in brain and inhibit seizure of rats [11]. In our experiment, the content of somatostatin in the hippocampus of rats treated with ear-point electrical stimulation was definitely reduced, accompanied with an improvement of myoclonic seizure-like episodes. This revealed there was a positive relationship between onset degree of seizure and somatostatin content in hippocampus. The seizure-related changes could be controlled by reducing somatostatin content in brain. We considered it was substantial evidence that somatostatin in hippocampus may be one substance of inducing epilepsy.

Excitotoxicity, resulting from the excessive release of excitatory amino acids, such as glutamate, is thought to contribute to a variety of neurological disorders, including epilepsy [13]. The results in our experiment showed that the contents of excitatory amino acids aspartic acid and glutamine were higher, inhibitory amino acids GABA, also higher in hippocampus of rat with penicillin-induced seizure, comparing to normal rats. Ear-point electrical stimulation could make a significantly lower release of aspartic acid and glutamine, and higher releases of glycine, taurine and GABA in rat hippocampus. Taurine and GABA had the function of anti-seizure. It was reported these two kinds of amino acids were positively correlated with epilepsy. Some neuroanatomical and neurochemical results had provided evidences that a large proportion of asp-,
glutamatergic-, somatostatin-immunopositive interneurons colocalized in brain \cite{14}. In addition, GABAergic neurons contain somatostatin-like immunoreactivity. Somatostatin and GABA are co-localized in some neurons in the CA1 area of the hippocampus, co-released and interactions \cite{15}, which meant there might have some functional relationship between amino acid neurotransmitters and somatostatin in brain. Someone discovered that somatostatin had a potent inhibitory effect on the function of GABAergic basket cells and GABA-mediated synaptic potentials which hyperpolarize pyramidal cells, and GABA could repress the somatostatin releases \cite{16,17}, meaning there were interaction between GABA and somatostatin. The increase of somatostatin release might induce a release increase of GABA for self-protection. Penicillin is a competitive antagonist at the receptor with GABA. Therefore, the increase of GABA in hippocampus of rats with penicillin-induced epilepsy did not suppress the onset of the epilepsy.

In conclusion, these findings demonstrated that the ear-point electrical stimulation appears to be a potentially effective treatment for epilepsy. The possible mechanisms of ear-point electrical stimulation against seizures were due to suppressing the releases of somatostatin and excitatory amino acid neurotransmitters aspartic acid and glutamine, promoting releases of inhibitory amino acids neurotransmitters glycine, taurine and GABA, modulating the firing rate of granule cells in hippocampus. The anticonvulsant effects of ear-point electrical acupuncture were certainly potent and persistent, and the operation was simple and easy. Therefore, if the ear-point electrical acupuncture was applied together with medicine in clinic, the therapeutic efficacy against generalized tonic-clonic and, to some extent, medically partial intractable epilepsy is probably much more affirmative. We will continually attempt to investigate it in future.

**REFERENCES**


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