The Anticonvulsant Effect of Transcutaneous Auricular Vagus Nerve Stimulation is Associated with Balancing the Autonomic Dysfunction in Rats

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ABSTRACT

Objective: The present study aims to investigate whether the anticonvulsant effect of transcutaneous auricular vagus nerve stimulation is associated with balancing the autonomic dysfunction in rats.

Methods: Healthy adult male Sprague-Dawley rats were anesthetized with an intraperitoneal injection of 10% urethane. Seizures were evoked by intraperitoneal injection of pentylenetetrazol (PTZ, 60 mg/kg). Femoral vein catheterization was performed for injection of sympathetic agonist and antagonists. Bipolar globe silver electrodes were utilized for epidural EEG recording. Three needles were inserted separately in subcutaneous muscles of left anterior limb, right anterior limb, and left hind limb to record ECG signals. ta-VNS was performed at auricular concha.

Results: In comparison with preictal state, the mean heart rate (HR) increased slightly during epileptic seizures (P<0.05). In comparison with ictal state, the mean HR decreased a little at postictal state (P<0.05). When continuous epileptic seizures in EEG traces occurred (in ictal state), vein injection of propranolol hydrochloride (sympathetic antagonist) suppressed the epileptic seizures. When epileptic seizures occurred rarely (in postictal state), vein injection of adrenaline hydrochloride (sympathetic agonist) exacerbated the epileptic seizures. In comparison with pre-stimulation, the integral of EEG traces after ta-VNS decreased (P<0.05), the mean HR decreased (P<0.05), and the high power (HF) of HRV increased (P<0.05) after ta-VNS.

Conclusion: The results showed that autonomic dysfunction occurred in epileptic rats characterized by enhanced sympathetic nerve activity. Epileptic seizures in EEG traces decreased, HR decreased and HF increased after ta-VNS, which indicated that ta-VNS may suppress epileptic seizures via balancing the autonomic dysfunction.

Key words: Transcutaneous auricular vagus nerve stimulation, Epilepsy, Heart rate variability, EEG

Abbreviations: SUDEP: sudden unexpected death; HRV: Heart rate variability; HR: heart rate; EEG: electroencephalograph; ECG: electrocardiograph; LF: low-frequency; HF: high-frequency; VNS: vagus nerve stimulation; ta-VNS: transcutaneous auricular vagus nerve stimulation; NTS: nucleus tractus solitaries; UCMS: unpredictable chronic mild stress.

INTRODUCTION

Epilepsy is a disease due to abnormal excessive or synchronous neuronal activity in the brain. Seizures often cause impaired breathing, cardiac dysfunction, and loss of consciousness. It has been reported that epilepsy is associated with autonomic dysfunctions[1-3]. And, seizures alter the function of different autonomic systems such as the respiratory, gastrointestinal, urogenital, and, cardiovascular system[2]. For example, higher sympathetic tone and vagal tone imbalance may induce early autonomic dysfunction and increase cardiovascular risk in patients affected by Temporal Lobe Epilepsy[3]. Seizure-induced cardiac and respiratory arrests were involved in sudden unexpected death (SUDEP) in epilepsy, and interictal autonomic dysfunction was sought to serve as a marker for patients at elevated risk for SUDEP[4].

Heart rate variability (HRV) is a reliable reflection of many physiological factors modulating the normal rhythm of the heart, which provide a powerful means of observing the interplay between the sympathetic and parasympathetic nervous systems. Hence, HRV analysis has become a popular noninvasive tool for assessing the activities of the autonomic nervous system[5]. Clinically, it has been observed that heart rate (HR) increased in all 29 right seizures and 42 of 48 left seizures. Onset time of HR increase in relation to ictal electroencephalograph (EEG) onset was significantly earlier in right seizures than in left seizures. Time of maximum HR was also significantly earlier in right seizures than in left seizures[6]. In another clinical study, using video-EEG-electrocardiograph (ECG) recordings, the changes in the HR of 81 patients during a total of 181 seizures were retrospectively assessed, in which HR increased rapidly within 10 seconds before seizure onset and ictus, and typically slowed to normal with seizure offset[7]. A systematic review and meta-analysis of HRV in epilepsy showed that epilepsy patients presented lower HRV than controls[8]. Sympathetic and parasympathetic
activity of HRV were assessed by low-frequency (LF) and high-frequency (HF) power spectrum, respectively. By using Video - EEG - ECG monitoring system, it was found that the values of mean HR, LF and LF/HF were greater in onset period of epilepsy in children[9].

As an alternative approach to cervical vagal nerve stimulation (VNS), auricular vagus nerve stimulation has been utilized as a complementary method for the treatment of epilepsy, including electric auricular stimulation[10], auricular acupuncture[11] and transcutaneous auricular vagus nerve stimulation (ta-VNS)[12-13]. In healthy participants, it has been observed that ta-VNS significantly increased HRV, manifested as increased parasympathetic nerve flow and reduced sympathetic nerve outflow[14]. Yet, whether ta-VNS plays a role in seizure suppression via modulating the autonomic dysfunction remains unclear.

The present study aims to investigate the relationship between the epileptic seizures and HRV, and the effect of ta-VNS on epileptic seizures, in order to provide further evidence for the anti-seizure effect of ta-VNS.

MATERIALS AND METHODS

Animals
Healthy adult male Sprague-Dawley rats (weight: 260 – 300 g) were kept in the animal house with a 12-hour light-dark cycle and free access to food and water for seven days before the experiment. Seizures were evoked by intraperitoneal injection of pentylenetetrazol (60 mg/kg; Sigma-Aldrich, U.S.A.). The rat was initially anesthetized with an intraperitoneal injection of 10% urethane (1.2 g/kg; Sigma-Aldrich, USA). Additional intraperitoneal injection of 10% urethane (0.3 g/kg) was administered if necessary to prolong the anesthetic state. Rats were placed on a heated surgical table to maintain rectal temperature at 37±1°C. Animals were sacrificed by an overdose of anesthetics after the study. The study was approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences, and was in accordance with National Institutes of Health guidelines.

Femoral vein injection of sympathetic agonists and antagonists
In supine position, an incision was made in the inguinal area (approximately 12 mm) along the natural angle of the hind leg. The connective tissue was separated until the femoral vein was exposed. Firstly, the distal femoral vein was ligated, and the proximal femoral vein was clamped by a bulldog clamp. Sympathetic agonists, adrenaline hydrochloride (10 μg/kg; Sigma-Aldrich, USA) and antagonists, propranolol hydrochloride (0.1 mg/kg; Sigma-Aldrich, USA) were extracted into two syringes. A polyethylene catheter full with saline was connected with the two syringes by a three-limb tube for drug delivery. Then the PE catheter was catheterized centripetally into the femoral vein and fixed. Finally, the incision was purse-string sutured.

EEG recording
After femoral vein catheterization, the head of the rat was fixed on the stereotaxic apparatus in prone position. After craniotomies, bipolar globe silver electrodes (diameter of 1 mm) were placed over the dura in two holes (distance to the bregma, AP: ±1.0 mm, ML: 2.0 mm) respectively for epidural EEG recording. EEG signals were amplified by Amplifier AM 1800 (A-M systems, Sequim, USA) with low frequency filter at 1 Hz and high frequency filter at 100 Hz. EEG signals were captured online and analyzed offline using the PowerLab data recording & analysis system (AD Instruments, Australia).

ECG recording
To collect ECG data in rats, three acupuncture needles (0.35*25mm, Hwato, China) were inserted separately in subcutaneous muscles of left anterior limb, right anterior limb, and left hind limb. Then the Acupuncture needles were connected to an amplifier (Amplifier AM 1800 (A-M systems, Sequim, USA) using of electrode wires. ECG signals were filtered and amplified by the amplifier, and were also captured online and analyzed offline using the PowerLab data recording & analysis system (AD Instruments, Australia). The simultaneous recording of EEG and ECG signals were shown in Figure 1.

Figure 1. Simultaneous recording of EEG and ECG signals. The upper lane marked with red color were EEG signals, the lower lane marked with blue color were ECG signals.
ta-VNS
Bipolar silver slice electrodes (diameter of 1.5 mm) being attached to the skin with adhesive tape were introduced for ta-VNS. The cathode of the electrodes was placed at the junction of cavity of the auricular concha and postero-inferior wall of the external acoustic meatus, the anode was placed at the cymba of auricular concha. For stimulations, the electrode leads were connected with stimulator (SEN-7203 Nihon Kohden, Japan). For electric stimulations, the acupuncture needles were connected with stimulator (SEN-7203 Nihon Kohden, Japan). Stimulation parameters were selected as: frequency, 20 Hz; pulse width, 0.5 ms; strength, 1.0 mA, duration: 5 min.

Data analysis
In EEG tracings, a seizure was shown as a highly synchronous and large-amplitude activity at least three times the amplitude of baseline. The integral of EEG traces was calculated in terms of area above baseline. HRV analysis was derived from ECG recording using a Power Lab software (HRV module for Chart5, AD Instruments, Colorado Springs, CO, USA). The mean HR and HRV were evaluated. Data were analyzed with SPSS 16.0 program (SPSS Inc. Chicago, IL). Values are presented as mean ± SEM. Kolmogorov-Smirnov of test was used to evaluate if groups fit normal distributions. Normally distributed groups were analyzed by parametric tests. Paired-T test were performed to compare data in the same group. One-way ANOVA with SNK post hoc test were performed to compare data between groups. P < 0.05 was considered significant.

RESULTS
1. Heart rate increased when epileptic seizures occurred after intraperitoneal injection PTZ
About 5 minutes after intraperitoneal injection pentylentetrazol, epileptic seizures occurred, which manifested as synchronized, high-amplitude traces in EEG. In comparison with preictal state, the mean heart rate (HR) increased during epileptic seizures (354.96±1.7 bpm vs 355.85±1.68 bpm, P<0.05). In comparison with ictal state, the mean HR decreased at postictal state (355.85±1.68 bpm vs 354.82±1.62 bpm, P<0.05). There is no difference of the mean HR between preictal state and postictal state (354.96±1.7 bpm vs 354.82±1.62 bpm, P>0.05).

2. Effect of vein injection of adrenaline hydrochloride and propranolol on epileptic seizures in EEG traces
As shown in Figure 2, the upper two lanes showed the changes of EEG traces after vein injection of propranolol hydrochloride (sympathetic antagonist). As manifested, when continuous epileptic seizures occurred (that is, in ictal state), vein injection of propranolol hydrochloride suppressed the epileptic seizures. The lower two lanes showed the changes of EEG traces after vein injection of adrenaline hydrochloride (sympathetic agonist). When epileptic seizures in EEG traces occurred rarely (that is, in postictal state), vein injection of adrenaline hydrochloride exacerbated the epileptic seizures. Yet EEG traces did not show obvious change when vein injection of adrenaline hydrochloride in ictal state or vein injection of propranolol hydrochloride in postictal state (data not shown).

3. ta-VNS suppressed epileptic seizures via balancing the autonomic dysfunction
In comparison with pre-stimulation, the integral of EEG traces after ta-VNS decreased from 222.79±7.75 V.s to 196.76 ±5.57 V.s (P<0.05) (Figure 3). In the meantime, we observed effect of ta-VNS on the HR and LF of the HRV in the rats. In comparison with pre-stimulation, the mean HR after ta-VNS decreased from 307.77±18.28 bpm to 291.43±15.95 bpm (P<0.05) (Figure 4). In comparison with pre-stimulation, the HF after ta-VNS increased from 1.45±0.77 ms² to 2.09±0.99 ms² (P<0.05) (Figure 5).

DISCUSSION
The results of the present study showed that autonomic dysfunction occurred in epileptic rats characterized by enhanced sympathetic nerve activity and HR. The integral of EEG traces decreased, HR decreased and HF increased after ta-VNS, which indicated that ta-VNS may suppress epileptic seizures via balancing the autonomic dysfunction.
Epilepsy is associated with near-fatal and fatal arrhythmias, and dysfunction of the autonomous nervous system causes arrhythmias. Recently it is newly proposed that HRV parameter changing prior to seizures could be used to predict future seizures. In children with epilepsy, HRV parameters such as the LF, HF or LF/HF could have potential to predict the seizures. In another study, the examination of HRV parameters during and before seizures revealed higher nLF and LF/HF ratio and lower nHF values in children with refractory epilepsy, which demonstrated increased sympathetic activity. Then, the author speculated that pre-ictal time can be considered as prediction time for early detection of seizure onset based on HRV. The results showed that autonomic dysfunction occurred in epileptic rats characterized by enhanced sympathetic nerve outflow to visceral organs and leading to SUDEP.

The mechanism of auricular vagus nerve stimulation remains unclear. It has been proposed that the auricular-vagal afferent pathway mediates the anticonvulsant effect of auricular vagus nerve stimulation, in which both the autonomic function and the central nervous system could be modulated by auricular vagal stimulation via projections from the ABVN to the NTS. Recently, anatomical, imaging, electrophysiological studies confirmed the correlation between the auricular branch of vagus nerve (ABVN) and the nucleus tractus solitarius (NTS), which provides evidence for the role of the auricular-vagal afferent pathway. Electroacupuncture at auricular concha region can elicit cardioinhibitory effect as VNS on the depressive status of unpredictable chronic mild stress (UCMS) rat, and significantly antagonized UCMS-induced depressive status. Auricular electroacupuncture improves rectal distention-induced gastric dysrhythmias via the vagal pathway in rats. Several studies have observed the antiseizure effect of auricular acupuncture on epilepsy. In our previous study, we compared the effect of electroacupuncture of different regions of the auricle on epileptic behaviors in awake rats, which showed that electroacupuncture of auricular concha has a better effect in suppressing epileptic behaviors than electroacupuncture at other auricular area. It was speculated that auricular acupuncture may plays a role in autonomic functions by regulating vagal activity.

Cervical VNS has been approved by FDA as a complementary method for the treatment of epilepsy and depression. Recently, auricular VNS has been suggested as an alternative approach instead of VNS. And, clinical studies have reported the utilization of auricular vagus nerve stimulation for the treatment of depression, cardiac safety, pain, and Atrial Fibrillation. Yet, much work should be supported to evaluate the efficacy and safety of ta-VNS for these neuropsychiatric disorders.

CONCLUSION

The results showed that autonomic dysfunction occurred in epileptic rats characterized by enhanced sympathetic nerve
activity and HR. Epileptic seizures in EEG traces decreased, HR decreased and HF increased after ta-VNS, which indicated that ta-VNS may suppress epileptic seizures via balancing the autonomic dysfunction.

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS
BZ conceived the study. WH, XYW, HS, YSS, XHJ and BZ participated in the study and contributed to the animal experiment. WH drafted the manuscript, and BZ participate in the revision. All authors contributed to the further final writing of the manuscript and approved the final version.

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REFERENCES


