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Effects of Applied Electrical Field on Biochemical Parameters and Erythrocytes of Rats

H. DEMIR*, T. CAKIR†, C. KARAKAYA‡, A. CEBI ILHAN**, N. ONURSAL†† and A. GÜR

Department of Chemistry, Art and Science Faculty, Yuzuncu Yil University, Van, Turkey
Fax: (90)(432)2251114; E-mail: halitdemir2005@yahoo.com

The purpose of this study was to determine whether there was any effect of some biochemical parameters and erythrocyte carbonic anhydrase on electrical field stimulation in female rat. The experiments were performed under the animals' scientific procedures and conform to National Institute of Health guidelines for the use of experimental animals. This study was carried out on female Sprague-Dawley rats (250–270 g). Comparasons were made using student-t tests. A p value less than 0.001 was considered statistically significant. It was demonstrated that alanine aminotransferase, aspartate aminotransferase, amylase and lactate dehydrogenase in serum were significantly affected by this electric field stimulation ($p < 0.001$). In addition, particularly carbonic anhydrase activity was affected by applied electrical field.

Key Words: Electric field stimulation, Carbonic anhydrase, Serum enzymes, Rat.

INTRODUCTION

In the area of biological effects and medical applications of non-ionizing radiation approximately 25000 articles have been already published during the past 30 years. Despite the feeling of some people that more research needs to be done, scientific investigation in this area is now more extensive than for most chemicals. Based on a recent in-depth review of the scientific studies, WHO concluded[1] that current evidence does not confirm the existence of any health consequences from exposure to low level electromagnetic fields. People may have attributed a diffuse collection of symptoms to low levels of exposure to electromagnetic fields at home. It is reported that symptoms include headaches, anxiety, suicide and depression, nausea, fatigue and loss of libido[1,2]. According to data, any scientific evidence does not support a link between these symptoms and exposure to electromagnetic fields. At least some of these health problems

†Department of Biochemistry, Department of Radiation Oncology, Medicine Faculty, Yuzuncu Yil University, Van, Turkey.
‡College Health Science, Yuzuncu Yil University, Van, Turkey.
**Department of Medical Biology, Medicine Faculty, Yuzuncu Yil University, Van, Turkey.
††Siirt Educational Faculty, Department of Physics, Dicle University, Diyabakr, Turkey.

may be caused by noise or other factors in the environment or by anxiety related to the presence of new technologies[3]. Carbonic anhydrase (CA) has been a well characterization pH regulatory enzyme in most tissues including erythrocytes. CA catalyses the reversible hydration of CO_2 to HCO_3^- and H^+. Seven distinct izozymes of carbonic anhydrase have been characterized from primary mammals[4,5]. The effects of *Crocus sativus* petals extract on blood in anaesthetized rats and also on responses of isolated rat vas deferens and guinea pig ileum induced by electrical field stimulation (EFS) has been investigated. The present results may suggest that the relaxatory action of *C. sativus* petals extract on contraction induced by EFS in the rat isolated vas deferens is a postsynaptic effect[6]. For individual variations in the biochemical character of animals as proved in the past, electrical fields are a very important phenomena to consider before making final conclusions[7].

In this study, the effects of electrical field of 380 volt have been investigated on rat erythrocyte carbonic anhydrase and rat serum ALT, AST, LDH and amylase.

EXPERIMENTAL

Chemicals and protein assay reagents were supplied by Sigma Chemical Co. (USA). The kits for analysis were obtained from Diagnostic Products Corporation (DPC, USA). Albino rats (Sprague-Dawley) weighing 200–270 g were provided by the animal house of the Medical School of Yuzuncu Yil University and housed in two groups, each of five rats. The animals were given standard rat pellets (Van Food Factory, Van, Turkey) and water *ad libitum* in stainless steel cages and received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health. Rats were lightly anesthetized with ether and a 0.5 mL sample of blood was withdrawn from the lateral tail vein. The animals were housed at 20 ± 2°C under a daily light/dark cycle.

Electrical field applied: The animals were leaved the main generator room which produced 380 V electrical potential.

Blood and serum collection: Serum samples were obtained from fresh rat blood. The blood samples were centrifuged at 1500 rpm for 15 min, plasma and buffy coat were removed. In addition, fresh rat blood collected in tubes with EDTA was centrifuged at 5000 rpm for 15 min. The plasma and leukocyte coats were removed. The packed red cells were washed with NaCl solution (0.9%) three times; the samples were centrifuged at 5000 rpm each time and supernatants were removed. The erythroctes were hemolyzed with 5 volumes of ice-cold water and centrifuged at 20,000 rpm at 4°C for 30 min to remove ghosts and erythroctes were obtained.

Measurement of carbonic anhydrase activity: CO_2^- hydratase activity of the enzyme was determined at 25°C in a veronal buffer (pH 8.15) with phenol red as indicator and saturated carbon dioxide solution as substrate at a final volume of 4.2 mL. The time taken for the solution to change from blue to green was measured. The enzyme unit is the enzyme amount resulting in 50% decrease of the non-enzymatic reaction time. Activity as enzyme unit was calculated by using the equation $(t_0 - t_c/t_c)$ where t_0 and t_c are times for pH change of the non-enzymatic and enzymatic reactions, respectively[8,9].

Protein determination: Protein determination was done by absorbance measurement at 595 nm with bovine serum albumin as a standard[10].

Measurement of biochemical parameters: Biochemical parameters were measured using an autoanalyzer (BNN/HITACHI-911) and the corresponding kit (DPC, Diagnostic Products Corporation, USA).

Analysis of date: Student's t-test was used for comparing groups. P values having $P < 0.001$ was considered significant. Statistical tests were performed using Statistica version 5.0 (SPSS/PC$^+$, Chicago, IL, USA).

RESULTS AND DISCUSSION

The levels of AST, ALT, LDH, amylase and CA were significantly different between the applied electrical field groups and control group of rats ($P < 0.001$). The applied electrical field caused significant increases in aspartate aminotransferase (AST), lactate dehyrogenase (LDH), amylase, whereas carbonic anhydrase (CA) and alanine aminotransferase (ALT) decreased. Our results are summarized in Table-1.

TABLE-1
EFFECTS OF APPLIED ELECTRICAL FIELDS ON BIOCHEMICAL PARAMETERS OF RATS

Parameters	Control ± SD	Applied electrical fields of rats	P
AST $(U/L)_{serum}$	166.4 ± 18.623	181.8 ± 15.750	$P < 0.001$
ALT $(U/L)_{serum}$	63.6 ± 4.336	48.4 ± 5.899	$P < 0.001$
LDH $(U/L)_{serum}$	900.4 ± 72.307	1004.0 ± 4.183	$P < 0.001$
Amylase $(U/L)_{serum}$	1483.8 ± 116.8	1783.64 ± 308.312	$P < 0.001$
Carbonic anhydrase $(EU/gHb)^{-1}_{erythrocyte}$	31600 ± 8.792	16640 ± 3.966	$P < 0.001$

Numerical computations are used to evaluate electric field dosimetry for high-resolution anatomically based in homogeneous models of a human male child and male and female rats and mice under exposure to 60 Hz uniform magnetic field sources of three perpendicular orientations[11].

With regard to the biochemical characteristics, we observed that the AST, LDH and amylase levels in the serum of the rats were higher than in that of the control group after applied electrical field (Table-1). Serum levels of liver enzymes were significantly improved in rats treated with dimethyl thiourea[12]. Literature studies indicate that some drugs possess an inhibitory effect on enzyme activity in serum, plasma, muscle and liver with the lapse of time[13]. After that will integrate results from cellular, animal and human health studies to allow as comprehensive a health risk assessment as possible. A holistic assessment of a variety of relevant and reliable studies will provide the most reliable answer possible about the adverse health effects, if any exist, of long term exposure to weak electromagnetic fields.

In this study, the inhibitory action of electrical field on levels of enzymes of serum and enzyme activity of erythrocyte in rats was investigated . Our data will provide a basis for further research, which would aim to understand the action of the applied electrical field damage of animals' biochemical parameters. In spite of the

proteolytic digestion of this external CA, a significant increase of enzymatic activity, as a function of increase in the diffusion rate of substrate across the lipid bilayer, was observed in the exposed samples. Investigations of CA-II enzyme activity on EFS have been done and observations recently reported that carbonic anhydrase was increased by combined 7 Hz magnetic fields[14]. The influence of low frequency (4–16 Hz), low amplitude (25–75 mu T) magnetic fields on CA enzyme activity have been investigated in rats. The studies suggest a plausible link between the action of extremely low frequency magnetic field on charged lipids and a change of membrane permeability of CA-I enzyme activitiy[15]. In addition, CA enzyme activity has been significantly inhibited by EFS in guinea pigs[16]. Further, biochemical studies are also needed to assess on different rat serum (ALT, AST, LDH and amylase) and rat erythrocyte CA.

As a result, in this study while AST, LDH and amylase increased, levels of ALT and CA decreased with EFS. In present literatures, it is stated that the levels of these enzymes decrease with high frequency and increase with low frequency. However, there have been no attempts to study many of the effects of EFS on rat serum (CA, ALT, AST, LDH and amylase) and further studies are required to investigate this. This study is the first to show its effect in serum and erythrocyte in 380 V electrical potential.

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