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Synaptosomal acetylcholinesterase activity variation pattern in the presence of electromagnetic fields

Ali Afrasiabi [a], Gholam Hossein Riazi [a,*], Shayan Abbasi [b], Ali Dadras [a], Behafarid Ghalandari [c], Hossein Seidkhani [a], Seyed Mohamad Sadegh Modaresi [d], Neda Masoudian [a], Amir Amani [e], Shahin Ahmadian [a]

[a] Institute of Biochemistry and Biophysics (I.B.B.), University of Tehran, Tehran, Iran
[b] Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
[c] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[d] Department of Biological Sciences, Kharazmi University, Tehran, Iran
[e] Department of Medical Nanotechnology, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Acetylcholinesterase (AChE) is the enzyme that controls the acetylcholine (ACh) concentrations in cholinergic synaptic clefts by hydrolyzing ACh to choline and acetate. Cholinergic synapses are involved in important functions such as learning, memory and cognition. In this study, we investigated the effects of a wide range of extremely low frequency electromagnetic fields (ELF-EMFs) on synaptic ACh concentrations through AChE enzyme activity assay. Synaptosome suspensions were prepared as a neural terminus from cerebral cortex of sheep brain. Prepared synaptosomes were exposed to ELF-EMFs with frequency ranging from 50 Hz to 230 Hz for duration between 15 and 120 min and flux intensity between 0.1 mT and 1.7 mT. Consequently, AChE activity was measured by Ellman method. Raw data were analyzed by neural network based software, Inform 4.02, to predict AChE activity pattern through nine 3D curves. These curves showed that AChE activity decreases when exposed to ELF-EMFs of 1.2 mT to 1.7 mT intensity and 50 Hz to 90 Hz frequency. Thus, it is proposed that exposure to fields of in this range of frequency–intensity would be effective in clinical treatments of cholinergic disorders to increase synaptic ACh concentration. However, more *in vivo* experiments are needed to develop this suggested treatment.

1. Introduction

There is a growing concern for the effects of electromagnetic fields (EMFs) on human health by enhancing and developing of electrical and telecommunication instruments in the urban environment such as cell phones, cordless phones, household wiring, and AC transmission lines etc. Many aspects of biological functions have been considered such as cognitive performance, immune system, signal transduction, cell proliferation responses and bimolecular structure in the presence of EMFs [1–7].

EMFs affects the cells through various mechanisms including modifications of biomolecules, changing membrane properties and ions currents, which leads to disturbed cellular signaling cascades [8,9]. Moreover, EMFs is able to induce oxidative stress by increasing the neuronal Ca^{2+} levels [8]. High and low frequency EMFs can affect the brain by altering the permeability of blood–brain barrier [10–12]. On the other hand, EMFs induces multiple variations in transportation and release of neurotransmitters such as serotonin, dopamine and acetylcholine (ACh) [13–15]. Lipid peroxidation development and nitric oxide production in brain especially basal forebrain and frontal cortex has been reported in mice that were exposed to ELF-EMFs (50 Hz, 0.5 mT) for seven days [16]. No cytotoxic and genotoxic effects have been reported for fields at 50 Hz and 1 mT radiating on neuroblastoma cells, however, a reduction in antioxidant homeostatic capacity of neural cells has been reported [17]. Several studies have shown that ELF-EMFs are capable of reducing the brain cognitive performance such as perception, cognitive behavior, attention and short-term memory [14,15,18–22]. On the other hand, some experiments have indicated that ELF-EMF has neuroprotective and nerve regeneration properties [23,24]. Despite many studies on the effects of ELF-EMFs on nervous system and cognitive performance, safe or unsafe areas of ELF-EMFs for human cognition health still remains unknown.

* Corresponding author. Neuro-organic Lab, Institute of Biochemistry and Biophysics (I.B.B.), University of Tehran, Tehran, Iran, P.O. Box 13145-1384.
Tel.: +98 21 61112473; fax: +98 21 66404680.
E-mail address: riazi@ibb.ut.ac.ir (G.H. Riazi).

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Recent studies on neurological effects of EMFs have been focused on the enzymatic activity, which is known to be involved in brain critical functions such as acetylcholinesterase (AChE) [25–27]. The enzyme AChE which appears in cholinergic synapses in both central and peripheral nervous systems (CNS and PNS), has a key role in sympathic and parasympathic function. AChE deactivates Ach, an excitatory neurotransmitter in cholinergic synaptic cleft by breaking it to acetate and choline [27,28]. Cholinergic systems are involved in essential functions of CNS such as learning, memory formation, synaptic plasticity and decision making etc [28–30]. Indeed, AChE dysfunction could lead to cognitive disorders such as Alzheimer disease (AD) or memory dysfunction [31–33].

A 42% decrement in AChE activity was reported in the presence of a 75 Hz field of 2.5 mT flux intensity [25]. Also, exposure of embryo homogenates to 75 Hz, 2.2 mT EMFs decreased AChE activity to 48% [34]. Moreover, sinusoidal ELF-EMFs of 50 Hz frequency and 0.74 mT intensity, decreases AChE activity about 27% [26].

To find out more details about the effects of ELF-EMFs on cholinergic synapses, we studied a wide range of ELF-EMFs (frequency range from 50 Hz to 230 Hz in the flux intensity range from 0.1 mT to 1.7 mT) effects on AChE activity. In present study, Synaptosomes were prepared from cerebral cortex of *Ovis aries* (sheep) brain as a model of neural terminus. Prepared synaptosomes were exposed to ELF-EMFs with desired frequencies and intensities for 15–120 min, and AChE activity was measured. Furthermore, we found more details about the effects of ELF-EMFs on cholinergic synapses through determining the 3D curves that indicate the AChE activity pattern. For this goal, Inform 4.02 software which is based on artificial neural networks (ANNs) was used.

2. Materials and methods

2.1. Animal

This study was carried out on extracted synaptosomes from sheep brain to investigate the effects of ELF-EMFs on cholinergic synapses. The study was approved by the University of Tehran and Animal Sciences Research Institute of Iran. One adult male sheep (Afshari Persian) with 67.350 Kg body weight and good body condition was decapitated. The process of decapitation was carried out in the presence of Animal Sciences Research Institute representative and The Iranian Society for The Prevention of Cruelty to Animals agents in Ehsan slaughterhouse (Shahr-e-Ray, Iran). The carcass was delivered to Ehsan slaughterhouse. Sheep skull was cracked and split by an axe. Whole brain was removed. Then, cerebral cortex was separated and kept in sucrose 0.32 M to be used in synaptosome preparation step.

2.2. Preparation of synaptosomes

Synaptosomes were prepared by sucrose gradient centrifugation using the method of Dodd et al. [35,36]. The cerebral cortex of sheep brain was applied to prepare synaptosomes. Extracted cerebral cortex was minced and homogenized with Motor-driven potter teflon-glass homogenizer at 800 rpm. The obtained homogenate was centrifuged at $3000 \times g$ for 30 min. Supernatant was loaded on top of sucrose 1.2 M. The sucrose gradients were centrifuged at 113,000 × g for 35 min. The soft middle white layer between the sucrose layers of 0.32 M and 1.2 M was then acquired and loaded on top of sucrose 0.8 M which was centrifuged at $113{,}000 \times g$ for 35 min. The resulting pellet, containing synaptosomes, was dissolved in sucrose 0.32 M solutions. Finally, synaptosomes were stored at −20 °C.

2.3. Transmission electron microscopy (TEM)

TEM micrographs were taken to verify morphology of synaptosomes [36,37]. The synaptosome suspension was centrifuged at $9000 \times g$ for 30 min, the supernatant was acquired and the resulting pellet was fixed in 2.5% glutaraldehyde for 1.5 h. The samples were rinsed twice by phosphate buffer for 5 min and were stained with 1% osmium tetroxide for 60 min. After dehydration by different concentrations of ethanol from 25% to 100%, the samples were mixed with agarose and sectioned by Richert Ultra microtome. Samples were stained with uranyl acetate and Pb citrate and were observed with Hitachi HU-12A electron microscope (Japan).

2.4. AChE activity assay

After incubation with EMFs, specific activity of synaptosomal acetylcholinesterase was measured by Ellman method. This method is based on NTB^{2-} (2-nitro, 5-thiobenzoic acid) production and its absorption at 412 nm [38]. The samples consisting of synaptosomal suspension (200 μg protein), acetylthiocholine 1.2 mM and 5′-dithiobis-2-nitrobenzoic acid (DTNB 5) 1 mM were prepared in phosphate buffer 50 mM pH 7.2. The enzyme activity was assayed at 37 °C. The protein concentrations were determined for enzyme specific activity using Bradford method [39].

2.5. Electromagnetic fields generator

EMFs exposure was generated with Helmholtz coil, containing two parallel coils with 35 cm distance and 35 cm internal diameter made of 1000 turns of coated 1.3 mm copper wire .The device is capable of generating EMFs characterized by a sinusoidal waveform with intensity of 0.1–1.7 mT and frequencies of 1–800 Hz. This device was supplied by a power generator, and EMFs frequency and intensities were monitored by an EMFs sensor connected to a digital multimeter and oscilloscope (DS20080A oscilloscope card, TNM ELECTRONICS, Iran). The full scheme of EMFs exposure system is shown in Fig. 1. A tube of synaptosomal suspension (1 mg protein) was placed in an area of the parallel coils where the magnetic field was uniform and magnetic field lines ran vertically. Mentioned area was determined by using a teslameter (13610.93, PHYWE, Germany) with a probe type of Hall Sound and plotted with MatLab software (GNU General Public License version 2.0) (Fig. 2). Next, samples exposed to EMFs with selected frequency and intensity. Control samples were run in the same experimental conditions as above, but in the absence of EMFs. Coils were located in the incubator maintained at 37 °C temperature. Fluctuations in temperature at the location of samples upon interaction with ELF-EMFs radiation were measured, and it was negligible. In other words, our selected fields have no significant thermal effect on the samples and environment.

2.6. ANNs studies

ANNs work based on brain data processing patterns. In other words, ANNs simulates model for data sets with non-linear relationships through processing inputs with their effective values and compute sum of these values for final output [40,41]. ANNs include computational units called artificial neurons, which organize at least three connected layers. First layer is input layer that take raw data, second layer contains the hidden layer(s) that compute the relationships between data series, and third layer is output layer that reports the final results [18]. INForm v4.02, Intelligensys, UK is the ANNs software that was used in this study [42]. The ability to predict a wide range of output values with only few random samples of inputs is one of the best properties of this software. At a minimum, two or three times of inputs required to develop a good