DOES ELECTRIC FIELD EFFECT COLLAGEN SYNTHESIS IN TISSUE ?

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SUMMARY : *This study is carried out to investigate the effect of electric field applied in different directions on collagen synthesis by determining the quantitative level of hydroxyproline in lung tissue. Ten guinea pigs were exposed to vertical electric field, while another 10 being exposed to horizontal eletric field with the intensity of 1.9 kV/m. Twenty guinea pigs were used as control, keeping in the same conditions without being exposed to any electric field. Exposure period was 9 hours / day for 3 days.*

A statistically significant increase was found in the lung tissue hydroxyproline level of vertical electric field applied group (p<0.001) and horizontal field applied group (p<0.05) compared to non-exposed controls. It was also observed that the increase in vertical electric field applied group was more than the horizontal field applied ones suggesting that the vertical electric field might be more effective than the horizontal.

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

INTRODUCTION

A very active period was between 1925 and the Second World War when a number of biophysicists applied potential theory originally developed for dielectrics to biological material (blood, tissue, nerve cells). About 1935, investigations began of the electrical properties of protein molecules by application of the concept of polar molecules originated by Debye (41). Of more fundemantal importance are studies of the electrical properties of tissue and cell suspensions related to :

a. Structural analysis of cellular organism (properties of cell membranes and cytoplasm).

b. Analysis of characteristics of protein molecules, such as dipole moment, shape, hydration. All of the apparatus and devices necessary in modern life such as power lines and electrical machinery are the sources of electric (E) fields which cause important effects on living organisms. Behavioral, cellular and pyhsiological functions of animals can be affected by e.m. stimuli (1. 12, 26). In recent years a body of data on the interactions of exogenous and endogenous e.m. fields with biological system has accumulated. As a result of these findings our understanding of biological functions has started to change.

The electric field of the Earth consists of a static component, which is dominant, and a time varying component, which is smaller than the static component by several orders of magnitude in the 50-60 Hz frequency range (38). The natural electric field near the Earth's surface is a static field of about 130 V/m (24).

Daily changes in the natural electric field are attributed to factors, such as thunderstorms, which have magnitudes of 3-20 kV/m (20).

Man-made sources of electric fields are high voltage transmission lines and all devices containing current - carrying wire, including equipment and appliances in the home and in industry.

Measurements of electric fields in a home ranges from 2 V/m to about 50 V/m at 30 cm from the appliances and can vary to 250 V/m near some appliances (36, 47). Living organisms interact with this environment of electric and magnetic fields. The question is did they adopt to this environment of electric and magnetic fields. I think, they did not yet, at least they didn't accomplish the process of adoptation.

The connective tissue protein, collagen, is the most abundant protein in higher animals. Collagen also provides the framework for parenchymal organs such as the liver, kidney, and spleen, either in its fibrous form or organized in basement membranes. Some tissues contain much more collagen than others : the collagen content of the skin accounts for about 70 % of the dry weight of skin whereas the collagen content of abdominal viscera is much lower and, in the case of the liver, amounts to about 4 % of the dry weight (32). Hydroxyproline is found almost exclusively in collagen, constituting about 14 % of the dry weight of the protein, and the amounts of this amino acid are relatively constant in collagens from various tissues in humans. The rate of hydroxyproline formation is therefore considered to be a good indication of the rate of collagen biosynthesis (22). The collagen content of a tissue is determined by measuring the content of protein bound hydroxyproline, an amino acid that is a marker of collagens (45).

The aim of this study is to examine protein synthesis under the effect of exogenous electric fields in different directions. Electric field was chosen with the magnitude in the order of electric fields of man made apparatus. For this aim 1.9 kV/m electric field in two different directions was applied to 20 guinea pigs and the effect of electric field was observed by determining the change in hydroxyproline level of lung tissue.

MATERIALS AND METHODS

Guinea pigs (10-12 weeks old) were exposed continuously to uniform electric fields of 1.9 kV/m generated between the parallel plates of a capacitor. The animals were separated into three groups. In Group I, 10 guinea pigs were exposed to vertical electric field while another 10 in Group II were exposed to horizontal electric field. Twenty guinea pigs were also kept in the cages at the same laboratory conditions and studied as the Control Group without any vertical and horizontal electrical field application. For 3 days the experimental animals were housed in wooden cages of 50 cm x 50 cm x 14 cm dimensions.

The simplified equation for the electric field between two parallel plates, the potential difference per distance, is widely used in biological experiments. This is, however, only true when the plate spacing is small enough in comparison with the dimensions of the plates. For that reason the plate spacing was 14 cm whereas the dimension of the plates was 50 cm x 50 cm x 0.1 cm. For 10-12 weeks old guinea pigs, this much spacing between the capacitor plates was the minimum possible distance with respect to dimensions of the animals.

Vertical Field Exposure Circuit : Copper plates 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 : Copper plates were mounted to the left and right faces of the cages. Positive probe of the power supply was always connected to the left plate and negative probe to the right plate for all of the cages.

Vertical and horizontal electric fields with the magnitude 1.9 kV/m were obtained from power supplies with 300 V DC potential and applied to the plates. Exposure period was 9 hours/day for 3 days throughout the study. Duration of electric field exposure was from 8^{00} am to 5^{00} pm, 9 hours/day.

During the study, all guinea pigs were housed in a room having a temperature of 23°C, and a light-dark cycle of 12 : 12.

Determination of Tissue Hydroxyproline : Lung tissue hydroxyproline contents of animals were determined with Stegemann-Stalder's Method (43). Among the methods for the determination of hydroxyproline the oxidation of this amino acid to a compound reacting with p-dimethylaminobenzaldehyde to form a chromophore is regarded as a relatively specific and sensitive assay. In the oxidation phase of hydroxypoline only Stegemann-Stalder and Woessner used chloramine-T, while others chosed hydrogen peroxide. It was shown that by using chloramine-T the same sample's hydroxyproline amount gave more sensitive result (23).

At the end of exposure period the lung was rapidly removed from guinea pigs after decapitation, and cut to small slices. The sample is placed in a pyrex tube; then heated so as to maintain a temperature of at least 70°C for at least 30 minutes. Four grams of the sample was taken into the hydrolysis flask. Hydrolysis was carried out in 6 N HCl at 107°C for 16 hours. Hot hydrolysate was filtered through filter paper into a 200 ml one-mark volumetric flask.

Flask and filter paper were washed three times with 10 ml portions of hot hydrochloric acid solution and the washings were added to the hydrolysate.Water was added and mixed, and 5 ml of the hydrolysate was diluted to 250 ml, so that the hydroxyproline concentration will be within the range 0.5 to 2 μg/ml. Two milliliters of the sample was mixed in a test tube with 1 ml chloramine-T solution both having a temperature of about 20°C.After 20 min, 1 ml of the perchloric acid solution is added and the mixture was shaked thoroughly. The tubes are immersed into a water bath at 60°C. After 15 min, the rack is cooled under tap water and within 45 min the color is read in Milton Roy UV-3000 Spectrophotometer at λ=560 nm. Hydroxyproline contents of the tissue samples were determined by using the standard curves which were plotted daily before each spectrophotometric reading by using the sample containing 0.5-2 μg/ml hydroxyproline (SIGMA H-1637).

RESULTS

Lung tissue hydroxyproline content of vertical and horizontal electric field applied groups were found from daily plotted standard curves. For each hydroxyproline determination two samples of the tissue taken from the homogenization step were studied seperately and the avarage of their spectrophotometric data was taken as the absorbance value of each sample. The avarage hydroxyproline concentration value was taken into consideration at the calculation step (Table 1).

Groups	X	sd	n
Control	1.374	0.539	20
Vertical electric field	2.306	0.814	10
Horizontal electric field	1.906	0.722	10

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

Statistical comparisons were made in two groups :

In the first group, hydroxyproline contents of lung tissues of electric field applied groups were compared with their controls with DUNCAN Test and the results were found as follows :

There is statistically significant difference between hydroxyproline contents of lung tissues of vertical electric field applied and control groups ($p<0.001$).Vertical electric field causes an increase in lung tissue hydroxyproline level :

Vertical = 2.306± 0.814

Control = 1.374 ±0.539

There is statistically significant difference between hydroxyproline contents of lung tissues of horizontal electric field applied and control groups ($p < 0.05$). Electric field also causes an increase in lung tissue hydroxyproline level :

Horizontal =1.906± 0.722

Control = 1.374 ± 0.539

It was observed that the vertical electric field increased hydroxyproline content of lung tissue more than the horizontal electric field.

In the second group, hydroxyproline contents of lung tissues of vertical electric field applied groups were compared with horizontal electric field applied group.

Statistically no significant difference is observed between hydroxyproline contents of lung tissues of vertical electric field group and horizontal electric field group ($p>0.05$).

DISCUSSION

The interaction of externally applied electric fields with living organisms has become an important subject for some decades. The fields can act only if physically present in or on tissue, and knowledge of their magnitude and distribution must be correlated with observed physiological responses before a deeper understanding of the interaction process itself can emerge. The structural complexity of even the simplest living systems, however, has prevented direct measurement of field distributions in tissue (33). Instead an approach to field distribution in tissue has been tried with modelling studies (11). The high resistivity of a cell membrane and the high conductivity of its fluid interior serve to concentrate the externally applied electric field across the portion of the membrane at right angles to the field. Some membranes carry structural surface charge which arises from the presence of ions on lipids, lipopolysaccharides, or proteins. Experimental results support the view that some properties of the bilayer membanes are influenced by electrostatic potential at the membrane. Such is the case with regard to membrane excitability, permeability, adsorption, adhesion and even membrane structure. The understanding and the theoretical description of these membrane properties, however, all depend on detailed knowledge of the electrostatic potential and of the electric field at and near the membrane surface (44).

Cellular organization and function can be described in terms of structural and regulatory interactions. On a molecular level interactions between proteins, lipids, carbonhydrates and other compounds can be described in terms of electromagnetic fields effects between participating molecules. Although the characteristics of the associated electromagnetic fields may be complex (particularly for large molecules, such as proteins), biological reactions are essentially electromagnetic. From this perspective, it is reasonable to consider that biological reactions may be influenced by electromagnetic fields (25). In vitro studies have shown that exposure to electric fields can increase cell proliferation and the results suggest that electric field may be transduced at the level of the plasma membrane (25).

Electromechanical force generated in the cell membrane by rapidly increasing electroforces can lead to temporary reversible electronic collapse of the membrane (40). DC fields of 10^3 V/m have been shown to move protein molecules along the surface of the membrane and through gap junctions (21, 39).

The localized electric field in saline-field scale models of human and animals are measured by Kaune and Forsythe. The vertical currents dominate and are fairly uniform across cross sections of the body. However there are small horizontal current components and in the axillae region the horizontal currents dominate. Measurements in saline-filled pig and rat models indicated increased horizontal current components arising from the horizontal orientation of the animal bodies (11).

In experimental studies on vertical and horizontal electric field applications on animals, since induced currents are not possible to measure ; mortality percentage, the changes in body weight and blood protein fractions, lipid peroxide levels, and protein synthesis are investigated (9, 10, 28, 29, 30, 31, 34, 35). In vivo studies on man, animal and bacteria with DC and AC applications also exist (2, 3, 4, 5, 6, 7, 8, 13, 14, 15, 16, 17, 18, 19).

The electrical properties of dried collagen and bone have been studied and both were shown to be piezoelectric (27). Nessler reported an increase in proline and hydroxyproline levels in rabbit flexor tendon by applying 7 µA DC along 7,14,21 and 41 days (37). DNA synthesis in epiphyseal cartilage cells increased in an electric field of 166 V/cm (40). Fitzsimmons observed the changes in hydroxyproline level in embryonic chick tibia with, 10^{-5} V/m the effect of electric field (25).

Marino found alterations in body weight, mortality, and blood protein fractions of mice under the effect of vertical and horizontal directions of exogenous electric field (34, 35). He found vertical E field application is more effective to blood protein fractions than the horizontal one although the difference was not found significant.

In this study vertical and horizontal application of E field with the intensity of 1.9 kV/m had been found to increase hydroxyproline content of lung tissue. The increase in hydroxyproline content of vertical E field application was more than the horizontal field although it was statistically insignificant, suggesting that the vertical E field might be more effective than the horizontal application.

Since the tissues like lung, kidney 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 which is a result of polar structure of water. This structure of lung might also been effective on the influence of E field on lung tissue (42).

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