RBC 2012

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Regional Biophysics Conference 2012
Kladovo-Belgrade, Serbia
September 03-07, 2012

PROCEEDINGS

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CYCLIC VOLTAMMETRY IN DIAGNOSIS OF ALS

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Abstract

The diagnosis of amyotrophic lateral sclerosis (ALS) based on ALS functional rating scale-revised (ALSFRS-R) scores is often shown to be unreliable and false. On the other hand, magnetic resonance imaging (MRI) studies of a brain of ALS patients showed the presence of iron deposits in the precentral gyruses of gray matter (PGGM) and also the potential leakage of iron to cerebro-spinal fluid (CSF) through compromised blood-brain barrier. The aim of this study was to determine if there is a specific feature in CSF that distinguishes patients with ALS from the control subjects. CSF obtained from ALS patients and normal controls were analyzed using the technique of cyclic voltammetry. The results show that, at the potential of 1.1 - 1.2 V vs. Ag/AgCl electrode, the plateau appeared and the potential of oxygen evolution was shifted toward more positive values for the ALS patients. These voltammogram features were not present for the control patients showing this technique to be promising candidate for evaluating new biomarkers for ALS.

Introduction

ALS is neurodegenerative disease which includes progressive degeneration of the motor neurons leading to weakness and atrophy. Although it is far from being curable [1], ALS is treatable, but reliable biomarkers are needed to follow up effects of potential drugs. Developing of ALS is most probably linked to the cell malfunctions and disturbed metabolism of reactive oxygen and nitrogen species which causes motor neuron cell damage [2,3]. As the chemical composition of CSF shows shortage of antioxidants and iron-binding proteins, CSF is highly exposed to Fenton-like chemistry reactions. Since nervous tissue spontaneously generates and releases H_2O_2 into the CSF [4,5], it is of primary interest to understand the iron metabolism of neural tissues. Iron deposits in the motor cortex and other brain regions of ALS patients have been observed two decades ago using MRI [6] and subsequently confirmed in number of studies indicating that preconditions for free radical reactions related to Fenton chemistry have been fulfilled. However, attempts to somehow quantify the free or helated iron content in CSF of ALS patients and correlate those results with ALSFRS-R scores, has proved to be rather difficult task [7]. Furthermore, the diagnosis of ALS based on the ALSFRS-R scores is often shown to be unreliable and false. Given that the redox activity of iron is related to the presence of specific ligands [8,9], there was a need for developing a new experimental approach which could expose the existence of ligands which could form a redox-active iron complexes in the CSF of ALS patients. In this paper, the technique of cyclic voltammetry was applied to determine if there is a specific feature in voltammogram of CSF that distinguishes patients with ALS from the control subjects.

Material and methods

Patients For this study, CSF of patients diagnosed with sporadic ALS was used. The ALSFRS-R scores were obtained from the participants who were informed that their CSF was to be used for our research

and provided written consent. The ALS group consisted of 32 males and 17 females. The control group consisted of 6 patients with other neurological disorders: tension headaches, lumbar disc herniations and migraines. CSF samples (1.5 ml) were obtained after an overnight bed-rest and fasting, by lumbar puncture. Samples were centrifuged (5000 g, 10 min at 4°C) and rapidly frozen and stored at - 80° C.

Method The electrochemical investigation was performed in a three-electrode all glass cell. A platinum rotating disc electrode was used as a working electrode. The reference electrode was Ag/AgCl in 1M KCl, while a platinum foil served as a counter electrode. The supporting electrolyte was 0.9 % NaCl. The device used for the electrochemical measurements was 757 VA Computrace Metrohm. The measurements were performed at potential rate of 50 mV/s, and rotation rate of 1000 rpm.

Results and Discussion

The results show that, at potential of 1.1 - 1.2 V vs. Ag/AgCl electrode, for the ALS patients, the plateau appeared and the potential of oxygen evolution was shifted toward more positive values (Fig. 1a). The plateau in cathodic sweep was better resolved then in anodic sweep. These voltammogram features were not present for the control patients (Fig. 1b). The measurements were performed for 49 samples of ALS patients and 6 control patients. The cathodic current response at 1.1 V varied in the range of 1.4 - 4 mA. However, the obtained results could not be correlated with ALSFRS-R scores. Furthermore, some patients with high ALSFRS-R scores had high current response and vice versa.

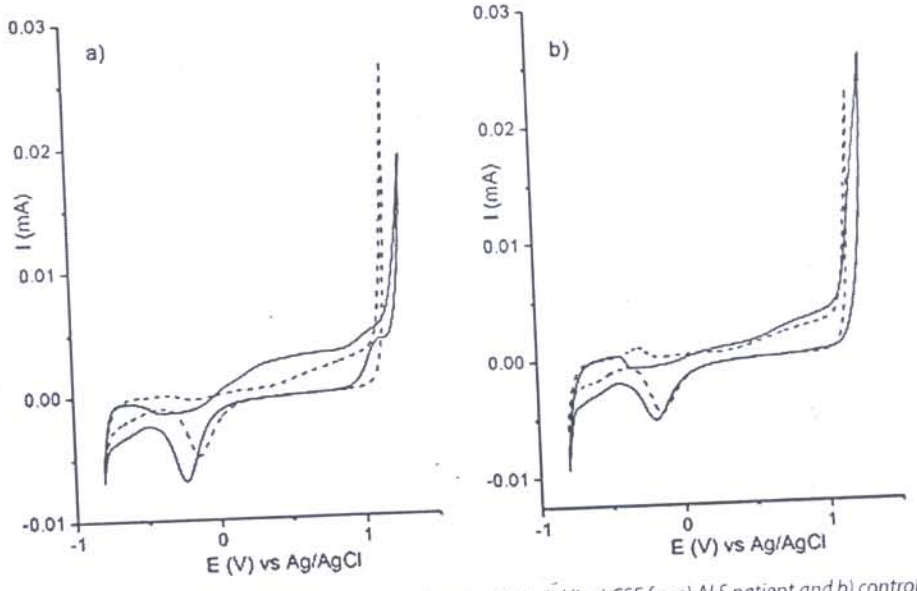

Figure 1. Cyclic voltammogram in 0.9 % NaCl without (dashed line) and with (solid line) CSF for a) ALS patient and b) control patient.

The present problem is the identification of the specie that causes the obtained response. Some preliminary results indicate that this specie could be some Fe^{2+}/Fe^{3+} binding protein or ligand. Since the signal disappeared from cyclic voltammogram upon addition of Fe^{2+}/Fe^{3+} ions in the solution, the calibration could be preformed allowing the estimation of the concentration of the unknown entity in the CSF sample (results not shown). Control measurements were performed to exclude the possibility that plateau derives form Fe^{2+}, Fe^{3+}, Ascorbate or Ascorbyl radical, which are shown to be present in CSF. The recordings performed on different polarization rate and rotation rate indicated that process of oxidation-reduction of this specie is reversible and controlled by mass transport.

Conclusions

Early diagnosis of ALS is difficult task because it may appear similar to several other neurological diseases. Up to date, the diagnosis of ALS is based on excluding other conditions using diagnostic

techniques like electromyography, nerve conduction studies, MRI, blood and urine tests and muscle biopsy. Possible reasons for developing ALS are still unknown and current studies suspect several possible causes such as gene mutation, disorganized immune response or chemical imbalance (especially related to the level of glutamate in CSF). However, we imply that the emergion of Fenton-like chemistry suitable environment in the CSF of ALS patients may be the main cause for ALS outbreak and progression. Accordingly, it is of primary interest to understand iron metabolism of neural tissues especially considering the presence of chelator agents that are likely to be responsible for possible formation of various redox active iron complexes, promoting the production of short-lived free radicals (especially $^{\bullet}OH$) in the Fenton chemistry of the CSF. The cyclic voltammetry is fast and inexpensive technique and appear to be promising candidate for evaluating new biomarkers for ALS. Up to date, there are no reports on using cyclic voltammetry for evaluating the presence of metal complexes or ligands in CSF of ALS patients. We have shown that cyclic voltammograms of CSF of ALS patients have distinguishing feature in comparison to CSF of control patients and suspect that this difference could derive from the existence of various chelator agents, ligands or complexes. However, further extensive investigations are necessary to confirm this hypothesis by detecting specific chemical features which are responsible for this observation.

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