THE INTERACTION OF ELECTRIC FIELDS WITH BIOLOGICAL SYSTEMS I : LIVER HYDROXYPROLINE

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SUMMARY : *The effect of electric fields in different directions on protein synthesis was studied on Guinea Pigs. Electric field which is obtained from 150 V potential difference applied in vertical and horizontal directions and their effects on collagen synthesis were analyzed by studying hydroxyproline level of liver tissue with Stegemann-Stalder's method.*

A statistically significant decrease was found in hydroxyproline levels in both of the horizontal and vertical E field applied groups. It was also noticed that the vertical E field was more effective than the horizontal.

The results showed that both of the electric fields-vertical and horizontal - decreased liver tissue hydroxyproline content but the vertical one is more effective than the horizontal electric field.

Key Words : *Electric Field, Hydroxyproline, Liver Tissue.*

INTRODUCTION

In recent years a body of data on the interactions of exogenous and endogenous electromagnetic fields (em) with biological systems has been gatherede. As a result of these findings our understanding of biological function has started to change. It would be incredible if em fields did not effect the electrochemical systems in living organisms. And since living organisms have only so recently found themselves immersed in this environment, they have not had an opportunity to adapt to it. This gives us, as biophysicists, the opportunity to use exogenous em fields to study the functioning of living systems. As in the other studies on living organisms including bacteria with em fields, the data collected is very few (2, 3, 4, 5, 6, 12, 13, 17) and are required to be repeated.

In this article it has hypothesized that the exogenous electric field has effect on collagen synthesis in exposed tissues and the effect of electric field in different directions - vertical and horizontal - on collagen synthesis was investigated by detecting the changes in hydroxyroline content of liver tissues of the exposed animals. The method used for detecting hydroxyproline amount of the tissue was Stegemann - Stalder modified method (33).

The first method used for hydroxyproline determination was Neuman - Logan's method (26). This method was modified by Barker et al, Martin and Axelrod, Wierbicki and Deatherage, Miyada and Tappel, Arenson and Elvehjem, Grunbaum and Glick, Antonacopoulos, Lollar, Laech Woessner, Stegemann and Stalder (26, 33, 34).

In the oxidation phase of hydroxyproline only Stegemann - Stalder and Woessner used chloramin - T, while others chosed hydrogen peroxide. Dahl - Persson showed that by using chloramin - T the same sample's hydroxyproline amount gave more sensitive result (15). For this reason Stegemann and Stalder's method was used in this study.

MATERIALS AND METHODS

Experiments were performed on 40 male, Guinea Pigs with an average weight of 350-400 g. They were separated into three groups. In Group I, 10 Guinea Pigs exposed to the vertical electric field while another 10 in Group II exposed to the horizontal electric field. 20 Guinea Pigs were also kept in the cages at the same laboratory conditions and studied as Control Group without any electric field exposure. Three animals in 3 different cages were studied simultaneously each time.

Vertical and horizontal electric fields with the magnitude of 580 V/m were applied to Guinea Pigs in wooden cages wih dimensions of 50 cm x 50 cm x 14 cm. Exposure period was 9 hours / day for 3 days throughout the study.

Potential differences applied to the cages were 150 V and obtained from high intensity power supplies. Voltage was kept fixed and controlled continuously with a voltmeter in the exposure circuit.

Vertical Field Exposure Circuit

Copper plates with dimensions of 50 cm x 50 cm x 0.1 cm were mounted on the top and the bottom faces of the cages.

Positive probe of the power supply was always connected to the upper plate and negative probe to the lower plate for all of the cages.

Horizontal Field Exposure Circuit

The plates were mounted to the left and right faces of the cages.

Positive probe of power supply was always connected to the right plate and negative probe to the left plate for all of the cages.

Duration of electric field exposure was from 8^{oo} am to 5^{oo} pm, 9 hours / day. Wooden blocks (60 cm x 60 cm x 2 cm) were used under the cages for isolation.

Liver tissue hydroxyproline contents of the animals of exposure groups and control group were determined with Stegemann - Stalder's Method (33). The basic principle of this method is to get the hydroxyproline of the tissue by hydrolization of the sample after homogenization and measuring the optical density of the color formed by adding p-dimethylaminobenzaldehyde, perchloric acid and propan - 2 -ol at pH=8 and at λ = 560 nm. Hydroxyproline contents of the tissue samples were determined by using the standard curves which were plotted before each spectrophotometric reading by using the sample containing known concentrations of Hydroxyproline (SIGMA H-1637).

RESULTS

Two samples were studied for hydroxyproline determination after the homogenization of each liver tissue. The average hydroxyproline concentration value was taken into consideration at the calculation step in determining the hydroxyproline content of liver tissue.

Statistical comparisons were made in two groups :

1. For each group, hydroxyproline contents of lung tissues of the electric field applied groups and their controls were compared with DUNCAN test (Table 1) and the results were found as follows :

Groups	X	sd	n
Control	0.2615	0.1450	20
Vertical electric field	0.0779	0.0228	10
Horizontal electric field	0.1194	0.032	10

Table 1 : Liver tissue hydroxyproline levels (µg/g tissue) of vertical and horizontal electric field applied groups and control group.

- There is statistically significant difference between hydroxyproline contents of liver tissues of **vertical electric field applied and control groups** ($p<0.01$). Vertical electric field application led to decreased liver tissue hydroxyproline level

($Hyd._{E.Vertical} = 0.0779 \pm 0.0228$; $Hyd._{Control} = 0.2615 \pm 0.1450$).

- There is statistically significant difference between hydroxyproline contents of liver tissues of **horizontal electric field applied and control groups** ($p<0.05$). Horizontal electric field application resulted in significantly decreased liver tissue hydroxyproline level

($Hyd._{E.Horizontal}$=0.1194 ± 0.032; $Hyd_{Control}$ = 0.2615±0.1450).

2. A comparison was made between the vertical and horizontal field groups to specify the effective direction of the electric field application : Vertical electric field decreased hydroxyproline content of liver tissue more than the horizontal electric field although the difference was not statistically significant ($p>0.05$).

DISCUSSION

The effect of electric field in different directions on collagen sythesis was investigated by detecting the variations in tissue hydroxyproline content. Both the vertical and horizontal E fields created by 150 V potential difference led to decreased liver tissue hydroxyproline levels, whereas vertical E field was found to be more effective than the horizontal.

There are researches that E fields are effective on tissues (6, 17, 25, 27, 28, 30, 35).

Internal E fields existing in tissues and external E fields transfer energy on the tissue in two different ways (19) :

1. In the tissues under the effect of E fields, positive charges moves in the same direction with E field while the negative ones move in the opposite direction, causing the tissue polarized.

2. Dipole moment vectors of polar molecules like H_2O, rotate to get the same direction with the externally applied E field. As a result an internal E field arises and its interaction with the external E field lessens the net E field in the tissue.

External E field applied living organisms are being examined in the senses of different E fields that arises on body surface and inside of the body, current intensity created in the body and the net electric current occuring along the whole body.

In the modelling studies for human and animals, it has been possible to determine the E field and electric current values arising inside and on the surface of the model by the effect of an externally applied field with the help of the developments in the biomedical electrode techniques. Using human, rat and pig models the values of surface electric fields and current densities generated with a vertical E field of 10 kV/m, and 60 Hz frequency are determined. The maximum sensitivity is localized at the head (180 kV/m) for the human, and at the upper body for the pig (67 kV/m) and for the rat (37 kV/m). Different tissues have different resistances and dielectric properties, and the external E field will always be greater than the induced E field inside of the body (9, 20, 21, 22).

It has been possible to direct collagen under E field. If a collagen solution soluble in acid is applied 1 µA direct current, collagen form a concave band near cathode (16, 18, 32). This finding shows that collagen acts as a cation under E field.

Proteins having a net electrical charce attain a movement under the influence of electric field. Since macromolecules such as proteins have different moleculer structure and shapes, they are exerted different frictional forces in a medium. For these reasons different protein molecules have different velocities when they are inserted in the same electric field. Amino and carboxyl groups are responsible from the net charge of a protein molecule. Secondly, proteins may gain positive or negative charge or they may have no charge according to the pH value of the medium. In the latter case proteins do not have the capability of movement in a electric field. If pH of the medium is less than the isoelectric pH of protein, then it posses a net positive charge and moves in the direction of electric field, whereas it gain a negative charge and move in the opposite direction with the applied electric field in the case that the pH value of the medium is to be greater than the isoelectric pH of the protein (14). The DC electric field with the strenght of 10 kV/m has the ability to move the protein molecules along the membrane surface (8).

When low intensity direct current is applied to tissues, O_2 is consumed at cathode. Tissue O_2 tension decreases and an increase occurs in the number of hydroxyl radicals present (7, 10, 11). Free oxygen radicals have toxic effect on proteins and DNA giving result to structural and functional defects in the cell. Amino acid compositions of proteins specify the extent of influence from free radicals. Unsaturated molecules and sulphure containing molecules are highly sensitive to free radicals. Besides the oxidation of amino acids, free radicals give rise to hydrolysation of peptide bonds, disulfide bonds, and cross bindings resulting the defects in functions of enzymes, disturbing ion seperation in and out of the cell, and causing serious injury of cell. The increase in radicals can be traced with the variation in malondialdehyde (MDA) level (37). In our another study carried out parallel to this study, it was found that both of the vertical and horizontal E fields produced by 150V potential difference cause an increase in MDA levels of medullar glands of guinea pigs (6), whereas a decrease was observed in the hydroxyproline levels of the liver tissue in this study.

The finding of a decrease in hydroxyproline level with the effect of electric field caused a decrease in tissue MDA level. Molecular O_2 could be transformed to free radicals as the result of energy transfer of E field to the applied tissue area. Therefore the increase in the radicals might give rise to a decrease in molecular O_2 required in hydroxyproline synthesis. This in turn might result in a slow down in the functioning of proline hydroxylase and α-keto-glutarate enzymes required in the synthesis of hydroxyproline.

Having the values of tissue conductivities and dielectric constants is important in determining the distribution of external electric fields and currents in the tissues. Since the tissues like lung and liver contain 20 % - 25 % protein and 70 % - 80 % water of their weights, they exhibit much more lower resistance and higher dielectric constant with respect to fatty tissues. Furthermore, increasing water percentage, increases conductivity. This property is a result of polar structure of water. This structure of liver has also been effective on the effect of E fields in liver tissue (1, 29, 31, 36).

It has also reported that the direction of E field was important. In 1983 Marino applied 19.7 V/m E field in vertical and horizontal directions to mice, and a big decrease in serum protein fractions was observed in vertical E field group while a slight change in horizontal E field group was observed (24). In his another study, Marino applied 15kV/m vertical and 10 kV/m horizontal E field to mike and observed an increase in the mortality on vertical E field applied group with respect to horizontal field applied ones (23).

This research has shown two results : One is the finding that electric field of 580 V/m has the ability to direct collagen in liver tissue in both of the horizontal and vertical directions. And the other is vertical electric field is more effective on collagen syntesis than the horizantal one. This result is in parallel with Marino's unique study on serum protein fractions and mortality.

This study was the first on the effect of elctric field on liver tissue hydroxyproline level. The thing that is required is more and more studies on the topic. The next step of our study will be the effect of electric field on liver tissue MDA levels.

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