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ORIGINAL ARTICLE

Serum NT-pro CNP levels in epileptic seizure, psychogenic non-epileptic seizure, and healthy subjects

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Abstract

Purpose Epileptic seizure is the result of uncontrollable neural excitation in the brain. The C-type natriuretic peptide is a member of natriuretic peptide hormone family and is synthesized by brain and blood vessels in CNS. NT-pro CNP is an amino-terminal fragment of C-type natriuretic peptide and is more stable compared to its predecessor. In this study, we aimed to evaluate the role of NT-pro CNP in psychogenic non-epileptic seizures, epileptic seizures, and normal subjects.

Methods Thirty-three patients with epilepsy and 43 patients with psychogenic non-epileptic seizures were enrolled in this study. The control group consisted of 28 healthy subjects. Post-ictal serum levels of NT-pro CNP were acquired from all participants. Statistically significant differences between patient groups and controls regarding serum levels of NT-pro CNP were sought.

Results NT-pro CNP levels were significantly lower in the epilepsy group than the psychogenic non-epileptic seizure group and control group with no significant difference between the psychogenic non-epileptic seizure and control group ($p < 0.05$).

Conclusion Post-ictal serum NT-pro CNP levels were lower in epileptic seizures compared to psychogenic non-epileptic seizures as well as healthy controls. We think that such a difference is associated with C-type natriuretic peptide-related neural mechanisms such as altered microcirculation, increased brain-blood barrier permeability, and synaptic stabilization.

Keywords Epilepsy · Psychogenic non-epileptic seizure · NT-pro CNP · Biomarker

Introduction

Unlike an epileptic seizure (ES), which is the result of uncontrollable neural excitation in the brain, psychogenic non-epileptic seizure (PNES) is a psychological entity that lacks abnormal neuronal discharge [1]. Seizure semiology is helpful in distinguishing ES from other entities. Furthermore, neuroimaging studies and EEG offer complementary information especially for the classification of epilepsy [2]. Other methods such as video electroencephalography (V-EEG) can be used to differentiate ES from non-epileptic conditions [3]. V-EEG is the gold standard to discriminate epileptic from non-epileptic seizures. Such a discrimination is often possible with the detection of non-epileptic seizure activity during recording that yields negative results if the occurrence of the event is infrequent [4]. In epileptic seizures, the use of biomarkers helps establish a diagnosis, locate the responsible injury site, reveal the severity of the injury, and finally monitor the treatment response [5]. For this purpose, various biomarkers in serum and cerebrospinal fluid (CSF) have been investigated [6, 7]. C-type natriuretic peptide (CNP) is a member of the family of natriuretic peptide hormones. It is produced by various tissues, such as endothelial cells and bone cells [8]. In the central nervous system (CNS), CNP is synthesized in brain and blood vessels [9, 10]. The acting mechanism of CNP is local and it has a very low level in the circulation [11]. Furthermore, the plasma half-life of CNP is very short, being only about 2.6 min [12]. As a result, detection of CNP in plasma is challenging, and assays have been developed for the amino-terminal fragment of CNP [13]. The plasma half-life of NT-proCNP remains unknown [14].

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In this study, we aimed to evaluate the role of NT-proCNP in ES by determining and comparing the serum levels in ES and PNES patients, as well as in healthy controls.

Methods

This study was approved by our institutional ethics committee, and written informed consent was obtained from all subjects.

Patient selection

In this study, 133 patients that presented to our emergency department with seizures between January 2014 and October 2015 were initially evaluated. After applying the inclusion and exclusion criteria (Fig. 1), 33 consecutive epilepsy patients with proven diagnosis were selected for the ES group and 43 patients for the PNES group. Additionally, 28 healthy subjects were recruited as the control group.

V-EEG assessment

V-EEG studies were performed using a Nihon Kohden EEG system at 500 Hz (Nihon Kohden Corporation, Tokyo, Japan). Audiovisual recordings lasted 24 h with at least one provocation test being applied. The provocation test consisted of verbal suggestion followed by 2 ml saline injection. In addition to the V-EEG studies, all patients were evaluated according to the criteria specified by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV).

Immunoassay analysis

Fasting blood samples were collected from the control group. In the ES and PNES groups, the blood samples were taken within 3 h after the seizure event. The samples were first stored for coagulation and then centrifuged for 15 min at 4000×*g* at + 4 °C. The serum samples obtained were divided into aliquots and stored in a deep freezer at − 80 ° until analysis. The human NT-proCNP levels in the serum samples were measured using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) with a "Human NT-proCNP ELISA Kit" (Eastbiopharm, Lot: 20141023, China) following the manufacturer's instructions. The colored reaction product was measured using an automated ELISA reader at 450 nm. The results were expressed as picomol per liter (pmol/L).

Statistical analysis

The summary statistics of all groups were obtained by mean ± standard deviations. Data regarding the NT-proCNP levels were reported as medians and 95% CI for medians. Distribution of normality was assessed with the D'Agostino-Pearson test. Categorical variables were assessed with the chi-square test. Continuous variables with a non-normal distribution within the groups were compared using the Kruskal-Wallis test

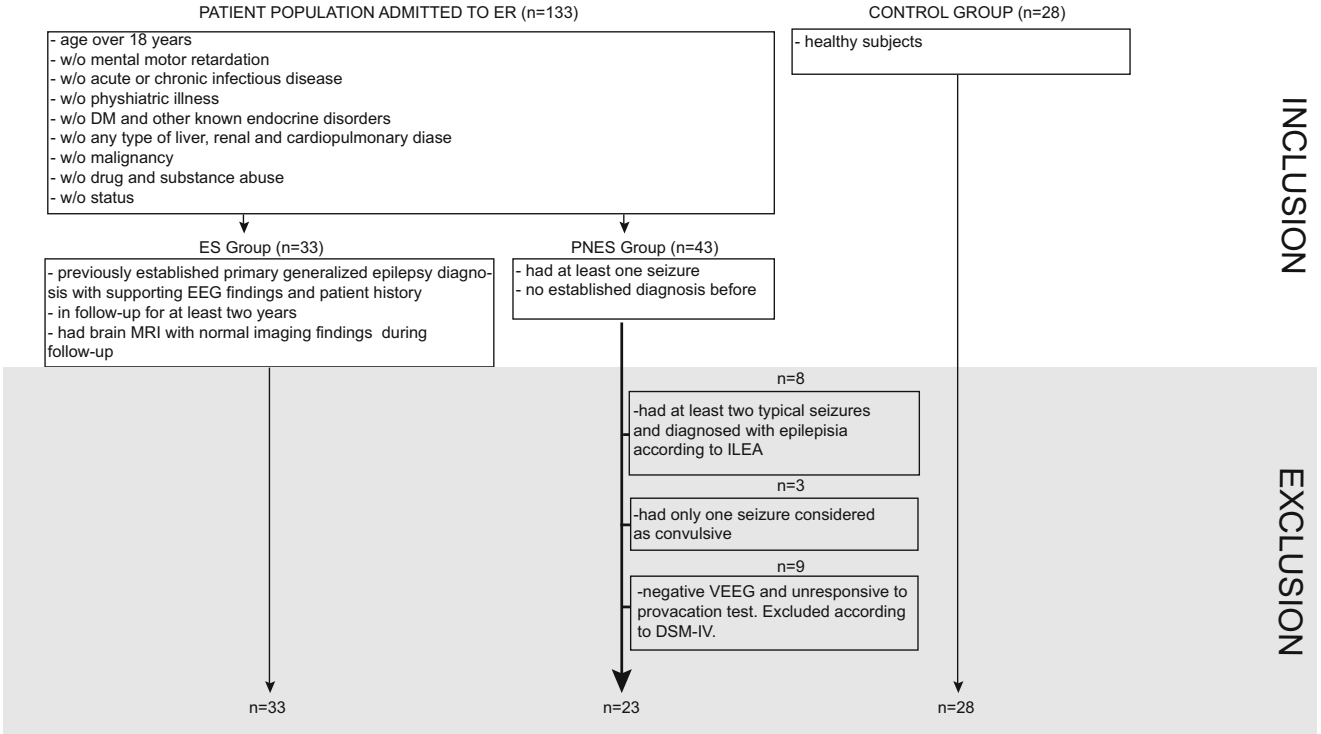

Fig. 1 Inclusion and exclusion criteria with the numbers of patients who met

whereas those with a normal distribution were assessed with one-way ANOVA. A two-tailed p value of < 0.05 was considered statistically significant. All statistical analyses were performed using MedCalc statistics software (MedCalc, version 12.2.1.0, Mariakerke, Belgium).

Results

The mean ages of the ES, PNES, and control groups were 35.6 ± 19.3, 34.7 ± 16.3, and 38.6 ± 9.6 years, respectively. The percentage of male participants in the three groups was 57.5%, 47.8%, and 42.9%, respectively. There was no statistically significant difference between the groups regarding age or gender. The mean disease duration of the ES group was 4.7 ± 1.2 years.

The three study groups were further divided into subgroups according to gender. The data of the serum NT-proCNP levels in all groups and the corresponding p values are shown in Table 1. This data is also presented as a box-and-whisker plot in Fig. 2. The NT-proCNP levels were significantly lower in the ES group than in the PNES and control groups with no significant difference between the latter two groups. The subgroup analysis showed no relationship between NT-proCNP levels and gender.

Discussion

In this study, we found that the serum NT-proCNP levels were significantly lower in ES patients compared to PNES patients and controls. This difference was not associated with gender.

Few studies in the literature aimed to establish the role of various biomarkers in ES. One of these biomarkers is prolactin, which was reported to be elevated in patients with generalized and complex partial seizures [15]. In another study, the authors found significantly high levels of HSP70, neuron-specific enolase (NSE), and S100B in epileptic patients with frequent seizures [16]. Furthermore, S100B and NSE were found to be associated with brain trauma-related epilepsy. Biomarkers such as glial fibrillary acidic protein and ubiquitin carboxyl-terminal hydrolase L1 were also elevated in status epilepticus [6, 7]. The presence of these biomarkers in the serum was the result of a common mechanism in many brain pathologies. A transient dysfunction of the blood-brain barrier may be responsible for the transportation of these biomarkers to the bloodstream [5].

The acting mechanism of CNP was identified as a receptor binding process by three receptors known as natriuretic peptide receptors (NPRs). NPR-B is located in the brain and veins and mainly binds CNP [17]. NPR-C is a clearance receptor and binds all natriuretic peptides [17]. Neutral endopeptidase (NEP) also catabolizes CNP via hydrolysis [18]. In contrast to CNP, NT-proCNP is not hydrolyzed by NEP or NPR-C; it is the main tracer of CNP in plasma and CNS [15]. It is worth mentioning that the levels of CNP in plasma and CSF differ, and CNP is abundantly present in the latter. Furthermore, it was reported that the concentrations of CNP in CSF and plasma were inversely related. However, the transfer mechanism of CNP from CNS to plasma remains controversial [13]. Nevertheless, positive arteriovenous gradients of CNP forms across the head and neck have been shown in human and animal studies [19, 20].

The association of epilepsy with altered brain microcirculation has been investigated for decades. In the past, SPECT imaging was used to assess the ictal and postictal perfusion changes in the brain [21]. Recently, advanced MRI techniques such as arterial spin labeling are used to demonstrate changes in cerebral perfusion after epileptic seizures [22]. CNP possibly alters the cerebral (micro) circulation based on different mechanisms, e.g., (i) secretion from cerebral capillaries might be an indication of a vasodilator action [23], (ii) the presence of CNP and receptors in the choroid plexus might be an indication of the indirect effect on circulation via the alteration of the CSF volume [14], and (iii) CNP might increase the blood-brain barrier permeability according to the results of animal studies [24]. In addition to its connection with cerebral circulation, CNP was also found to be associated with neurogenesis [25], synaptic plasticity [26], and axonal branching [27]. Among these, the association with synaptic plasticity was also confirmed by our findings. In another study, the authors found CNP-reduced action potential in the CA1 cells of the hippocampus. The CNP

Table 1 Concentrations of NT-proCNP in epilepsy, PNES, and control group

	Epilepsy			PNES			Controls		
	No	Median pmol/L	95% CI for median	No	Median pmol/L	95% CI for median	No	Median pmol/L	95% CI for median
Total	33	24.1*	22.3–26.7	23	27.9	23.2–45.1	28	27.8	24.5–36.9
Gender									
Male	22	24.3	20.5–26.6	10	26.9	23.3–50.1	10	25.8	21.7–38.8
Female	11	23.9	21.9–28.2	13	29.3	20.6–57.3	18	29.2	24.1–50.3

PNES, psychogenic non-epileptic seizure

*$p = 0.0458$ epilepsy vs. PNES and control group

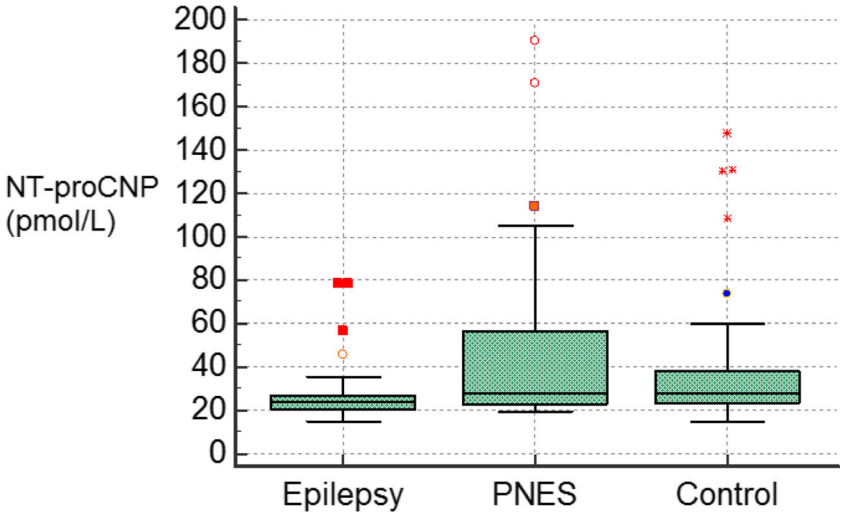

Fig. 2 The box plot of the concentrations of NT-proCNP in epilepsy, PNES, and control groups

bathing of the specimens also increased the excitatory postsynaptic potentials of those cells [25]. Considering the role of CNP in synaptic stabilization and the reduced serum levels of NT-proCNP in ES patients, we suggest that there might be a cause and effect relationship between CNP and seizure.

Our findings indicate that CNP can be used as a neurostabilizer agent against epileptic seizure and targeted by antiepileptic drug therapy. If seizures emerge while CNP levels are low, sustaining a pre-determined blood or CSF level of CNP might protect the patients from epileptic attacks.

Some limitations of this study should be noted. First, the study population was relatively small. Second, we only evaluated primary generalized epilepsy in the seizure group. Thus, our results should be verified by studies conducted with patients having secondary and partial seizures. Third, we did not stratify the serum NT-proCNP levels according to the ictal duration of seizures. Apart from status epilepticus, the serum levels of NT-proCNP could differ in patients with different seizure durations. Furthermore, we measured NT-proCNP levels at a single time point, which may have negatively affected the reliability of the results. Lastly, we did not record the exact timing of the blood test, especially in the ES group because of the patients undergoing a seizure at that moment. For this reason, the times of drawing blood from the ES group might be later than the time the blood samples were obtained from the PNES and control groups.

Conclusion

In patients with primary generalized epileptic seizures, the postictal serum NT-proCNP levels were lower than those with PNES and healthy controls. We postulate that this decrease is associated with CNP-related neural mechanisms, such as altered microcirculation, increased blood-brain barrier permeability, and synaptic stabilization.

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