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Epileptic seizures induce structural and functional alterations on brain tissue membranes

Sevgi Turker [a,d], Mete Severcan [b], Gul Ilbay [c], Feride Severcan [d,*]

[a] Department of Biology, Kocaeli University, 41300 Kocaeli, Turkey
[b] Department of Electrical and Electronics Engineering, Middle East Technical University, 06800 Ankara, Turkey
[c] Department of Physiology, Medical School of Kocaeli University, 41300 Kocaeli, Turkey
[d] Department of Biological Sciences, Middle East Technical University, 06800 Ankara, Turkey

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ABSTRACT

Epilepsy is characterized by disruption of balance between cerebral excitation and inhibition, leading to recurrent and unprovoked convulsions. Studies are still underway to understand mechanisms lying epileptic seizures with the aim of improving treatment strategies. In this context, the research on brain tissue membranes gains importance for generation of epileptic activities. In order to provide additional information for this field, we have investigated the effects of pentylenetetrazol-induced and audiogenetically susceptible epileptic seizures on structure, content and function of rat brain membrane components using Fourier transform infrared (FT-IR) spectroscopy. The findings have shown that both two types of epileptic seizures stimulate the variations in the molecular organization of membrane lipids, which have potential to influence the structures in connection with functions of membrane proteins. Moreover, less fluid lipid structure and a decline in content of lipids obtained from the ratio of CH_3 asym/lipid, CH_2 asym/lipid, C═O/lipid, and olefinic═CH/lipid and the areas of the PO_2 symmetric and asymmetric modes were observed. Moreover, based on IR data the changes in the conformation of proteins were predicted by neural network (NN) analysis, and displayed as an increase in random coil despite a decrease in beta sheet. Depending on spectral parameters, we have successfully differentiated treated samples from the control by principal component analysis (PCA) and cluster analysis.
In summary, FT-IR spectroscopy may offer promising attempt to identify compositional, structural and functional alterations in brain tissue membranes resulting from epileptic activities.

1. Introduction

Epilepsy is a heterogeneous collection of neurological disorders that have common recurrent hypersynchronous activation of neurons in focal areas or in the whole brain [1]. Even though epilepsy is a clinically well-known neurological disorder, there is no single treatment strategy to prevent epileptic conditions. To accomplish new advances for this concept, the identification of seizure-induced changes correlating with their pathology has been aimed in most of the studies [2]. However, the research in human needs invasive intervention, therefore; various animal models have been developed.

Pentylenetetrazol (PTZ)-treated animal models have been widely used in epilepsy research. Single and repeated injection of PTZ causes generalized tonic–clonic seizures, which result in similar alterations observed in human epilepsy [3]. Another model includes a subpopulation of some WAG/Rij (Wistar albino Glaxo from Rijswijk) rats, which are susceptible to audiogenic (convulsive) seizures. In response to audiogenic stimulation WAG/Rij rats show motor seizures involving wild running followed by clonic convulsion and/or catalepsy. Since they display a dual pathology (coexistence of nonconvulsive and convulsive seizures), such pattern offers mixed form of epileptic model [4].

Fourier transform infrared (FT-IR) spectroscopy has the ability to investigate the composition, structure and function of biomolecules, to detect the changes in these parameters induced by any pathological condition [5–15]. Therefore, over the years, this method has been widely addressed for identification of disease-conditions in various biological samples such as isolated membranes and their constituent lipids [6,11,12]. However, its application in epilepsy and epileptic conditions is scarce with limited number of reports. We previously investigated the effects of pentylenetetrazol-induced seizures on whole rat brain by FT-IR spectroscopy [15]. In other studies, synchrotron radiation Fourier transform infrared (SRFT-IR) micro-spectroscopy was applied for analysis of whole rat brain tissue [16] and hippocampus [17] as well as for examination of accumulated creatine in hippocampus [18] upon pilocarpine-evoked epilepsy. The effects of epileptic seizures on rat femur and tibia bone tissue were also detected by FT-IR microspectroscopy [19]. Kumar, et al. [20] reported the pathological changes in the IgG samples taken from people suffering from epilepsy by using the same technique.

* Corresponding author. Tel.: +90 312 210 51 66; fax: +90 312 210 79 76.
E-mail address: feride@metu.edu.tr (F. Severcan).

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It has been well-documented that proper function of membrane is largely correlated with its structure. And, membrane structure is fully mediated through physical properties of fatty acids, polar head groups of lipid membrane proteins, as well as lipid order, lipid fluidity and content of membrane components [21]. When brain tissue membrane functions are considered, the determination of seizure-stimulated changes on brain tissue membranes has great importance in understanding the generation of epileptic conditions. This was suggested by previous studies focusing on alterations induced by epileptic seizures on subcellular membrane compartments such as mitochondrial, lysosomal and microsomal membranes [22–24]. Under the light of such background, the aim of the study is concerned with the role of membrane structure and function in development of non-spontaneous PTZ-induced and audiogenetically susceptible seizures. We designed our experiment to investigate acute effects of epileptic seizures on membrane compartments, all of which may have potential role to generate epileptogenesis.

For this purpose, we used Fourier transform infrared (FT-IR) spectroscopy. Although FT-IR spectroscopy gives global information about lipids and proteins rather than providing information about specific types of lipids and proteins, it is an effective technique to study disease-induced early compositional and structural alterations rapidly and sensitively without need for isolation of particular biomolecules [5–15]. We have also predicted the structural changes in membrane proteins using neural networks (NNs) based on FT-IR spectral data as previously used [15,25]. Finally, both principal component analysis (PCA) and cluster analysis have been performed to discriminate treated groups from the control, based on their spectral variations.

2. Experimental

2.1. Chemicals

All chemicals were used without further purification. PTZ, sucrose, trizma base, ethylene diamine tetra acetic acid (EDTA), phenylmethylsulfonylfluoride (PMSF), buthylatedhyroxytoluene, magnesium chloride, pepstatin, and aprotonin were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). Trichloroacetic acid and hydrochloric acid were obtained from Merck.

2.2. Animal studies

All procedures were performed in accordance with welfare guidelines approved by Ethics Committee (KOU-44543) and all treatment processes were applied by following the literature.

Adult male Wistar rats weighing 200–250 g were housed in a room under a constant 12-h light/dark cycle with humidity of 10–50% having free access to standard rat food and tap water. Three groups as control ($n = 6$), PTZ-induced ($n = 6$) and audiogenetically susceptible group ($n = 5$) were designed. All animals were treated once a day for five days. After each injection, the monitored seizures were scored based on literature, for both treated groups [26,27]. Since all animals come from the same strain of Wistar rats, only one group of control consisting of wild type Wistar rats was used as carried out in earlier studies [26]. This group received intraperitoneal (i.p.) physiological saline and key ringing and, no epileptic seizure was observed. The PTZ-group was intraperitoneally injected by convulsant dose (60 mg/kg) once a day. PTZ-induced convulsions were scored according to Racine 26] as follows: stage 0; no response, stage 1; ear and facial twitching, stage 2; convulsive waves through the body, stage 3; myclonic jerks, stage 4; clonic–tonic seizures, and stage 5; generalized clonic–tonic seizures. For the entire PTZ-group, the seizures were interpreted as 4.2 ± 0.3, which reflects stage 4 and stage 5, and lasted 400 ± 30 s. To induce audiogenetically susceptible seizures, audiogenetically susceptible WAG/Rij rats were placed in a testing chamber and sound stimulation was provided by a short manual shake of a bunch of keys (6–10 metal door keys on a metal key-ring) held at 50 cm above the floor of the box. The frequency and intensity of the sound were measured by Biopac MP36 Data Acquistion System (St Barbara, CA,USA) and by sound level meters Lutron SL-4012 (Taipei, Taiwan). The peak frequency of sound stimulation was approximately 6.7 kHz with a wide range of 2–14 kHz. The intensity of sound ranged from 80 to 90 dB. Upon this stimulus, several phases of seizures occur. The intensity of audiogenic seizures was estimated with four level scale as proposed by Krushinski and Molodkina [28]. Stage 0; lack of audiogenic seizures, 1; wild running, 2; clonic seizures with the rat lying "on its" belly, 3; continuation of clonic seizures with the animal turning on its side, and 4; end of seizures with tonic phase. The seizures were scored as 3.2 ± 0.6 and lasted 79 ± 5 s. Subsequently, at the end of five days following the observation of the last seizure, the animals were sacrificed for FT-IR spectroscopic study, and the brains were quickly dissected out.

2.3. Sample preparation for FT-IR study

For the isolation of plasma membrane from rat brain, a method optimized by Scott and co-workers [29] was followed. The solutions were prepared in advance:

(A) 0.25 M sucrose, 10 mM Tris–HCl, 1 mM $MgCl_2$, pH 7.4, density 1.03 g/mL (1.3450)
(B) 0.25 M sucrose, density 1.03 g/mL (1.3450)
(C) 2.0 M sucrose, 10 mM Tris–HCl, 1 mM $MgCl_2$ pH 7.4, density 1.26 g/mL (1.4297)

Briefly, each brain tissue was chopped in Reagent A containing pepstatin, aprotinin and PMSF and homogenized with a tissue homogenizer using a loose-fitting Teflon pestle. Then, 10,000 lbs pressure to homogenate was applied by French pressure cell (Thermo, Electron). After filtration, the homogenate was diluted with Reagent A, and centrifuged for 10 min at 300 $\times g$ and 0–2 °C. The supernatant was collected and pellet was resuspended in Reagent A, then, it was centrifuged again. The supernatants from two extractions were pooled and centrifuged for 15 min at 1500 $\times g$, 0–2 °C. The resulting supernatant was resuspended in Reagent A and was homogenized by 10-second strokes of the loose-fitting pestle. Then, suspension volume was increased using Reagent C, transferred to a centrifuge tube, and carefully overlaid with 4 mL of Reagent B. Afterwards, the sample was centrifuged at 104,000 $\times g$ for a max of 75 min at 2 °C. Membrane structures, which formed a layer at the interface, were collected and resuspended in Reagent B and homogenized as before with strokes of the loose-fitting pestle. Finally, the membrane fractions were centrifuged at 1500 $\times g$ for 20 min. The resulting pellet was resuspended in Reagent B and manually homogenized using Teflon glass homogenizer. The suspension was composed of the membranes originating from various membrane components. This membrane preparation was stored at −80 °C till FT-IR study.

2.4. FT-IR spectroscopic study

IR spectra were acquired using Perkin Elmer Spectrum 100 FT-IR spectrometer (Perkin Elmer, Norwalk, CT, USA) equipped with a deuterated triglycine sulfate (DTGS) detector. The samples were continuously purged with dry air. The interference of atmospheric water vapor and carbon dioxide was automatically removed by subtraction of background from the sample spectra. To obtain the best quality spectrum, scanning parameters were optimized by following the earlier studies with mammalian tissue samples [6,12,15,25,30]. 15 μL samples were placed between ZnSe windows with a spacer to obtain 12 μm sample thickness. All spectra were recorded at the wavenumber range of 4000–900 cm^{-1}. Interferograms were averaged for 200 scans at 2 cm^{-1} resolution at 25 °C. For each scan, the spectra of three independent aliquots from the same sample were recorded to minimize any variability, and to check the precision of the absorbance values. These replicates belonging to the same source